Contributions to Microbiology
Editors: A. Schmidt, H. Herwald
Vol. 13

Infection and Inflammation: Impacts on Oncogenesis

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T. Dittmar
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KARGER

Contributions to Microbiology

Vol. 13

Series Editors

Axel Schmidt *Witten*
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Infection and Inflammation: Impacts on Oncogenesis

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26 figures, 6 in color, and 5 tables, 2006

KARGER

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Contributions to Microbiology
formerly 'Concepts in Immunopathology' and
'Contributions to Microbiology and Immunology'

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Library of Congress Cataloging-in-Publication Data

Infection and inflammation : impacts on oncogenesis / volume editors, Thomas Dittmar, Kurt S. Zaenker, Axel Schmidt.
p. ; cm. – (Contributions to microbiology, ISSN 1420-9519 ; v. 13)
Includes bibliographical references and index.
Memoriam contains some German text.
ISBN 3-8055-8064-9 (hard cover : alk. paper)
1. Carcinogenesis. 2. Inflammation. 3. Cancer–Immunological aspects. 4. Microbial carcinogenesis.
[DNLM: 1. Neoplasms–etiology. 2. Cell Transformation, Neoplastic. 3. Infection–complications. 4. Inflammation–complications. QZ 202 I42 2006]
I. Dittmar, Thomas. II. Zaenker, Kurt S. III. Schmidt, Axel, 1962- IV. Series.
RC268.5.I49 2006
616.99′4071–dc22

2005035136

Bibliographic Indices. This publication is listed in bibliographic services, including Current Contents® and Index Medicus.

www.karger.com
Printed in Switzerland on acid-free paper by Reinhardt Druck, Basel
ISSN 1420–9519
ISBN 3–8055–8064–9

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In Remembrance of Rudolf Virchow (1821–1902)

In 1863, Rudolf Virchow postulated in his well-recognized comprehensive publication ‘Die krankhaften Geschwülste – Malignant Neoplasias’ that inflammation is one of the predisposing factors of tumor genesis. He also noted that infectious diseases such as syphilis and tuberculosis show signs of a ‘tumor

process' and were often difficult or even impossible to separate from a 'genuine' malignant and/or benign tumor process. Virchow's hypothesis has almost been forgotten and ignored for more than a hundred years, but experienced a renaissance in the past 10 years.

Axel Schmidt

Foreword

Obtaining knowledge on the etiopathology of neoplasias and trying to elaborate a consistent explanation for neoplastic syndromes is a scientific and public issue which might be as old as mankind itself.

In this current volume of the Karger book series *Contributions to Microbiology*, we give an up-to-date overview about the aspect of the connection between inflammation and cancer. This connection was originally postulated by the German physician and pathologist Rudolph L.C. Virchow in 1863 in his well-recognized comprehensive publication 'Die krankhaften Geschwülste – Malignant Neoplasias'. Virchow recognized inflammation to be one of the predisposing factors of tumor genesis. He also noted that infectious diseases such as syphilis and tuberculosis show signs of a 'tumor process' and were often difficult or even impossible to separate from a 'genuine' malignant and/or benign tumor process.

Virchow's hypothesis has been almost forgotten and ignored for more than a hundred years, but has experienced a renaissance in the past 10 years. Because of the increasing knowledge about the inflammatory micro-environment, it is now generally accepted that carcinogenesis is more than a simple summation of mutation events in single cells. In fact, cancer is the result of a sustained proliferation of cells embedded in an environment rich in inflammatory cells, DNA-damage-promoting agents, cytokines, and chemokines, and which can be followed from a chronic infection with pathogens such as *Helicobacter pylori* or *Schistosoma haematobium*. Moreover, it is becoming clearer and clearer that the chronic inflammatory microenvironment does not exert its transforming

capacity on differentiated tissue cells, but rather on undifferentiated cells. In other words: Cancer might be a stem cell-based disease. Recent results substantiate this hypothesis by showing that bone marrow-derived stem cells can give rise to gastric cancer in the presence of a chronic *Helicobacter pylori* infection. Thus, Virchow's hypothesis has received new impact, which will definitely have implications on future pathological research and therapeutic options which are based on the use of bone marrow-derived stem cells for tissue function restoration.

However, increasing knowledge on the inflammatory microenvironment and on the dynamic interplay of growth factors and chemokines in the growth, migration, and organ-specific spreading of tumors is starting to have implications in both cancer prevention as well as cancer treatment. Clinical trials are currently underway and the results are encouraging. Anti-inflammatory-based strategies are efficacious in preventing neoplastic progression and malignant conversion, and inhibition of the interplay of chemokines and their receptors reduces metastasis.

We are glad that so many internationally recognized experts accepted our invitation to contribute to this exciting volume. We sincerely thank them all for their interest in this important topic and that they, despite their other duties and responsibilities, found the possibility to present us with excellent and comprehensive overviews of the most important recent findings in their field of scientific engagement within this topic.

We further thank Mr. *T. Nold* and Mr. *F. Brian* from Karger Publishers for their helpful assistance and excellent collaboration with this challenging project.

We hope that this volume may encourage new scientific approaches within this interdisciplinary field of oncology/tumor pathology, immunology, inflammation, and infectious agents as well as closer interdisciplinary collaboration on this fascinating and important medical and pathophysiological issue in the future.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 1–15

In Memoriam of Rudolf Virchow: A Historical Retrospective Including Aspects of Inflammation, Infection and Neoplasia

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Abstract

Rudolf Ludwig Carl Virchow (1821–1902) studied medicine and received his academic degree 'Dr. med.' in 1843. In 1856 he was appointed as head of the institute of pathology at the University of Berlin. In 1859, he became a member of the Berlin town council and later additionally a member of the Prussian and the German parliament. With his probably most important publication '*Cellularpathologie*' he introduced pathology to a cellular rationale. This was the major basis for his research in oncology. Virchow further studied aspects of inflammation, despite only few links to tumor pathology were drawn. The few links from infection and inflammation to tumor pathology have almost been forgotten or ignored and have never been evaluated and discussed sufficiently. Virchow recognized that inflammation is a pre-disposing factor for tumor genesis. Furthermore, infectious diseases such as syphilis and tuberculosis had elements of a 'tumor process' and were therefore often difficult or impossible to separate from a 'genuine' tumor process, which was recognized by him. He further tried to explain tumor dissemination by an 'infectious' process. Additionally, there were ideas for a coherent explanation of tumor etiology in form of a common bacterial pathogen ('Krebsbacillus').

Note of Comment on Rudolf Ludwig Carl Virchow

A great physician, a revolutionary politician, an outstanding scientist and a hidden philosopher – Rudolf Ludwig Carl Virchow: The reformer and revolutionary ascetically serving mankind, forcefully straight forward and without any concessions. After extensive studies of many biographies of outstanding persons, not only within medicine, Virchow convinces as a brilliant of human career and character.

Who Was Rudolf Ludwig Carl Virchow?

Rudolf Ludwig Carl Virchow was born on October 13th 1821 in Schivelbein/Pommern (part of 'former Germany'; today: Swidwin/Poland) and died on September 5th 1902. In the time period between 1839 and 1843 he studied medicine at Berlin and received his academic degree 'Dr. med.' in 1843. In 1844, he became an assistant to the pro-sector in Berlin and took over responsibilities as a pro-sector at Berlin Charité University in 1846. In 1847, Virchow became a certified university teacher and lecturer ('Habilitation' and 'Privatdozent'). Due to Virchow's liberal political activities, he was suspended from this position and was appointed as head of the institute for pathological anatomy at Würzburg University in the same year. Virchow married in 1850 and went back to Berlin in 1856. He was appointed head of the new institute of pathology at Berlin University within this year. In 1893 Virchow visited the United Kingdom and gave a presentation to the 'Royal Society'. Virchow broke his hip after jumping from a tram in a hurry in Berlin in 1902. He never recovered from this tragic accident and died on September 5th 1902 [1–6]. Virchow's attitudes to medicine, pathology and sciences were strongly influenced by his teacher and pathologist, Johannes Müller [7–15]. Virchow's publication '*Cellularpathologie*' (1858) [16] is today recognized as his most outstanding scientific publication. This contribution opened a new era of scientific insights into pathological processes which brought older approaches to a cellular-orientated approach. This was one of the most important changes in paradigm of scientific insights especially in the rapidly developing medicine at this time.

Virchow coined the scientific career of many famous and highly recognized scientist such as Wilhelm His (1831–1904), Friedrich Daniel von Recklinghausen (1833–1910), Edwin Klebs (1834–1913), Ernst Haeckel (1834–1919), Georg Eduard von Rindfleisch (1836–1908), Nicolaus Friedreich (1825–1882), Adolf Kussmaul (1822–1902), and Hugo Wilhelm von Ziemssen (1829–1902) [2].

Virchow had a broad spectrum of interests. His main topic was research in pathology on the cellulary orientated basis [16, 17]. He used the insights especially for his studies in tumor pathology [18–30]. Monumental is his three volume book '*Die krankhaften Geschwülste*' (The malignant neoplasias, lectures from 1862/1863) [18–20]. Of further scientific significance are Virchow's insights in inflammation [31, 32]. In addition to pathology, hygiene, Public Health issues and bacteriology were a scope of Virchow's scientific career [33–39]. Virchow understood himself as a physician and was very active and engaged also with his obligations as a practicing physician as he always highlighted that only the combination of clinics and pathology can give sufficient insights into the etiopathology of diseases [40–43]. Virchow further brought the medical terminology – especially within the field of pathology – to a new rationale [44–46].

In the second half of his life, Virchow did research on the fields of 'anthropology' and archaeology. His personal understanding of 'anthropology' was rather distinct and covered aspects of anthropometry, ethnology, anthropogeny, archaeology, and paleonthology especially of primates [47–55]. His major experimental focus within this field was anthropometry and performing diverse archaeological excavations at pre-historical sites. Virchow accompanied Heinrich Schliemann (1822–1890) and Wilhelm Dörpfeld (1853–1940) on several of his archaeological expeditions (e.g. Troja 1879, Egypt 1888) [56–61]. Overall, Virchow did significant contributions to the history of medicine and sciences all over the world [62–73].

Virchow's importance in politics was not minor to that in medicine and sciences. His 'real political career' started when he retuned to Berlin in 1856 [2, 74–77]. He understood his activities as an inner 'political mission and concern'. This is reflected that he was elected as a member of the Berlin town council in 1859 where his major responsibilities were on health issues and statistics. Further, in 1861 he was additionally elected as a member to the Prussian parliament where he rapidly took over the key function as head of the budget commission. In 1861, he was enthusiastic in founding the new liberal political party, the 'Deutsche Fortschrittspartei'. This understanding of Virchow's towards a liberal state brought him into deepest conflicts of interests with the German chancellor and minister of the exterior, Fürst Otto von Bismarck (1815–1898). This public dispute culminated in Virchow's talk to the Prussian parliament in 1865, where he articulated his doubts whether Bismarck was standing behind the interests of the German Nation. Virchow became so obsessed with this issue, that a duel between Virchow and Bismarck could only be prevented by the Prussian defense secretary. Virchow also pinpointed the 'holy' encyclica 'Quanta Cura', released by pope Pius IX in 1864, which significantly hindered the development process in modern liberalism, understanding of democracy, and consecutively also with sciences.

Virchow's political aim was to contribute to a united Germany, and showed this in being a co-founder of the German 'Nationalverein'. He fought for pacifism and for the significant reduction of the military budget in order to shift this budget to, in his view, more important areas improving traffic, Public Health issues, medicine, and education. This idea conflicted with the opinion of Bismarck and the German Emperor and Prussian King Wilhelm I (1871–1888), which made Virchow an extremely unpopular and uncomfortable person for both of them. Virchow's aversion against Bismarck lasted life-long and was even not diminished after Bismarck's unpopular suspension from all his political obligations by the German Emperor and Prussian King Wilhelm II (1859–1941) on March 18th 1890. For the time period between 1880 and 1893 Virchow was additionally active as an elected member of the German parliament and articulated his concerns about the senselessness of German colonialism

('Schutzgebiete'). He articulated the appeal to a liberalization of politics and the strengthening of activities in sciences and medicine in Germany. Additionally, Virchow was active as a publicist [78], most obviously with his revolutionary medical newspaper 'Die Medizinische Reform' (The Medical Reform), a publication he founded in 1848. Overall, becoming older and approaching the end of his life, Virchow was full of disappointment and had growing inner conflicts especially with politics. An overview about further literature on and original documents from Virchow is given by Kirsten [79].

Virchow and Tumor Pathology

It is out of the scope of this chapter to review Virchow's extensive research activities in the field of tumor pathology. Details about his achievements in tumor pathology are already extensively reviewed [25–30]. Instead, exemplarily, a case of tumor pathology (neurofibromatosis von Recklinghausen; fig. 1), described by Virchow [18, pp 325–327], including the corresponding figures, will be presented in the following part. To cite an original case as evaluated by Virchow appears relevant as it exemplarily reflects the style of how Virchow approached and evaluated cases in pathology and reflects the medical standard in oncology at this time:

Eine 47jährige Frau trug auf ihrem ganzen Körper zerstreut eine grosse Masse kleinerer und grösserer Gewächse, welche sich seit Jahren langsam entwickelt hatten. Viele von ihnen waren ganz klein, erbsen- bis kirschkerngross, rund und von glatter Haut bedeckt; andere waren grösser, wallnussgross und darüber, übrigens von gleicher Beschaffenheit. Das grösste sass links in der unteren Rippengegend mit breiter Basis auf; es hatte 48 Zoll vom Rückgraht. Es hing von da tief nach unten über die Hüfte herab. An seiner Oberfläche und in seinem Umfange trug es mehrere kleine Secundärknoten; im Ganzen war die es bedeckende Haut aber glatt und verhältnismässig dünn. Dabei fühlte es sich weich, fast fluktuirend an. Nachdem es (von Herrn Kreisphysikus Dr. H e y l a n d in Guben) exstirpirt war, wog es 32½ Pfund. Neun Jahre früher war es Kindskopfgross gewesen.

Die Untersuchung ergab auch hier wieder ein sehr saftreiches, im Allgemeinen nur wenig gefässreiches, lockeres Bindegewebe, welches hauptsächlich die Region des alten Panniculus adiposus einnahm. Aus ihm liess sich eine grosse Menge gelblicher, eiweissreicher Flüssigkeit mit Leichtigkeit ausdrücken. Das Gewebe zeigte selbst schon für das blosse Auge eine gewisse Ungleichmässigkeit. Derbere, weissliche Züge, in welchen etwas grössere Gefässe verliefen, umschrieben grössere Räume (Areolen), welche ihrerseits wieder von einem feinmaschigen Fasernetz durchzogen waren und, von demselben umschlossen, den ausdrückbaren Saft enthielten. Bei einer schwachen Vergrösserung zeigte sich diese Anordnung überaus deutlich ('Fig. 57'; fig. 2). Die feineren Fasernetze gingen mit breiteren Ansätzen aus den dichteren und breiteren Faserzügen der Umgebung hervor, und es entstand so eine Art von lappiger Anordnung, welche auf die Entstehung dieser Maschen aus den früheren Fettlappen hinwies. Bei stärkerer Vergrösserung fand sich nur Bindegewebe mit beträchtlich gewachsenen Körperchen vor.

Fig. 1. Title photogravure (‘Titelkupferstich’): Fibroma molluscum multiplex. Reprint of the front illustration (photogravure) of volume 1 ‘The Malignant Neoplasias’ (‘Die malignen Geschwülste’) showing a patient with a tumor disease (fibromatosis) entitled ‘Fibroma molluscum contagiosum’ deriving from the 13th lecture on tumors (‘Molluscum’). This case reflects an elephantiasis neuromatosa with plexiform neurofibromatas in a case of a neurofibromatosis von Recklinghausen [80]. Original size.

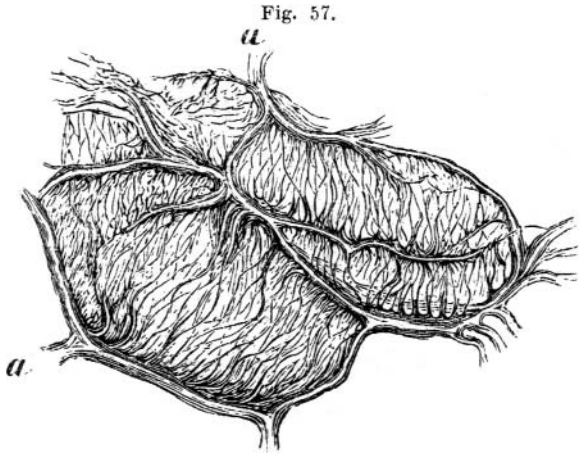

Fig. 2. Wood-cut engraving of this case. Entitled 'Fig. 57. Fibroma molluscum. Von dem auf dem Titelkupfer abgebildeten Falle; ein bei 20 facher Vergrösserung gezeichneter Durchschnitt aus der inneren Substanz der grossen, hängenden Geschwulst. ***a***, ***a*** grössere Balken mit Gefässen; dazwischen das maschige Fasernetz von bald dichteren und breiteren, bald feineren und weiten Balken (Präparat No. 32. vom Jahre 1862).' (Entitled Fig. 57. Fibroma molluscum. Deriving from the clinical case presented on the front cover: Cut through the inner substance of the extensively hanging tumor. Magnification ×20. a = Big barks with vessels; in between fibers of dense to broad until fine to separated barks (pathological archive #32/1862). Original size.)

Die kleineren Knoten der Oberfläche ergaben sich bei Einschnitten als ganz unabhängige, mit den grossen Gewächsen in gar keinem Zusammenhange stehende Gebilde. Sie lagen theils in der Tiefe, zum grossen Theil aber ganz oberflächlich in der Cutis selbst. Manche gingen offenbar von der äussersten Schicht der Cutis aus, denn sie berührten beinahe das übrigens unveränderte Rete Malpighii, während sie von dem Unterhautfettgewebe noch durch eine gewisse Derma-Lage getrennt waren ('Fig. 58'; fig. 3). Sie hatten frisch ein blassgelbröthliches, weiches und feuchtes Ansehen; das Mikroskop zeigte darin ein zellenreiches, in voller Wucherung begriffenes Granulationsgewebe.

Vergleicht man diese Bildung mit der Elephantiasis der Genitalien, so leuchtet die Analogie ein, nur stimmt der in der Regel ganz fieber- und entzündungsfreie Verlauf nicht. Denn die Entwickelung erfolgt meist ganz langsam und unmerklich. Trotzdem lässt sich eine Grenze nicht ziehen, da auch die Elephantiasis vulvae nicht selten in ähnlicher Weise verläuft. Nichtsdestoweniger habe ich nichts dagegen einzuwenden, wenn man diese Form abtrennen will; der passende Name würde dann F i b r o m a m o l l u s c u m sein.

(The body of a woman, 47 years of age, was totally covered by a huge mass of small-to-large efflorescences which developed slowly within years. Many of them were very small, the size of a pea or a cherry-stone. They were round and covered by a smooth cutis. Other efflorescences were larger, of the size of a walnut or even larger and of the same consistency. The location of the largest

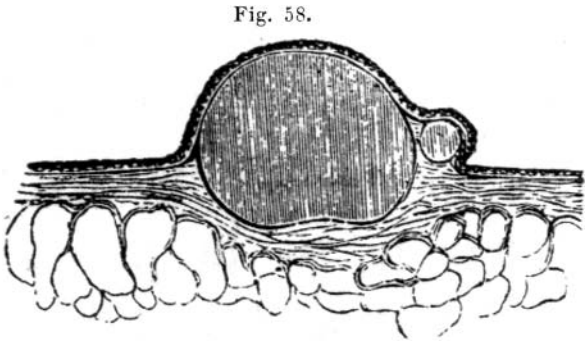

Fig. 3. Wood-cut engraving of this case: Entitled 'Fig. 58. Fibroma molluscum. Zwei accessorische Hautknoten, inmitten der Cutis entwickelt. Natürliche Grösse. Von demselben Fall wie Fig. 57.' (Entitled Fig. 58. Fibroma molluscum. Two accessory cutaneous lymphnodules within the cutis. Original size. Derived from the same case as Fig. 57.) Original size.

one was on the left side within the lower rib region and had a broad basis. The size was 48 inches when measured from the spinal cord. The efflorescence hung downwards lower than the hip. On its surface and on its circumference it was covered by multiple secondary nodules. In total, the covering cutis was smooth and relatively thin. When examined by palpebration, it was smooth and had almost a fluctuating consistency. After resection (by the responsible surgeon Dr. Heyland in Gruben) it had a weight of 32.5 pounds. Nine years before, the tumor had a size of a child's head. The pathological examination again showed a loose connecting tissue which contained a lot of liquid but only a small amount of vessels. This seemed to be the region of the former 'Panniculus adiposus'. Under pressure, a huge amount of yellow, protein containing liquid was released easily. Larger cavities ('Areolen') were formed by stronger, white tissue fibers, in which the larger vessels were located. These were penetrated by a dense network of smaller fibers containing the expressible liquid. This organization could be excellently studied under smaller magnification ('Fig. 57'; fig. 2). The smaller network of fibers developed with a broad basis out of the dense and stronger tissue fibers of the surrounding tissue. In total, the efflorescences had a lobular habitus which made it most probable that these lobes developed out of the former fatty tissue lobes. Under stronger magnification, connecting tissue with significantly enlarged bodies became evident. The smaller nodules of the surface – when histologically cut – showed to be not related to the large efflorescences. Some were located in deeper areas, despite most of them being located superficially within the cutis. Some of them seem to have developed from the outer part of the cutis as they almost reached the unchanged 'Rete malpighii' and were separated from the subcutaneous fatty

tissue through parts of the cutis (‘Fig. 58’; fig. 3). In the fresh material they were of pale yellow to red color, smooth and moist. Microscopically, they contained a cell-rich, massively proliferating granulomatous tissue. This efflorescence shows a strong homology with the elephantiasis of the genital region despite of the difference that this case does not show any signs of fever or inflammation as the development occurred very slowly and almost unremarkable. Nevertheless, a clear differentiation is not possible in so far as the ‘Elephantiasis vulvae’ may also develop in a comparable manner. Despite all of this, I see this case as a separate disease. The appropriate nomenclature would be ‘Fibroma molluscum’.)

Virchow’s Theories about the Impact of Infection and Inflammation on Oncogenesis

Lots of historical studies have focused on Virchow’s achievements in tumor pathology and with minor impact also on the field of inflammation, despite Virchow’s ideas on the impact of infection and inflammation on tumor pathology have almost been forgotten or ignored and have never been evaluated and discussed sufficiently. Four different key issues of infection and inflammation in Virchow’s tumor theory became evident to us and are reflected below.

Inflammation

Virchow published in 1863 [18, p 65]:

‘Ferner wissen wir, dass an Schleimhäuten am häufigsten die Geschwülste gerade an solchen Stellen vorkommen, welche vorher der Sitz einfach entzündlicher Erkrankungen waren, die ausreichten, um nach und nach die natürliche Structur der Theile zu verändern. Aus der einfach entzündlichen Hyperplasie des chronischen Katarrhs geht die Bildung von Polypen hervor und die Polypen können später wieder der Sitz krebsiger oder kankroider Entwickelung werden.

(Furthermore, we know that most of the tumors develop on formerly simple mucocutaneous lesions, where a normal inflammation occurred prior to it. Parts of these tissues were altered by the inflammatory process. Out of the ‘normal’ inflammatory hyperplasia of a chronic ‘catarrh’, polypous alterations may develop. Theses efflorescences may later on convert into cancer.)

Syphilis and Tuberculosis

Virchow [18, pp 76–78]:

Das am meisten charakterisierte Beispiel, das wir dafür besitzen, bietet uns wohl die Geschichte der Syphilis, welche hier um so mehr in Betracht zu ziehen ist, als bekanntermassen im Laufe der Lues wirkliche Geschwülste entstehen, die unter Umständen überaus schwer zu unterscheiden sind von anderen Geschwülsten, daher nicht ganz selten Veranlassung zu falschen Diagnosen geben und auch leicht zu einem falschen praktischen Handeln führen können. (. . .) Ganz ähnlich verhält es sich auch mit anderen, sogenannten Dyskrasien. Ich erinnere an die Tuberculose, bei der freilich die Dyskarasie nicht

unmittelbar nachweisbar ist, wo ihr Bestehen aber wenigstens nach einer Art von Consensus omnium als selbstverständlich angenommen wird.

(The most outstanding example, we possess thereof, is the history of syphilis. This disease is in so far of major importance as we know that under this disease real tumors may develop, which are often only very difficult to differentiate from other tumors and therefore may lead to wrong diagnoses and therapeutic approaches. (. . .) Nevertheless, a comparable issue is with other diseases ('Dyskrasien') such as tuberculosis. Despite in case of tuberculosis, characteristic tumor lesions ('Dyskrasien') cannot be directly determined; their existence can be anticipated as a 'Consensus omnium'.)

Generalization and 'Infection'

Virchow [18, p 127]:

Je ärmer eine Geschwulst an Gefässen ist, um so mehr wird sie nur die Nachbarschaft inficiren; je reicher sie aber an Blut- und Lymphgefässen ist, je mehr Blut und Lymphe hindurchströmt, je mehr das Blut in Berührung kommt mit den Parenchymsäften, um so leichter wird die Infection eine allgemeinere werden können.

(The less a tumor is vascularized, the more the tumor will only infect the surrounding tissue. The more blood circulates and lymphatic vessels are within a tumor, that means, the more blood and lymphatic liquid circulates through the tumor, and the more the blood comes into contact with the parenchymal liquids, the easier the infection will become generalized.)

Further, he published concerning this issue [18, pp 51–52]:

Meiner Ansicht nach ist gerade das Stadium der localen Vergrösserung der Knoten einer der entscheidendsten Beweise für die infectiöse Natur der Stoffe, welche in der Geschwulstsubstanz entstehen; und die Bildung dieser neuen Heerde, oder, was man kurzweg das Wachsthum der Geschwulst genannt hat, das ist für mich genau dasselbe, wie die Erkrankung der Lymphdrüsen und entfernter Organe im Laufe der Generalisation. In allen drei Fällen haben wir eine A n s t e c k u n g, eine Art von Contagion, wo ein Ansteckungsstoff, eine infectiöse Substanz, ein 'Miasma' von dem Ort der ersten Bildung aus sich verbreitet, theils auf dem Wege der direkten Imbibition, der einfachen Endosmose in die Nachbarschaft, theils auf dem Wege der Lymphströmung zu den nächsten Lymphdrüsen, theils auf dem Wege der Blutcirculation durch die Venen.

(To my concern especially the locally restricted growth of the tumor mass on its own is one of the most important criteria and proofs for the 'infectious' nature of the substances which are released by this tumor mass. Growth of new lesions and the growth of the primary tumor seem to be of similar origin to me as well as the affection of the lymphatic glands and other organ dissemination during the stage of generalization. In all three cases we have a kind of 'infection', a kind of 'Contagion', where an 'infectious' agent or an 'infectious' substance, a so-called 'Miasma', is generalized from the original, primary tumor site. This may happen by direct distribution into the surrounding tissue, through lymphatic dissemination or through hematogenous dissemination via the veins.)

The 'Krebsbacillus'

In 1888, Virchow [24, pp 18–19] published:

Eine ganz andere Bedeutung würde es haben, wenn es gelänge in dem Krebs irgend eine andere Substanz zu entdecken, welche geeignet wäre, die alten Vorstellungen von einer specifischen Schädlichkeit wieder aufleben zu lassen. Die seit einer Reihe von Jahren immer zahlreicher werdenden Nachweise parasitärer Mikroorganismen in krankhaften Theilen haben bei Vielen die immer zuversichtlicher auftretende Hoffnung erregt, es werde sich auch ein Krebsbacillus finden lassen. Bis jetzt sind die Ergebnisse auch der eifrigsten Forschung noch nicht in einer überzeugenden Demonstration vorgelegt worden. Indess ist die Möglichkeit eines solchen Vorkommens nicht einfach abzuweisen; ja, man kann zugestehen, dass mit dem Auffinden eines specifischen Bacillus ein wichtiger Fortschritt in der Diagnose und Prognose des Carcinoms gemacht werden würde. Der Versuch, alle Erscheinungen der Krebswucherung bis zur Dissemination und Metastase auf die Verbreitung von Krebszellen zurückzuführen, ist keineswegs durch anatomische oder experimentelle Feststellungen so sicher unterstützt, dass für einen anderen Modus der Erklärung kein Raum übrig bliebe. Umgekehrt ist aber auch das Bedürfnis nach einem Krebsbacillus kein so grosses, dass wir ohne denselben jeder Möglichkeit eines Verständnisses beraubt sein würden. Thierische oder menschliche Zellen besitzen ebenso gut, wie Bakterien, die Fähigkeit, auf den Stoffwechsel bestimmend einzuwirken und wirkungsfähige Secretstoffe der verschiedensten Art zu erzeugen. Warum sollten wir gerade diese Fähigkeit bei den Krebszellen bestreiten, welche in vielen und gerade den schlimmsten Fällen in so ausgeprägtem Maasse den Habitus von Drüsenzellen an sich tragen?

(The identification of a specific substance responsible for cancer induction would have significant impact. This would allow us to continue our old visions about a specific cause within malignant diseases. In the past years a rising amount of parasitic micro-organisms have been detected in affected tumor sides. This encouraged many of us that it might be possible to find a specific micro-organism ('Krebsbacillus') responsible for it. Despite intensive research attempts on this issue, there has been no evidence for this hypothesis until now. Nevertheless, we also cannot reject the possibility of a prevalence of such a micro-organism. Finding such a specific micro-organism would be a significant innovation concerning diagnosis and prognosis of cancer. From the anatomical point of view, and as known from experimental approaches, there is no certainty that all pathophysiological effects observed in neoplasias – including dissemination and metastasation – have to be due to the tumor cell on its own. There is, of course, enough space for alternative explanations. On the other hand, the necessity for a specific responsible micro-organism is not that big that we would not have a chance for understanding the etiopatology of neoplasias without of it. Human cells and cells of other mammalian origin can comparably adapt, like bacteria, to change metabolism and are able to secrete substances of different origin. Why should we have doubts concerning cancer cells not to have these abilities, as especially their habitus is very much in common with glandular cells?)

Discussion

In the recent literature, it is cited: 'In 1863 Virchow hypothesized that the origin of cancer was at sites of chronic inflammation, in part based on his hypothesis that some classes of irritants, together with the tissue injury and ensuring inflammation they cause, enhance cell proliferation' [81] or 'It was in 1863 that Rudolf Virchow noted leucocytes in neoplastic tissues and made a connection between inflammation and cancer. He suggested that the 'lymphoreticular infiltrate' reflected the origin of cancer at sites of chronic inflammation' [82].

What is right about these statements in recent literature? Were they really so detailed as cited? Or is it the result of a more than hundred years' citation cascade in introductions of scientific papers?

It is only fact that Virchow recognized that inflammation is one of the predisposing conditions for a tumor process. He identified more than ten of those factors and did not put any outstanding or specially detailed emphasis on the aspect of inflammation in oncology out of these different factors [18]. He saw the 'tumor process' within syphilis and tuberculosis, and recognized that it was sometimes impossible to make a clear cut between a 'genuine tumor' and a tumor process associated with an infection [18]. Furthermore, the termini 'dissemination' and 'infection' were unfortunately almost simultaneously used by him for the aspect of metastazation. Virchow even postulated that an 'infection' may be the reason for tumor dissemination and not the tumor or tumor cell on its own, which was unfortunately even a form of regression towards Galen's theory of 'Humoralpathology' [25]. In this case the definition of the word 'infection' from Virchow's perspective remains unclear. Most evident, Virchow speculates on 'substances released by the tumor mass' ('*Contagion*', '*Miasma*') despite these speculations on generalization/metastazation are overall ranging from responsible micro-organisms to specific tumor cells on their own. The speculation about a specific pathogen ('Krebsbacillus') as a common reason or agent for all neoplasias nowadays appears to be nothing more than a form of 'capitulation' in Virchow's late tumor pathology research approaches and the hope and wishes for an easy mono-causal and comprehensive explanation of tumor etiopathology in 1888 [24]. This publication in 1888 [24] was indeed Virchow's last significant contribution to pathology and oncology, a time when he had already put his key interests on other topics.

Acknowledgements

As the original citations deriving from Virchow cannot be translated into the recent German or English language without significant changes of their meaning, it showed to be un-avoidable to include the original citations and wording as published by Virchow within this chapter. Big efforts have been undertaken in order to translate these citations into an

English version, which meets best with the version as formulated by Virchow. Nevertheless, changes within the contents were unavoidable by the translation procedure. In questionable cases, the reader should attempt to refer to the original, old German version in order to avoid misunderstandings.

Herewith, we further want to express our outstanding thanks to all the numerous private persons and institutions who gave us access to historical documents which are in private ownership. Outstanding thanks shall also be addressed to the staff of 'The Library of Congress', Washington, D.C.

References

1 Lubarsch O: Rudolf Virchow and his opus (Rudolf Virchow und sein Werk). Berl Klin Wochenschr 1921;58:1345–1349.
2 Schipperges H: Rudolf Virchow, ed 2. Reinbeck, Rowolt, 2003.
3 Vasold M: Rudolf Virchow. The Famous Physician and Politician (Rudolf Virchow. Der große Arzt und Politiker). Stuttgart, Enke, 1988.
4 Ackerknecht EH: Rudolf Virchow: Physician, Politician and Anthropologist (Rudolf Virchow: Arzt, Politiker, Anthropologe). Stuttgart, Enke, 1957.
5 Diepgen P: The universality of Rudolf Virchow's opus (Die Universalität von Rudolf Virchows Lebenswerk). Virch Arch 1952;322:221–232.
6 Schipperges H: Rudolf Virchow and the reformation of medicine (Rudolf Virchow und die Reform der Medizin). Wege neuen Heilk 1978;1:142–153.
7 Virchow R: Johannes Mueller: A Remeberance Talk on His Funeral on July 24th 1858 (Johannes Müller: Eine Gedächtnisrede bei der Todtenfeier am 24. Juli 1858). Berlin, Hirschwald, 1858.
8 Virchow R: Relationship between pathological anatomy and clinical disciplines (Ueber die Stellung der pathologischen Anatomie zu den klinischen Disciplinen). Verh Dtsch Path Ges 1899;1:1–6.
9 Virchow R: Approaches deriving from natural sciences influencing therapeutic approaches (Die naturwissenschaftliche Methode und die Standpunkte in der Therapie). Arch Path Anat 1849;2:3–37.
10 Virchow R: The position of pathology among biological studies. Proc R Soc 1893;53:114–129.
11 Virchow R: The necessity and validity of a medical approach with mechanical orientation (Über das Bedürfnis und die Richtigkeit einer Medizin vom mechanischen Standpunkt). Virch Arch 1907;188:1–21.
12 Löhlein M: Rudolf Virchow and the development of aetiology based research approaches (Rudolf Virchow und die Entwicklung der ätiologischen Forschung). Virch Arch 1921;235:225–234.
13 Rath G: Struggle between 'Zellularpathologie' and 'Neuralpathologie' within the 19th century (Der Kampf zwischen Zellularpathologie und Neuralpathologie im neunzehnten Jahrhundert). Dtsch Med Wochenschr 1957;82:740–743.
14 Schmidt MB: Virchow's pathologic-anatomic reasearch approaches concerning diseases of the sceletal system (Virchows pathologisch-anatomische Forschungen über die Erkrankungen des Skelettsystems). Virch Arch 1921;235:273–297.
15 Pagel W: Virchow and the philosophy of pathology. Bull Hist Med 1945;18:1–43.
16 Virchow R: Cellular Pathology (Cellularpathologie). Reprint of the edition from 1858. Hildesheim, G. Olms, 1966.
17 Virchow R: Handbook of Special Pathology and Therapy (Handbuch der speciellen Pathologie und Therapie). Erlangen, Enke, 1854.
18 Virchow R: The Malignant Neoplasias. Thirty Lectures Hold Within the Winter-Semester 1862–1863 at Berlin University. Lectures 1–16 (Die krankhaften Geschwülste. Dreissig Vorlesungen gehalten während des Wintersemesters 1862–1863 an der Universität zu Berlin. Vorlesungen 1–16). Berlin, A. Hirschwald, 1863, vol 1.
19 Virchow R: The Malignant Neoplasias. Thirty Lectures Hold Within the Winter-Semester 1862–1863 at Berlin University. Lectures 17–21 (Die krankhaften Geschwülste. Dreissig

Vorlesungen gehalten während des Wintersemesters 1862–1863 an der Universität zu Berlin. Vorlesungen 17–21). Berlin, A. Hirschwald, 1864/1865, vol 2.
20 Virchow R: The Malignant Neoplasias. Thirty Lectures Hold Within the Winter-Semester 1862–1863 at Berlin University: Strumata, Myomata, Neuromata, Angiomata. Lectures 21–25 (Die krankhaften Geschwülste. Dreissig Vorlesungen gehalten während des Wintersemesters 1862–1863 an der Universität zu Berlin: Strumen, Myome, Neurome, Angiome. Vorlesungen 21–25), vol 3, first half ('Erste Hälfte'; second half has never been published). Berlin, A. Hirschwald, without year of publication within the volume.
21 Virchow R: Ätiopathology of cancer and theories about fat-genesis within animals including forms of pathological resorption (Zur Entwicklungsgeschichte des Krebses nebst Bemerkungen über Fettbildung im thierischen Körper und pathologische Resorption). Arch Pathol Anat 1847;1:94–204.
22 Virchow R (reported by Liman F): Miscellanious (Miscellen). Sitzungsprot Gesell Wiss Med Berlin 1852;April 19th 1852:286–287.
23 Virchow R: About catharalic neoplasias (Ueber katarrhalische Geschwüre). Berl Med Wochenschr 1883;8:109–112.
24 Virchow R: Diagnosis and prognosis of the carcinom (Zur Diagnose und Prognose des Carcinoms). Archiv Path Anat Physiol Klin Med 1888;111:1–24.
25 Lubarsch O: Virchow's theory on oncogenesis and its further development (Die Virchowsche Geschwulstlehre und ihre Weiterentwicklung). Virch Arch 1921;235:235–261.
26 Kaiserling C: Virchow's impact on oncology (Virchows Bedeutung für die Lehre von den Geschwülsten). Dtsch Med Wochenschr 1921;47:1191–1192.
27 David H: Rudolf Virchow and modern aspects of tumor pathology. Path Res Pract 1988;183: 356–364.
28 Hauptmann S, Schnalke T: Rudolf Virchow's view of malignant proliferation (Rudolf Virchows Sicht der malignen Geschwülste). Pathologe 2001;22:291–295.
29 Bauer AW: '… Impossible to find anything specific in it'. Rudolf Virchow and tumor pathology ('… unmöglich, darin etwas Specifisches zu finden'. Rudolf Virchow und die Tumorpathologie). Medizinhist J 2004;39:3–26.
30 Keil G: The evaluation of Virchow's necropsy protocols deriving from his time in Wuerzburg (Zur Überlieferung von Virchows Würzburger Sektionsprotokollen). Sudhoffs Arch 1980;64:287–297.
31 Virchow R: On inflammation (Über die Entzündung). Dtsch Klin 1857;4:286–287.
32 Lubarsch O: Virchow's theory on inflammation and its development until now (Virchows Entzündungslehre und ihre Weiterentwicklung bis zur Gegenwart). Virch Arch 1921;235:186–211.
33 Hesse E: Rudolf Virchow and Public Health issues (Rudolf Virchow und die öffentliche Gesundheitspflege). Virch Arch 1921;325:399–417.
34 Beneke R: Virchow's importance for Public Health issues and welfare (Von Virchows Bedeutung für die öffentliche Gesundheitspflege und Wohlfahrt). Dtsch Med Wochenschr 1921;47:1192–1195.
35 Jacob W: Rudolf Virchow's achievements in Public Health issues. Medicine as applied science of the human being (Aus dem sozialmedizinischen Erbe Rudolf Virchows. Medizin als Wissenschaft vom Menschen). Janus 1965;52:218–240.
36 Jacob W: Recent significance of Rudolf Virchow's engagement in Public Health issues (Die gegenwärtige Bedeutung der Sozialmedizin Rudolf Virchows). Dtsch Med Wochenschr 1965;90:2113–2116.
37 Ackerknecht EH: Rudolf Virchow and Public Health (Rudolf Virchow und die Sozialmedizin). Sudhoffs Arch 1975;59:247–253.
38 Virchow R: Medical advice for soldiers in case of warfare (Gesundheitsregeln für die Soldaten im Felde). Arch Path Anat 1870;51:127–136.
39 Orth J: Virchow and bacteriology (Virchow und die Bakteriologie). Dtsch Med Wochenschr 1910;36:1937–1939.
40 Ebstein W: Rudolf Virchow as a physician (Rudolf Virchow als Arzt). Stuttgart, Selbach, 1903.
41 Munk F: Virchow as therapeut (Virchow als Therapeut). Virch Arch 1948;315:32–45.
42 Brinkhous KM: Why Virchow became a physician. Arch Pathol 1968;85:331–334.
43 Ringert R: Virchow, a physician with social and scientific responsibilities (Virchow, Arzt in sozialer und wissenschaftlicher Verantwortung). Ber Phys Med Ges Würzburg 1972;80:121–136.

44 Virchow R: Barbarisms in medical terminology (Barbarismen der medicinischen Sprache). Arch Path Anat 1883;91:1–11.
45 Virchow R: New terms and terminology within pathology (Neue Namen und Begriffe in der Pathologie). Berl Klin Wochenschr 1900;37:1–3.
46 Simon F: Rudolf Virchow as terminologist (Rudolf Virchow als Terminologe). Medizinhist J 1991;26:329–336.
47 Virchow R: Examinations on the Neandertal skull (Untersuchung des Neanderthal-Schädels). Verh Berl Anthrop Ges 1872;4:157–165.
48 Ivanhoe F: Was Virchow right about Neandertal? Nature 1970;227:577–578.
49 Virchow R: Gypsum mask of the skull of Haydn and photographies of the skulls of Schubert, Beethoven and Haydn (Gypsabguss des Schädels von Haydn und ein Blatt mit Photographien der Schädel Schuberts, Beethovens und Haydns). Verh Berl Anthrop Ges 1887;19:408–413.
50 Virchow R: The skulls from Canstatt (Die Schädel von Canstatt). Corr Dtsch Anthrop Ges 1892;32:90–94.
51 Virchow R: Recent issues of ancient anthropological research (Die heutigen Probleme der anthropologischen Alterthumsforschung). Corr Dtsch Anthrop Ges 1893;9.
52 Hemmeter JC: Rudolf Virchow's conntributions to scientific anthropology (Rudolf Virchows Leistungen auf dem Gebiet der wissenschaftlichen Anthropologie). Janus 1907;12:549–557.
53 Luschan v J: Rudolf Virchow as an anthropologist (Rudolf Virchow als Anthropologe). Virch Arch 1921;325:418–443.
54 Schrenk M: Rudolf Virchow initiating anthropological concepts within pathology (Anthropologische Konzepte in der Pathologie seit Rudolf Virchow). Ber Phys Med Ges Würzburg 1972;80:143–147.
55 Andree C: Rudolf Virchow as a pre-historian (Rudolf Virchow als Urzeitforscher). Waage 1980; 19:122–128.
56 Virchow R: Rememberences on Schliemann (Erinnerungen an Schliemann). Gartenlaube 1891; 39:66–68.
57 Virchow R: Rememberences on Schliemann (Erinnerungen an Schliemann). Gartenlaube 1891; 39:104–108.
58 Virchow R: Rememberences on Schliemann (Erinnerungen an Schliemann). Gartenlaube 1891; 39:299–303.
59 Virchow R: Countryside and people in the old and new Egypt (Land und Leute im alten und neuen Ägypten). Verh Ges Erdk Berl 1888;15:428–442.
60 Virchow R: Journey to Egypt in 1888, Rudolf Virchow's letters to his wife (Aegyptenreise 1888, Rudolf Virchows Briefe an seine Frau). Waage 1974;13:1–20.
61 Virchow R: Medical rememberences deriving from a journey to Egypt (Medizinische Erinnerungen an einer Reise nach Ägypten). Arch Path Anat 1988;113:361–385.
62 Wagner D: Rudolf Virchow as a medical historian (Rudolf Virchow als Medizinhistoriker). Z Ges Hyg Grenzgeb 1972;18:442–447.
63 Müller M: Rudolf Virchow as a historian (Rudolf Virchow als Historiker). Sudhoffs Arch 1941;34:137–145.
64 Bier A: Rudolf Virchow following up the systematic approach and his contributions as philosopher (Rudolf Virchow als Systematiker und Philosoph). Virch Arch 1937;300:517–533.
65 Jacoby A: Rudolf Virchow and his influence on medicine in America (Rudolf Virchow und die amerikanische Medizin). Berl Klin Wochenschr 1901;38:1046–1047.
66 Ewing J: Virchow's influence on medical sciences in America (Der Einfluss Virchows auf die medizinische Wissenschaft in Amerika). Virch Arch 1955;325:444–452.
67 Pye-Smith PH: The influence of Virchow on pathology in England. Berl Klin Wochenschr 1901;38:1036–1038.
68 Sacharoff GP: Rudolf Virchow's impact on medicine in Russia (Rudolf Virchow und die russische Medizin). Virch Arch 1921;235:329–378.
69 Salomonsen CJ: Rudolf Virchow's impact on medicine in Denmark (Rudolf Virchow und die dänische Medizin). Berl Klin Wochenschr 1901;38:1045–1046.
70 Sundberg C: Rudolf Virchow's impact on pathology in Sweden (Rudolf Virchow und die schwedische Pathologie). Berl Klin Wochenschr 1901;38:1044–1045.

71 Stokvis BJ: Virchow's impact on medicine in The Netherlands (Virchow und die niederländische Medizin). Berl Klin Wochenschr 1901;38:1044–1045.
72 Winau R: Rudolf Virchow and changes in medicine in the 19th century (Rudolf Virchow und der Wandel der Medizin im 19. Jahrhundert). Verh Dtsch Ges Path 1997;81:28–34.
73 Dhom G: Rudolf Virchow's impact on recent medicine after 100 years (Rudolf Virchows Spuren in der Medizin nach 100 Jahren). Pathologe 2003;24:1–8.
74 Bussmann W: Rudolf Virchow and the state (Rudolf Virchow und der Staat). Eigenverlag München – Wien, Festschrift für Theodor Schieder 1978, pp 267–285.
75 Bauer A: 'Bottomline, politics is nothing more than medicine in general'. Rudolf Virchow as pathologist, reformer and person with visions ('Die Politik ist weiter nichts als die Medicin im Grossen': Rudolf Virchow als Pathologe, Reformer und Visionär). Immunol Akt 2000;1:40–48.
76 Lohse M: No duell with Otto von Bismarck (Kein Duell mit Otto von Bismarck). News (Mainpost); 18.01.2002.
77 Kümmel W: Rudolf Virchow and antisemitism (Rudolf Virchow und der Antisemitismus). Medizinhist J 1968;3:165–179.
78 Heischkel E: Rudolf Virchow as a publicist (Rudolf Virchow als Publizist). Med Rundschau 1947;1:230–233.
79 Kirsten C: Documents about Rudolf Virchow in the 'Zentralarchiv der DAW' and in the 'Archiv der Humboldt-Universität zu Berlin' (Quellen über Rudolf Virchow im Zentralarchiv der DAW und im Archiv der Humboldt-Universität zu Berlin). Z Ges Hyg Grenzgeb 1972;18:426–429.
80 Goldblum JR, Weiss SW: Enzinger and Weiss's Soft Tissue Tumors, ed 4. St. Louis, Mosby, 2001, pp 1130–1132.
81 Coussens LM, Werb Z: Inflammation and cancer. Nature 2002;420:860–867.
82 Balkwill F, Mantovani A: Inflammation and cancer: back to Virchow? Lancet 2001;357:539–545.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 16–44

Aneuploidy and Cancer: From Correlation to Causation

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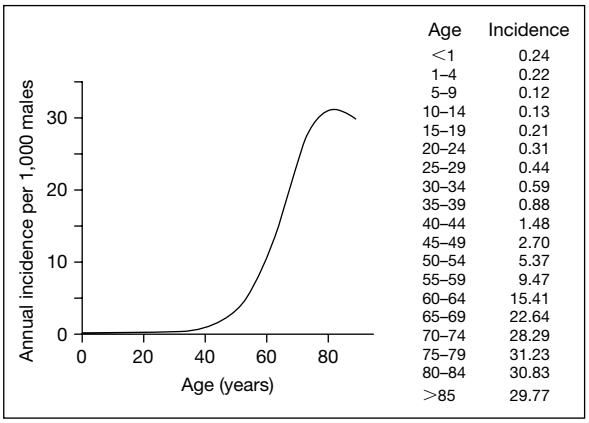
Abstract

Conventional genetic theories have failed to explain why cancer (1) is not found in newborns and thus not heritable; (2) develops only years to decades after 'initiation' by carcinogens; (3) is caused by non-mutagenic carcinogens; (4) is chromosomally and phenotypically 'unstable'; (5) carries cancer-specific aneuploidies; (6) evolves polygenic phenotypes; (7) nonselective phenotypes such as multidrug resistance, metastasis or affinity for non-native sites and 'immortality' that is not necessary for tumorigenesis; (8) contains no carcinogenic mutations. We propose instead that cancer is a chromosomal disease: Accordingly, carcinogens initiate chromosomal evolutions via unspecific aneuploidies. By unbalancing thousands of genes aneuploidy corrupts teams of proteins that segregate, synthesize and repair chromosomes. Aneuploidy is thus a steady source of karyotypic–phenotypic variations from which, in classical Darwinian terms, selection of cancer-specific aneuploidies encourages the evolution and subsequent malignant 'progressions' of cancer cells. The rates of these variations are proportional to the degrees of aneuploidy, and can exceed conventional mutation by 4–7 orders of magnitude. This makes cancer cells new cell 'species' with distinct, but unstable karyotypes, rather than mutant cells. The cancer-specific aneuploidies generate complex, malignant phenotypes, through the abnormal dosages of the thousands of genes, just as trisomy 21 generates Down syndrome. Thus cancer is a chromosomal rather than a genetic disease. The chromosomal theory explains (1) nonheritability of cancer, because aneuploidy is not heritable; (2) long 'neoplastic latencies' by the low probability of evolving competitive new species; (3) nonselective phenotypes via genes hitchhiking on selective chromosomes, and (4) 'immortality', because chromosomal variations neutralize negative mutations and adapt to inhibitory conditions much faster than conventional mutation. Based on this article a similar one, entitled 'The chromosomal basis of cancer', has since been published by us in Cellular Oncology 2005;27:293–318.

Despite over 100 years of cancer research, the cause of cancer is still a matter of debate [1–26]. We propose here that the problem of cancer is still



Age	Incidence
<1	0.24
1–4	0.22
5–9	0.12
10–14	0.13
15–19	0.21
20–24	0.31
25–29	0.44
30–34	0.59
35–39	0.88
40–44	1.48
45–49	2.70
50–54	5.37
55–59	9.47
60–64	15.41
65–69	22.64
70–74	28.29
75–79	31.23
80–84	30.83
>85	29.77

Fig. 1. Age-specific incidence of invasive cancers of males in the United States in 2001. The dominant contributors to the total number of invasive cancers are solid tumors. The growth is approximately exponential until about age 70 and then levels off. Data for the figure, shown in the table at the right, are from the National Program of Cancer Registries at http://www.cdc.gov/cancer/npcr/index.htm.

unsolved, because this debate has been monopolized by conventional genetic theories, which hold that cancer is a 'genetic disease' [27–35]. But these genetic theories cannot explain any of the following properties of carcinogenesis:

Cancer Is Not Heritable

The best news about cancer is that we and other animals are all born cancer-free and typically acquire cancer, if at all, only at advanced age [34, 36–40]. This bias of cancer for old age is exponential, increasing the cancer risk 300-fold with age, from near-zero rates in newborns and adolescents to rates of 1 in 3 in the last third of a human or animal life span (fig. 1).

In view of the prevailing gene-based cancer theory, however, this age bias is paradoxical. This theory holds that cancer is caused by clonal expansion of one single cell that has accumulated about four to seven complementary mutations during the lifetime of a patient [1, 12, 34, 38, 41, 42]. If this theory is correct, cancer should be common in newborns. For example, a baby, which inherits 3 colon cancer mutations from his mother and 2 from his father, out of the presumably 6 that are thought to cause colon cancer [1, 34], should develop cancer at a very young age from just one more spontaneous mutation in any one of the billions of its colon cells. Indeed, many hypothetical cancer-causing mutations, including those thought to cause colon cancer, are heritable in transgenic mice (Appendix) and also in humans. According to Vogelstein and Kinzler [43], "one

of the cardinal principles of modern cancer research is that the same genes cause both inherited and sporadic (noninherited) forms of the same tumors".

But there is no colon cancer in newborns (fig. 1). Thus, cancer is somatically generated and not a heritable disease.

Long Neoplastic Latencies

Experimental or accidental carcinogenesis, and the age bias, demonstrate that cancer is a late product of a gradual evolution of somatic cells that may be 'initiated' either by carcinogens or spontaneously [1, 10, 38, 40, 44, 45]. Once initiated, this evolution is autonomous but very slow, generating cancer cells only after lengthy and uneventful 'neoplastic latencies' [40, 45]. These latencies last many months to years in carcinogen-treated rodents and decades in accidentally exposed humans [40, 45–48]. For example, (1) the solid cancers, which developed in human survivors only 20 years after the explosion of atomic bombs in Japan in 1945 [38]; (2) the breast cancers, which developed only 15 years after treatments of tuberculosis with X-rays in the US in the 1950s [49], and (3) the lung cancers, which developed in workers of a mustard gas factory only 30 years after it was closed in Japan in 1945 [50]. The exponential increase of the spontaneous cancer risk of humans with age even implies neoplastic latencies of up to 50 years from a near zero-risk at birth to a one in three risk in the last three decades of a human lifespan of about 80 years (fig. 1). The primary cancer cells that appear after these lengthy pre-neoplastic evolutions continue to progress independently within individuals tumors to form evermore 'polymorphic' [51] and malignant cancers with evermore exotic karyotypes and phenotypes [45].

These long latencies of carcinogenesis, however, are incompatible with the immediate effects of conventional mutation [2, 31, 35, 52]. It is for this reason that Cairns wrote in Cancer: Science and Society: 'The conspicuous feature of most forms of carcinogenesis is the long period that elapses between initial application of the carcinogen and the time the first cancers appear. Clearly, we cannot claim to know what turns a cell into a cancer cell until we understand why the time course of carcinogenesis is almost always so extraordinarily long' [38].

Non-Mutagenic Carcinogens Cause Cancer

Both mutagenic and non-mutagenic carcinogens cause cancer. Examples of non-mutagenic carcinogens are asbestos, tar, mineral oils, naphthalene, polycyclic aromatic hydrocarbons, butter yellow, urethane, dioxin, hormones, metal ions such as Ni, Cd, Cr, As, as well as spindle blockers such as vincristine and colcemid, extranuclear radiation and solid plastic or metal implants (Appendix). Conventional genetic theories, however, fail to explain carcinogenesis by non-mutagenic carcinogens.

Karyotype-Phenotype Variations at Rates that Are Orders Higher than Mutation

During the neoplastic phase of carcinogenesis, cancer cells gain or lose chromosomes or segments of chromosomes (fig. 2) and change phenotypes at rates that far exceed those at which genotypes and phenotypes are changed by conventional mutation [53–55]. For example, highly aneuploid cancer cells become drug resistant at rates of up to 10^{-3} per cell generation [53, 54, 56–58] or become metastatic at 'high rates' [59, 60]. As a result of this inherent chromosomal instability most cancers are enormously heterogeneous populations of nonclonal and partially clonal, or sub-clonal cells [13, 61]. Thus, cells from the same cancer differ from each other in 'bewildering' phenotypic and chromosomal variations [62] and in mutations – even though most cancers are derived from a common, primary cancer cell and thus have clonal origins [38, 45, 51, 56, 61, 63–67].

By contrast, the karyotypes of normal cells are stable despite mutational or developmental phenotype variations [31, 34, 52, 68]. And phenotypic variation of normal cells by conventional gene mutation cells is limited to 10^{-7} per cell generation for dominant genes and to 10^{-14} for pairs of recessive genes in all species [6, 47, 52, 57, 68, 69]. Even the mutation rates of most cancers are not higher than those of normal cells [6, 19, 20, 47, 66, 70–75]. Thus, phenotypic variation in cancer cells can be four to eleven orders faster than conventional mutation.

Cancer-Specific Aneuploidies

Despite the karyotypic instability and heterogeneity of cancer cells partially specific or nonrandom aneuploidies have been found in cancers since in the late 1960s [61, 62, 76–87]. Since the 1990s, many more nonrandom aneuploidies have been detected in cancers by the use of comparative genomic hybridization, rather than by identifying specific aneusomies cytogenetically [61, 88–96]. The term aneusomy is used for a specific, aneuploid chromosome. Specific aneuploidies have even been linked with specific stages of carcinogenesis and with specific phenotypes of cancers such as: (1) Distinct stages of neoplastic transformation in human [62, 89, 95–99] and in animal carcinogenesis [84]; (2) invasiveness [97, 98, 100]; (3) metastasis [101–106]; (4) drug-resistance [53, 69, 107]; (5) transplantability to foreign hosts [108]; (6) distinct cellular morphologies [109]; (7) abnormal metabolism [62, 110], and (8) cancer-specific receptors for viruses [62, 109].

Cancer-specific, nonrandom aneuploidies, however, are inconsistent with the conventional mutational theories of cancer. In fact they are a direct challenge of the mutation theory, because specific aneusomies have the potential to generate cancer-specific functions (Appendix). The Down syndrome-specific functions of trisomy 21 are a confirmed model [111–114].

Karyotypes of clonal cultures of the near-diploid human colon cancer line HCT 116 and the hyper-diploid human colon cancer line SW480

HCT 116, mn=45

Chrom.	Metaphases 1 to 29	30
1	2	2
2	2	2
3	2	2
4	2	2
5	2	2
6	2	2
7	2	2
8	2	2
9	2	2
10	1	1
11	2	2
12	2	2
13	2	2
14	2	2
15	2	2
16	1	1
17	2	2
18	1	1
19	2	2
20	2	2
21	2	2
22	2	2
X	1	1
Y	0	0
M1 10^{+}	1	1
M2 8/16	1	1
M3 17/18	1	1
M4 12^{-}	0	***1***

SW480, mn=57

Chrom.	Metaphases 1 to 6				10					15					19
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
7	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	***0***
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	3	3	3	3	3	3	3	3	3	3	***2***	3	3	3	3
12	1	1	1	1	1	1	1	1	***0***	1	1	1	1	1	1
13	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
14	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
15	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
16	2	2	2	2	2	2	2	2	2	2	2	2	***1***	2	2
17	3	3	3	3	3	3	3	3	3	3	***4***	3	3	3	3
18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
21	3	3	3	3	3	3	3	3	3	***2***	3	3	3	***1***	
22	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
X	2	2	2	2	2	2	2	2	***1***	2	2	2	2	2	2
M1 2/12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M2 3/12/10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M3 9/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M4 Ω9/1	1	1	1	***0***	***0***	***0***	***0***	***0***	***0***	***0***	1	1	1	1	
M5 3^{+}	1	1	1	1	1	1	***0***	1	1	1	1	1	1	***0***	
M6 8/9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M7 7/14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M8 5/20/7	1	1	1	1	1	1	1	1	1	1	1	1	1	***0***	
M9 5/20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M10 Ω5/20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M11 3^{-}	1	1	1	1	1	1	1	1	***0***	1	1	1	***2***	1	
M12 12^{-}	1	1	1	1	1	1	1	1	1	1	1	1	***2***	1	
M13 19/8/19/5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
M14 19/8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M15 15/18	2	***1***	***1***	2	1	2	2	2	***1***	2	2	2	2	2	
M16 16/14/13	0	0	0	0	0	0	0	0	0	***1***	0	0	0	0	
M17 9/5	0	0	0	0	0	***1***	0	0	0	0	0	0	0	0	
M18 2/8	0	0	0	0	0	0	***1***	0	0	0	0	0	0	0	
M19 9/1/11	0	0	0	0	0	0	0	***1***	0	0	0	0	0	0	
M20 12/1	0	0	0	0	0	0	***1***	0	0	0	0	0	0	0	
M21 21/11	0	0	0	0	0	0	0	***1***	0	0	0	0	0	0	

a

Fig. 2. Karyotypes of clonal cultures of human colon cancer and Chinese hamster cell lines. ***a*** Karyotypes of clonal cultures of the near-diploid human colon cancer cell line HCT 116 (modal chromosome number = 45) and of the hyper-diploid human colon cancer cell line SW480 (modal chromosome number = 57). The karyotype of only 1 out of 30 cells of the clonal culture of the near-diploid HCT 116 line was non-clonal, containing an extra, partially deleted chromosome 12, termed marker M4 12^{-} (***bold italic*** number). By contrast, 13 (***bold italic*** numbers) out of 19 cells of the clonal culture of the hyper-diploid SW480 line had nonclonal karyotypes. All 13 nonclonal karyotypes differed from the modal karyotype of this line in the numbers of one or more chromosomes. Four of these 13 nonclonal cells also contained new structurally altered chromosomes, labeled M16 to 21 (***bold italic*** numbers). Chromosomal constituents of the marker (hybrid) chromosomes are indicated following their

Karyotypes of clonal cultures of the near–diploid, hyper-diplod and near-triploid Chinese hamster cells

			Normal chromosomes												Altered chromosomes	
Clone	Meta	Chr No.	1	2	3	4	5	6	7	8	9	10	X	Y	Partially clonal	Non-clonal
B69–1	1 to 17	23										1			ac1–2	
mn = 23	18	24										1			ac1–2	ac101
	19	24										1			ac1–2	ac102
	20	48	4	4	4	4	4	4	4	4	4	2	2	2	ac1[2]–2[2]	ac101[2]
D1	1	30		4			3	4	4						ac1	
mn = 29	2	29		4			3	3	4						ac2	
	3	28		4	3			3	3						ac1	
	4	29		4			3	3	4						ac2	
	5	30		3			3	4	4						ac1–2	
	6	30		4	3			4	4						ac1	
	7	29		4			3	4	4				0		ac1	
	8	29		4				4	4						ac1	
	9	28		4				3	3						ac1–2	
	10	29		4				4	4							ac101
	11	30		4			3	4	4						ac1	
	12	33		4			3	4	4						ac2	ac102–104
	13	30		4				4	4			3			ac1	
	14	27		4				3	3		3					
	15	32		4		3	3	4	4	3						ac105
	16	30		4			3	4	4						ac1	
	17	30		4				4	4						ac1	ac106
	18	29		4			3	4	4							
	19	32		4	4			4	4							ac102, 107
	20	29		4		1	3	4	4						ac1	
B2	1	33							3	3	3				ac1, 5, 7, 12, 21–22	ac201–202
mn = 35	2	34								3					ac1–2, 4–5, 7, 21, 23	ac203–206
	3	34				1									ac1–2, 4–5, 11, 21–22	ac207–212
	4	34													ac1–2, 4–5, 7, 12, 22, 24	ac213–216
	5	33					3								ac2, 4, 5, 7, 22, 24	ac217–220
	6	38					3	1							ac1, 4–5, 7, 21–22	ac221–230
	7	34				3									ac1, 4, 7, 11–12, 22	ac231–235
	8	34			3	3									ac1–4, 7, 12, 21, 24	ac236–237
	9	36					3				3				ac2, 5, 7–8, 11–12, 22	ac238–242
	10	33													ac1, 3, 11–12, 22, 24	ac243–247
	11	32	1	1		1			1						ac1–2, 4–5, 7, 11, 21	ac248–254
	12	36					3		3						ac1–2, 4–5, 11, 21, 24	ac255–259
	13	37				3			3	3					ac1–5, 7[2]–8, 22	ac260–262
	14	38				3									ac1–2, 4–5, 7–8, 12, 21	ac263–269
	15	32				3	3								ac1, 4–5, 7, 22	ac270–272
	16	30	1	1											ac1, 5, 7, 21	ac273–278
	17	32			3										ac1, 4–7, 12	ac279–281
	18	34													ac2–5, 11, 21	ac282–287
	19	36				3					3	4			ac1–2, 4, 12, 21–22	ac288–291
	20	34													ac1–2, 4–5, 12, 21–22	ac292–296
	21 to 26	~66	too complex to analyze													

b

designation, e.g. M1 2/12 for a hybrid of chromosomes 2 and 12. ***b*** Karyotypes of clonal cultures of the near-diploid, hyper-diploid and near-triploid Chinese hamster cell lines B69–1 (modal chromosome number = 21), D1 (modal chromosome number = 29) and B2 (modal chromosome number = 35). No numbers signal normal chromosome numbers. It can be seen that only 3 of 20 cells of the near-diploid line B69–1 had nonclonal karyotypes. Each of these included one new structurally altered chromosome, termed ac101 and ac102. One of these three nonclonal karyotypes also had undergone tetraploidization. By contrast, there were no two identical cells in the clonal cultures derived from the hyper-diploid and near-triploid Chinese hamster cells. Nevertheless, the degrees of both numerical and structural variations were much higher in near-triploid than in hyper-diploid Chinese hamster cells.

Cancers Have Complex Phenotypes

The complexity of most cancer-specific phenotypes far exceeds that of phenotypes generated by conventional mutation. For example, the kind of drug-resistance that is acquired by most cancer cells exposed to a single cytotoxic drug is more complex than just resistance against the drug used to induce it. It protects not only against the toxicity of the challenging drug, but also against many other chemically unrelated drugs [56, 58, 115]. Therefore, this phenotype has been termed 'multidrug resistance'. Thus, drug resistance must be polygenic. The same is likely to be true for the other cancer-specific phenotypes such as grossly altered metabolism, invasiveness, metastasis, and immortality [40, 45], because all of these phenotypes correlate with altered expressions of thousands of genes [34, 87, 116–118] and with highly abnormal concentrations of thousands of normal proteins [16, 40, 51, 119]. Moreover, in highly aneuploid cancer cells the number of centrosomes is increased up to 5-fold – from a normal of two to around ten – and at the same time their structures are often altered [120–123].

The high genetic complexities of most cancer-specific phenotypes, however, are incompatible with accumulations of large numbers of gene mutations generated at conventional rates during the limited live spans of humans and animals. Indeed, it is virtually impossible that the up to 5-fold increased numbers of centrosomes that are observed in highly aneuploid cancer cells [17, 120, 121, 124], would be the result of mutations that increase the numbers of the 350 different proteins that make up centrosomes [125].

Nonselective Phenotypes of Cancer Cells

Cancer-specific phenotypes can be divided into two classes: Those, which are selective, because they advance carcinogenesis by conferring growth advantages to cancer cells such as invasiveness, grossly altered metabolism and high adaptability via high genomic variability [40, 45], and those, which are not selective for growth [73, 126]. The nonselective, cancer-specific phenotypes include metastasis, drug resistance and immortality. Metastasis is the ability to grow at a site away from the primary tumor. Therefore, it is not selective at the site of its origin [126]. Likewise, drug resistance is not a selective advantage for natural carcinogenesis in the absence of chemotherapy. Yet, a high percentage of cancers is a priori or intrinsically drug-resistant [127, 128]. Moreover, the majority of the drug resistances associated with multidrug resistance offer no selective advantages against the drug that induced it. Even immortality is not a selective advantage for carcinogenesis, because many types of human cells can grow over 50 generations according to the Hayflick limit [129], and thus many more generations than are necessary to generate a lethal cancer. Consider that 50 cell generations produce from one single cell a cellular mass equivalent of 10 humans with 10^{14} cells each [10]. Nonselective

phenotypes, however, are entirely inconsistent with conventional gene mutation-selection mechanisms.

No Carcinogenic Genes in Cancer

Numerous gene mutations have been found in cancer cells since the 1980s [1, 29, 42, 130–133], and the prevailing genetic theories of cancer postulate that these mutations are carcinogenic [29, 30, 33, 34, 42].

But none of the mutations found in cancers are cancer-specific [1, 134], and in cases where this information is available many, perhaps most, mutations are nonclonal [8, 134, 135] and are not detectably expressed in human cancer cells in vivo [8, 116, 136, 137]. Despite enormous efforts in the last 25 years, no mutant gene and no combination of mutant genes from cancer cells has been found that converts diploid human or animal cells into cancer cells [4, 5, 12, 13, 24, 73, 138]. Moreover, mouse strains with artificially implanted, hypothetical cancer genes, or with artificially deleted tumor suppressor genes have survived many generations in laboratories with either the same or slightly higher cancer risks than other laboratory mice (Appendix) [8, 24, 73].

In view of this, Vogelstein and Kinzler [1] closed a very influential review of the mutation theory in 1993 as follows: 'The genetics of cancer forces us to re-examine our simple notions of causality, such as those embodied in Koch's postulates: How does one come to grips with words like "necessary" and "sufficient" when more than one mutation is required to produce a phenotype and when that phenotype can be produced by different mutant genes in various combinations?' These and other inconsistencies between carcinogenesis and established genetic theories are the reasons why it is still debated, whether mutations or aneuploidies or epigenetic alterations cause cancer [1, 3–8, 10–14, 16–22, 24–26, 42].

A New, Chromosomal Evolution Theory of Carcinogenesis

In an effort to resolve the many discrepancies between carcinogenesis and conventional genetic theories listed above, we present here a new, chromosomal evolution theory of carcinogenesis. Our theory is based on: (1) the ubiquity of aneuploidy in cancer [61, 62, 65, 78, 139]; (2) our own data that aneuploidy changes the numbers and structures of chromosomes and phenotypes automatically much faster than and independent of mutation [53–55, 137, 140]; (3) an earlier chromosomal theory of cancer proposed by Boveri and von Hansemann over 100 years ago [141–143]. This theory, however, was abandoned in the 1950s and 1960s in favor of mutation, because instead of the expected cancer-specific aneuploidy, karyotypic heterogeneity was found in most cancers by the methods developed at that time [62, 144, 145]. Ever since, 'aneuploidy and other forms of

chromosomal abnormality' of cancer cells [56] are generally interpreted as 'secondary' events [24, 56, 61, 62, 146] – secondary to presumably primary gene mutations [15, 32, 64, 75, 147–153]; (4) cancer-specific aneuploidies discovered since the late 1960s by many laboratories including ours, particularly by comparative genomic hybridizations [84]. These discoveries, however, are not appreciated as chromosomal causes of cancer because of the prevailing genetic theories.

According to our new chromosomal evolution theory, carcinogenesis is the result of the following chain of events: (1) carcinogens and spontaneous mitotic errors induce unspecific aneuploidies; (2) aneuploidy corrupts teams of proteins that segregate, synthesize and repair chromosomes. Aneuploidy is thus a steady source of karyotypic-phenotypic variations from which, in classical Darwinian terms, selection of cancer-specific aneuploidies encourages the evolution and spontaneous 'progressions' of the malignant phenotypes of neoplastic cells. The rates of these variations are proportional to the degrees of aneuploidy; (3) this chromosomal evolution makes cancer cells new, inherently unstable cell 'species' with distinct, but unstable karyotypes, rather than mutant cells. Owing to this inherent chromosomal instability, cancers are uncertain combinations of random and of relatively specific or 'nonrandom' aneuploidies; (4) the cancer-specific aneuploidies generate complex, malignant phenotypes via abnormal dosages of thousands of genes. Down syndrome is a model for how aneuploidy generates complex, abnormal phenotypes, and (5) thus cancer is a chromosomal rather than a genetic disease.

Below, we offer a brief explanation of how aneuploidy generates new phenotypes, independent of mutation. According to this mechanism variations of chromosomes have the same effects on the phenotypes of cells as variations of the assembly lines of a car factory on the phenotypes of an automobile. If changes are made that do not alter the balance of components, e.g. moving the engine from the front to the rear, new, competitive car models are generated. Indeed, motor companies change their assembly lines to create a new car model. Likewise, phylogenesis generates new species by changing the numbers and structures of the chromosomes of existing species [154].

If unbalanced, i.e. aneuploid, changes are made, abnormal and defective products must be expected. The human trisomy 21, which causes Down syndrome, is a classic non-neoplastic example [113, 114]. Although trisomy 21 is only a tiny aneuploidy compared to that of most cancers, it generates 71 Down-specific phenotypes [111, 112]. Likewise, experimentally induced, congenital aneuploidies generate numerous abnormal phenotypes in drosophila, plants and mice, independent of gene mutation [155–157]. Thus, the complex aneuploidies of cancer cells can be expected to generate numerous new phenotypes.

By contrast, the power of changing the phenotypes of the cell by gene mutation is comparable to employing a few defective or overactive workers on

the assembly lines of a car factory. Neither of these variables will generate a new car model, except possibly to produce either a defective car or no car at all, if an assembly line comes to a stop [158]. For example, none of the 1.42 million point mutations that distinguish any two humans [159] have generated a new human species, nor have they even been sufficient to cause cancer in newborns.

Instead of being controlled by hypothetical oncogenes or tumor suppressor genes, alias 'gate keepers and caretakers' [75, 160], or being de-controlled by the corresponding mutations, most phenotypes of normal and cancer cells are controlled 'democratically' by hundreds of kinetically linked proteins [161]. Such cooperative assembly lines of gene products are buffered against mutations of single genes by the assembly line principle [161, 162]. According to this principle, unchanging supplies and demands of numerous unmutated genes from upstream and downstream of biochemical assembly lines buffer mutations in two ways. They automatically raise substrate concentrations upstream of slow-working, mutationally compromised genes and restrict by normal supplies of substrates mutationally activated genes [161, 163]. This is indeed the principle that buffers cells of all multicellular organisms against all but knock out mutations that occur during their long lifetimes.

Thus aneuploidization, upsetting the balance of thousands of normal genes, rather than mutation of a few genes, is necessary to generate the complex and dominant phenotypes of cancer cells.

In sum, the chromosomal evolution theory provides a coherent explanation of carcinogenesis that is independent of mutation, and that can explain each of the many idiosyncratic features of carcinogenesis that are paradoxical in view of the mutation theory. However, the chromosomal theory remains challenged by competing claims of the prevailing genetic theories of cancer. In the following we take up this challenge.

Testing Specific Predictions of the Chromosomal Theory against Competing Claims by Genetic Theories of Cancer

According to the prevailing genetic theories of cancer, 'carcinogens are mutagens' [164] initiating carcinogenesis by mutation, and 'initiated' cells then evolve into cancer cells via poorly defined sets of four to seven complementary mutations [1, 29, 34, 35, 38, 41, 42, 52, 134, 165]. Since these claims of the prevailing genetic theories of cancer have monopolized cancer research in the last decades, we have tested the most distinctive predictions of the chromosomal evolution theory: (1) carcinogens initiate carcinogenesis by aneuploidisation; (2) aneuploidy is inherently variable and thus sufficient to catalyze the evolution of cancer-specific chromosome patterns, and (3) carcinogenesis is independent of somatic mutation.

Carcinogens Function as Aneuploidogens

This prediction has been confirmed previously by others [4, 10, 44, 67, 70, 73, 158, 166–168] including Boveri, who first demonstrated that X-rays, several chemicals, heat and physical stress generate aneuploidy, but failed to observe cancer in experimental animals [142, 143]. However, since these studies did not establish pre-neoplastic aneuploidy as the cause of carcinogenesis [6, 7, 24, 25], we have recently retested the question whether carcinogens cause aneuploidy experimentally, using mutagenic [84] and nonmutagenic carcinogens [169, 170], and by reviewing the literature [4, 10, 25, 73, 158]. These tests have shown that mutagenic carcinogens generate aneuploidy either by breaking and rearranging chromosomal DNA or by chromosome nondysjunction owing to alterations of the spindle apparatus. By contrast, nonmutagenic carcinogens would induce aneuploidy primarily via de-polymerization of the proteins of the spindle apparatus or even via physical interference with mitosis as by asbestos [80]. Polycyclic aromatic hydrocarbons and vincristine are examples of carcinogens that cause aneuploidy by depolymerizing protein polymers of the spindle apparatus [70, 158].

Moreover, carcinogens, particularly radiations and mutagenic chemical carcinogens, induce aneuploidy without delay, and thus long before cancer [170–174], as postulated by the chromosomal theory. Most importantly, our own studies have shown that among the many effects that carcinogens have on cells [40], aneuploidy is the one that consistently segregates with subsequent carcinogenesis [84, 170].

A series of recent studies, aiming at the definition of mutations that might 'initiate' carcinogenesis, have instead all pointed to chromosomal initiation [67, 73, 174]. Based on the dosage of a carcinogen delivered to cell cultures, the percentages of 'initiated' cells were found to be >1,000-fold larger than expected for the target gene [73]. Markers identified for the initiation of carcinogenesis were either aneuploidy or chromosomal destabilization or immortalization or 'delayed reproductive death' [67] or transformation of cells in vitro [73]. Since an average human chromosome contains about 1,500 genes – 35,000 genes divided by 23 chromosomes [154] – it follows that the chromosome is the target for the initiation of carcinogenesis [73]. We conclude that carcinogens function as aneuploidogens as postulated by the chromosomal theory.

Aneuploidy Is Inherently Variable and Thus Sufficient to Catalyze the Evolution of Cancer-Specific Chromosome Patterns

We have tested this critical prediction of the chromosomal evolution theory, by measuring the rates at which karyotypes of cancer cells vary spontaneously per cell generation. For this purpose clonal cultures of cancer cells with different degrees of aneuploidy were prepared and the fraction of nonclonal karyotypes in these cultures was determined. The rates of karyotype alteration

per cell generation are then calculated by dividing these fractions by the number of generations of the clonal culture.

Using this method we found karyotypic variation at rates of near 10^{-2} per generation in the hyper-diploid – modal chromosome number = 57 – human colon cancer cell line SW480 [53]. This rate was calculated from the data shown in figure 2a as follows: 6 of the 19 karyotypes were identical and are thus considered the 'stemline' [62] or modal karyotype of this line. But, 13 of 19 'clonal' SW480 cells had non-clonal karyotypes, differing from the predominant 'stemline' in numerical and structural aneusomies, which are identified by bold italic numbers in figure 2a. Since the clone was about 23 generations old by the time it was analyzed, having grown from a single cell to about 10^7, the average rate of karyotype variation per cell per generation is about 3% (13:19:23). Indeed, this is a minimal estimate, because many random chromosomal variations are not viable. A comparison of the karyotypes of an SW480 cell with a normal human foreskin cell is shown in figure 3. The karyotypes were prepared from metaphase chromosomes hybridized in situ with color-coded chromosome-specific DNA probes, as described by us recently [53].

Even higher rates of over 1 chromosomal variation per cell generation were observed in the hyper-diploid and near-triploid Chinese hamster cell lines D1 (modal chromosome number = 29) and B2 (modal chromosome number = 35) [55, 140] (fig. 2b). The normal chromosome number of the Chinese hamster is 22. Not even two of these highly aneuploid Chinese hamster cells were the same [55]. This means that the rates of karyotype variations per cell generation were at least 4% (100%: 23), but probably higher, because most random variations are likely to be lost as fast as they are generated. However, in the case of the near-triploid B2 line the rates of structural chromosomal rearrangements were at least 100% per generation, because each metaphase contained several unique structural chromosome alterations, numbered ac201-ac296 in figure 2b.

As predicted by the chromosomal theory, much lower rates of karyotype variations were observed at low degrees of aneuploidy, namely in the near-diploid human colon cancer cell line HCT 116 (modal chromosome number = 45) and in the near-diploid Chinese hamster line B69–1 (modal chromosome number = 23) [55, 140]. Only 1 of 30 clonal HCT 116 cells contained a new, structurally altered chromosome, again identified by a bold italic number in figure 2a, which corresponds to a rate of only 0.15% karyotypic variations per cell generation. Not even one purely numerical variation was detected in 30 metaphases. Likewise only 3 of 20 clonal B69–1 cells had nonclonal karyotypes (fig. 2b), which corresponds to a rate of 0.65% karyotypic variations per cell generation.

It follows that the degrees of both numerical and structural chromosomal instability of human and Chinese hamster cells are proportional to the degrees

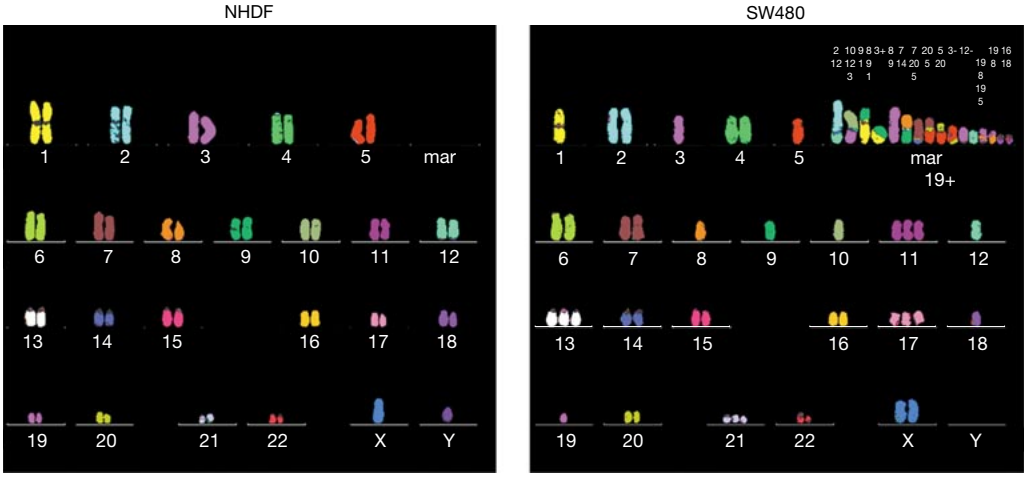


Fig. 3. Metaphase chromosomes of a normal human foreskin cell and of a cell from the human colon cancer cell line SW480. Cytogenetically intact chromosomes are identified by numbers. The group labelled 'mar' (for marker chromosome) shows structurally abnormal chromosomes, which are either rearranged intra-chromosomally or inter-chromosomally to form various hybrid chromosomes. The numbers above these marker chromosomes identify the chromosomal origins of hybrid chromosomes in their relative order or the basis of intra-chromosomal alterations, e.g. 3+ for an amplification of chromosome 3. A comparison of the two karyotypes shows that the cancer cells differ from the normal cell in numerous numerical and structural chromosomal alterations or aneusomies. See online version for color.

of aneuploidy, as postulated by the chromosomal theory. Others have recently described very similar correlations between chromosomal instability and degrees of aneuploidy in human cancer cells including some of those used by us [175–177].

However, the fact that chromosomes are destabilized in proportion to the degree of aneuploidy could also be explained by a series of independent mutations. But, this mutation argument is unlikely, because it is very unlikely that two inherently different kinds of mutations, those that alter the structures and those that alter numbers of chromosomes, would both be equally proportional to the degrees of aneuploidy in all cancers, considering that specific mutations are very rare, even in cancer cells (Appendix). In other words, this argument predicts some cancers with high numerical and no or low structural instability, and others with the opposite distribution, but so far no such cancers have been described.

In sum, the conclusion can be drawn that the inherent variability of aneuploidy is the cause of the chromosomal and phenotypic instabilities of cancer cells and the resulting cellular heterogeneities of cancer, as predicted by the

chromosomal theory. This aneuploidy-specific, chromosomal uncertainty principle had become the nemesis of the Boveri-von Hansemann theory in the 1950s and 1960s.

Carcinogenesis Independent of Somatic Mutation

Cancer coincides with aneuploidy as well as with mutations [6, 7, 10, 13, 24]. In the words of a recent review in *Science*, 'Cancer cells are chock-full of mutations and chromosomal abnormalities' [6]. Therefore, it can be argued that spontaneous and carcinogen-induced aneuploidization is sufficient for the initiation and autocatalytic evolution of carcinogenesis, as we did here. But, it could also be argued that the initial aneuploidization and its subsequent evolution depend on somatic mutations, as others have done recently [13, 14, 26, 150–153, 178].

However, the following 4 arguments indicate that carcinogenesis (of normal cells in normal organisms) is independent of somatic mutation [25]. In fact, cancer cells, via their specific aneuploidy, are even protected against the negative effects of mutation: (1) Initiation of carcinogenesis by aneuploidy, generated by mutagenic carcinogens fragmenting or eliminating chromosomes, is about 35,000 times more likely than by aneuploidy, generated by mutation of a specific mammalian 'aneuploidy-gene' [6]. This is because mammals contain about 35,000 genes, and thus only 1 in 35,000 specific mutations would generate an 'aneuploidy gene' [25, 154], but any mutation leading to a chromosome break or rearrangement generates aneuploidy. Using nonmutagenic carcinogens to generate initiating aneuploidy via the spindle apparatus is in fact infinitely more efficient than via the nontarget gene. Thus, initiation of carcinogenesis is independent of somatic mutation. (2) Generating the complex, cancer-specific phenotypes by chromosomal variation is about 1,500 times more efficient than by mutation. Indeed, it would be almost impossible to generate the complex, polygenic phenotypes of cancer cells in a lifetime of a cancer patient by mutating many genes, considering the complexity of cancer-specific phenotypes and the low rates of spontaneous mutation in normal and most cancer cells (Appendix). By contrast, chromosomal variation is a mechanism that automatically alters the dosages and expressions of thousands of genes. Therefore, aneuploidization is infinitely more efficient in generating the complex phenotypes of cancer cells than mutation. Thus, carcinogenesis is independent of somatic mutation in generating complex, cancer-specific phenotypes. (3) The high rates of cancer-specific karyotype-phenotype variations are irreconcilable with the low rates of conventional mutation. New, cancer-specific phenotypes appear or old ones disappear in highly aneuploid cancer cells at rates of up to 10^{-3} per cell generation, which is four to eleven orders faster than conventional gene mutation (Appendix). Thus phenotype variation in cancer cells is independent

of mutation. (4) The relevance of somatic mutations for carcinogenesis is uncertain. Cancer-specific aneuploidy can generate gene mutations by the same mechanism that varies the structures of chromosomes. In addition, aneuploidy renders DNA synthesis error-prone by unbalancing nucleotide pools [179]. Thus, the simplest explanation of the many mutations of cancer cells would be that these mutations are consequences of aneuploidy and thus not necessary for carcinogenesis. This hypothesis explains why the mutations found in cancer cells are frequently nonclonal in cancers [8, 135], and why they do not transform normal cells to cancer cells and do not breach the livelihood of transgenic mice (Appendix). Indeed, cancer cells are immortal, because frequent, aneuploidy-catalyzed karyotypic variations neutralize all potentially negative mutations at much higher rates than they can be generated.

We conclude that carcinogenesis is independent of somatic mutation, because aneuploidy is much more likely to be generated and varied at the chromosomal level than by mutation. In response to this it has been argued that cancers associated with heritable cancer-disposition syndromes prove that carcinogenesis is dependent on mutation. Examples are the retinoblastoma, xeroderma, Bloom syndrome, and mosaic variegated aneuploidy syndromes [32, 34, 180, 181]. However, these heritable – rather than somatic – mutations are not direct causes of cancer. Instead they initiate carcinogenesis by aneuploidization at much higher rates than it would occur in normal cells by spontaneous or carcinogen-induced aneuploidization [181–183]. According to the chromosomal theory these mutations are genetic equivalents of carcinogens that induce aneuploidy at high rates. This view is supported by the presence of aneuploidy in such patients prior to carcinogenesis, as for example in mosaic variegated aneuploidy patients [183, 184], Bloom patients [182] and xeroderma patients [185], and by the presence of aneuploidy in the cancers of patients with retinoblastoma [186–189], mosaic variegated aneuploidy [183, 184], xeroderma [185, 190] and Bloom patients [182].

We conclude that the abnormally high rates of carcinogenesis in heritable cancer disposition syndromes are dependent on abnormally high rates of aneuploidizations that are generated by these heritable genes. Thus carcinogenesis encouraged by certain heritable mutations confirms and extends the chromosomal theory of carcinogenesis, but does not show that carcinogenesis in normal cells depends on conventional mutation.

Explanatory Value of the Chromosomal Theory of Cancer

In table 1, we have summarized how the chromosomal cancer theory explains each of the idiosyncratic features of carcinogenesis that are paradoxical

Table 1. Features of carcinogenesis

Genetic paradox	Chromosomal solution
1 Cancer not heritable	aneuploidy is not heritable
2 Long neoplastic latencies	autocatalyzed evolution of cancer-specific aneusomies
3 Non-mutagenic carcinogens	carcinogens function as aneuploidogens
4 High rates of karyotype-phenotype variations and the origin of 'immortality'	aneuploidy catalyses karyotype-phenotype variations, including resistance to otherwise lethal conditions, at high rates
5 Cancer-specific aneuploidies	cancer-specific aneuploidies generate cancer phenotypes
6 Complex phenotypes	cancer-specific aneuploidies alter dosages and functions of thousands of genes
7 Nonselective phenotypes	nonselective genes hitchhiking with selective, cancer-specific aneusomies
8 No carcinogenic genes in cancer	cancer is caused by specific aneuploidies

in terms of conventional genetic theories. In the following we offer further commentary on items 1, 2, 5, 6 and 7 listed in table 1, because they are not sufficiently explained by the table and the preceding arguments.

Cancer Is Not Heritable

The chromosomal theory predicts no cancer in newborns, because aneuploidy is not heritable. Aneuploidies are not heritable, because they corrupt embryogenic developmental programs [113, 114], which is usually fatal [157, 191] as originally shown by Boveri [142]. Only some very minor congenital aneuploidies, such as Down syndrome and syndromes based on abnormal numbers of sex chromosomes, are sometimes viable, but only at the cost of severe physiological abnormalities and of no or very low fertility [31, 65, 68, 192]. Thus, ontogenesis is nature's checkpoint for normal karyotypes. The postnatal exponential increase of the cancer risk with age would then reflect the gradual accumulation of non- or preneoplastic aneuploidy with age, multiplied by the relatively slow, nonselective replication of aneuploid, preneoplastic cells (figs 1, 2).

However, it is as yet unclear, why after initiating doses of carcinogens the neoplastic latencies are very species-dependent, namely much shorter in rodents than in humans [1, 46, 47, 193–195]. It is also unclear, why the increase of the cancer risk is proportional to the lifespan of an animal, i.e. is very low for decades in humans (fig. 1), but only for months in rodents [38, 47]. Still, this is unlikely to be due to species-specific mutation rates, because the rates of conventional mutations are highly conserved in all species [52, 68]. However, the

significantly higher chromosomal instability of aneuploid rodent cells compared to equally aneuploid human cells, shown here in figure 2, may offer a different explanation, namely that chromosomal stability of normal and cancer cells is different in different species.

Long Neoplastic Latencies

The chromosomal evolution theory predicts that carcinogenesis is initially very slow, because preneoplastic cells have no growth advantages compared to normal cells and are typically only little aneuploid (fig. 4). Therefore, they would not form large clonal populations that would increase the probability of further evolutions. The non-clonality of the pre-neoplastic aneuploidies also hides any abnormal phenotypes of pre-neoplastic cells, because phenotypes of single cells are hard to recognize. By contrast, neoplastic 'progression' of established cancer cells is predicted to be faster than during the pre-neoplastic phase for two reasons: (1) Neoplastic cells, through their selective phenotypes, will generate large 'clonal' populations with high probabilities of further variations. (2) The generally high degrees of most cancer-specific aneuploidies catalyze high rates of chromosomal variations, compared to those of preneoplastic cells (fig. 4).

The chromosomal theory also predicts a certain endpoint of chromosomal evolutions in carcinogenesis. This endpoint would be an equilibrium of aneuploidizations, which is reached once a cancer has maximized cellular variability and adaptability [73] and 'optimized its genome' for essential metabolic functions [196]. According to the chromosomal theory maximal chromosomal variability would correspond to near or above triploid chromosome numbers (>3n) [13, 73, 137]. Near triploid aneuploidy offers an optimal average redundancy of one spare for each normal chromosome pair, and thus sufficient redundancy to compensate for any losses or genetic mutations of a given chromosome [73]. Accordingly, it is the karyotype of most malignant cancer cells [10, 62, 65, 73, 146, 158, 178, 197].

High Rates of Karyotype-Phenotype Variations and the Origin of Immortality

The chromosomal theory attributes the high rates of karyotype-phenotype variations of cancer cells to the inherent variability of aneuploidy. On this basis, the chromosomal theory also explains the notorious immortality of cancer cells as already described in 1972 by the cytogeneticist Koller [62]: 'It seems that malignant growth is composed of competing clones of cells with different and continuously changing genotypes, conferring the tumor with an adaptable plasticity against the environment. The bewildering karyotypic patterns reveal the multipotentiality of the neoplastic cell; while normal cells and tissues age and die, through their inherent variability, tumor cells proliferate and survive.' Thus, cancers are immortal, because subspecies from within the zoos of their polyphyletic

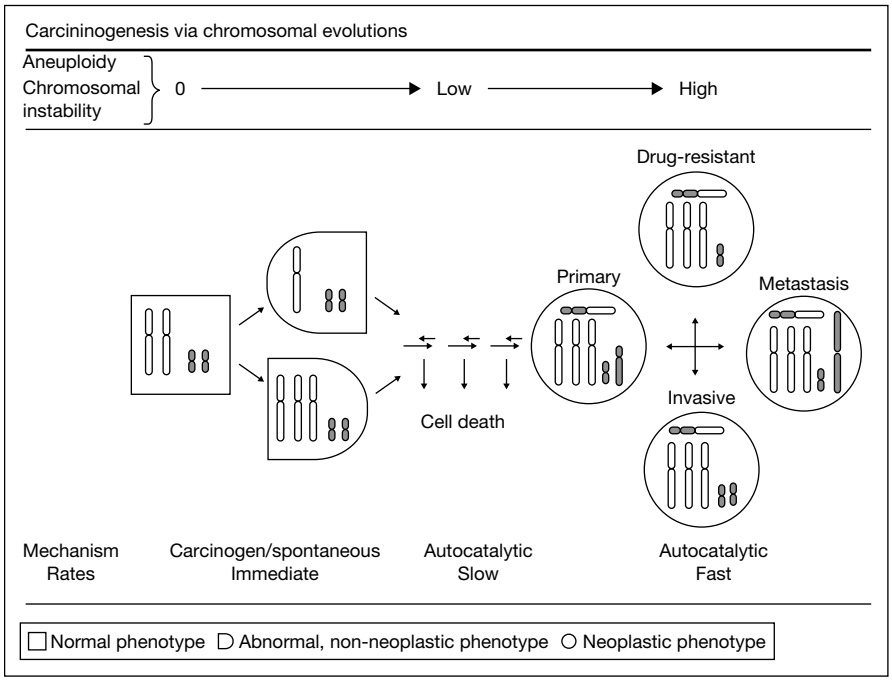


Fig. 4. Carcinogenesis via chromosomal evolutions. According to this mechanism carcinogenesis is initiated by unspecific aneuploidies induced either by carcinogens or spontaneously. Aneuploidy then alters the karyotype automatically at rates that are proportional to the degree of aneuploidy, because it corrupts teams of proteins that segregate, synthesize and repair chromosomes. Aneuploidy is thus a steady source of chromosomal variations from which, in classical Darwinian terms, selection would encourage the evolution and subsequent progressions of neoplastic cell 'species' with cancer-specific aneusomies. This evolution would be slow in the preneoplastic phase, because preneoplastic cells have no growth advantages over normal cells and because the degree of preneoplastic aneuploidy is typically low. By comparison the rate of karyotype variations of most cancer cells would be fast, because cancer cells form large populations by outgrowing normal cells and because the degrees of cancer-specific aneuploidy are typically high. Any kind of cancer could have as many specific aneusomies as there are chromosomes involved in the differentiation of its precursor cell in addition to random aneusomies. Thus cancer-specific phenotypes, such as invasiveness, metastasis, and drug-resistance, are generated by the abnormal dosages of thousands of normal genes. Since aneuploidy is inherently unstable, cancer-specific phenotypes, such as drug-resistance, can be reversible or convertible to other specific phenotypes at the same rates at which they are generated. The chromosomal model predicts the heterogeneous phenotypes and karyotypes of cancers as consequences of independent evolutions of the inherently unstable cancer cells. Since aneuploidy causes dedifferentiation, the model further predicts that the degrees of malignancy of cancer cells are proportional to the degrees of aneuploidy.

cell populations [110] – species are defined by karyotypes – survive conditions that are lethal to the mortal majority of the cells, as for example toxic drugs.

Cancer-Specific Aneuploidies

The presence of cancer-specific or nonrandom aneuploidies is directly predicted by and thus correlative proof for the chromosomal theory in terms of Koch's first postulate. Functional proof that cancer-specific aneuploidy generates malignancy could be derived from evidence that the degree of malignancy is proportional to the degree of aneuploidy. Indeed, numerous correlations have confirmed the principle that the degree of malignancy of cancer cells is proportional to their degree of aneuploidy since the 1930s [10, 45, 62–64, 97, 198–204]. Moreover, other studies have shown that maximal malignancy is, indeed, achieved at maximally stable, near-triploid or hypertriploid aneuploidy [65, 178, 197, 205, 206]. The parallel evolutions of aneuploidy and malignancy in cancer cells are thus functional proof for the chromosomal evolution theory of cancer in terms of Koch's third postulate.

Complex Phenotypes

Conventional genetic theories cannot explain the generation of the polygenic cancer-specific phenotypes such as multidrug resistance, polymorphism, metastasis to non-native sites, and transplantability to heterologous species [108] based on conventional rates of mutation and selection in the lifespan of a human or animal. By contrast, the chromosomal theory of cancer explains the complexity of cancer-specific phenotypes by the complexicity of the genetic units that are varied, namely chromosomes with thousands of genes. Accordingly, the complex phenotypes of cancer cells have recently been shown to correlate with over- and underexpressions of thousands of genes [34, 87, 116–118, 136]. Likewise, cancer cells over- and underproduce thousands of normal proteins [16, 40, 51, 119].

Nonselective Phenotypes`

Conventional genetic theories explain the evolution of cancer cells by cancer-specific mutations and Darwinian selections. But this mechanism cannot explain the nonselective phenotypes of cancer cells, such as metastasis, drug resistance and 'immortality'. By contrast, the chromosomal theory of carcinogenesis attributes nonselective phenotypes such as metastasis and intrinsic multidrug resistance to nonselective genes hitchhiking with selective, cancer-causing aneusomies, because they are all located on the same chromosomes. The same would be true for that part of acquired multidrug-resistance, which is not directed against the selective drug that induced it. The nonselective phenotype immortality has been explained above.

Conclusions

We conclude that the chromosomal theory provides a coherent explanation of carcinogenesis and can resolve all features of carcinogenesis that are paradoxical in terms of the prevailing genetic theories of cancer. In addition, the theory stands out for making new, clinically testable predictions, as for example the prediction that cancer could be detected prior to malignancy via preneoplastic aneuploidy and that chemotherapy could be based on the presence or absence of resistance-specific aneusomies. Thus, if confirmed, the chromosomal theory should become beneficial for cancer research and therapy.

Appendix

The Achilles Heels of the Mutation-Cancer Theory

The currently prevailing cancer theory postulates that cancer is caused by clonal expansion of one single cell that has accumulated about four to seven complementary mutations during the lifetime of a patient [1, 12, 34, 38, 41, 42]. However, the mutation theory is hard to reconcile with the following list of facts.

1 *Nonmutagenic Carcinogens.* Contrary to the mutation hypothesis, many carcinogens are not mutagens, including some of the most potent ones. Examples are asbestos, tar, mineral oils, naphthalene, polycyclic aromatic hydrocarbons, butter yellow, urethane, dioxin, hormones, metal ions such as Ni, Cd, Cr, As, spindle blockers such as vincristine and colcemid, extranuclear radiation and solid plastic or metal implants [40, 44, 67, 70, 73, 158, 166, 168].

2 *No Transforming Genes.* Despite years of efforts no genes or combinations of genes from cancers have been shown to transform normal cells to cancer cells [4, 5, 138] or mice carrying such genes in their germ lines into polyclonal tumors [1, 24, 56]. Accordingly, many, presumably cancer-specific mutations are not detectably expressed in cancer cells [8, 116, 136, 137].

3 *Dependence of Cancer on Unrealistically High Rates of Mutation.* The mutation hypothesis explains the exponential increase of the cancer risk with age by the low probability of four to seven specific mutations [1, 41, 42]. However, in order to maintain the integrity of the genome, spontaneous mutation rates in all species are naturally restricted to about 10^{-7} per dominant gene and to about 10^{-14} per recessive gene per cell generation [6, 47, 52, 57, 68]. Thus, based on these conserved mutation rates cancer via four to seven mutations would not even exist [10]. For example, based on just 4 specific dominant mutations cancer would occur only once in 10^{12} human lifetimes. This is calculated as follows: Since the spontaneous mutation rate per specific, dominant gene is about 10^{-7}, it takes 10^{28} cells to generate one human cell with 4 specific mutations. The expected cancer rate per human lifetime of 1 in 10^{12} is then obtained by dividing 10^{28} by 10^{16}. 10^{16} is the number of cells that correspond to an average human lifetime [10, 38]. Thus, in order to explain the current cancer risk of Americans and Europeans of about 1 in 3 lifetimes [39] (fig. 1), the mutation hypothesis has to assume mutation rates, which are 10^3 [$(10^3)^4 = 10^{12}$] times higher than in conventional mutation.

4 *No Explanation for the Long 'Neoplastic Latency' in Carcinogenesis Induced by a Critical Dose of Carcinogen.* The mutation hypothesis has no answer to the question why, after a critical dose of carcinogen, carcinogenesis would only occur after exceedingly long 'neoplastic latencies' of years to decades [1].

5 *Dependence of Phenotype Alterations in Cancers on Unrealistically High Rates of Mutation.* The mutation hypothesis has to assume mutation rates of up to 10^{-3} per cell generation to explain the frequent, spontaneous variation of phenotypes in highly aneuploid cancer cells. Examples are the 'high rates', compared to mutation, at which some cancers generate metastatic cells [59, 60], or generate drug-resistant variants [53, 54, 56, 58]. But the mutation rates of most cancers are not higher than those of normal cells [6, 19, 20, 47, 66, 70–74].

6 *Heritable Cancer Genes, but no Heritable Cancer.* The four to seven gene mutation hypothesis predicts that subsets of cancer causing mutations should be heritable. Indeed, proponents of the mutation hypothesis have demonstrated that several of the six mutations thought to cause colon cancer [1] can be introduced into the germ line of mice without breaching the viability of these animals. According to one study, animals with one of these mutations, namely ras, were found 'without detectable phenotypic abnormalities' [207]. Another study reports, "surprisingly, homozygosity for the Apc1638T mutation is compatible with postnatal life" [208]. Thus subsets of colon cancer genes are heritable. Therefore, colon cancer should be common in newborns, which are clonal for inherited subsets of these six mutations (like transgenic mice). But there is no colon cancer in newborns [38, 39].

Acknowledgments

We thank Harvey Bialy (Institute of Biotechnology, Autonomous National University of Mexico, Cuernavaca, Mexico), Tom Bethell (Washington, D.C.), Alecia DuCharme (UC Berkeley), Siggi Duesberg, George Miklos (Secure Genetics Pty Limited, Sydney, Australia) and Rainer K. Sachs (Departments of Mathematics and of Physics, UC Berkeley) for critical and constructive reviews of the manuscript. We also thank Rainer K. Sachs for figure 1 and David Rasnick (Pretoria, South Africa) for valuable information. We are especially grateful to Robert Leppo (philanthropist, San Francisco) for support and for the fluorescence microscope used for karyotyping of human cancer cells, and the Abraham J. and Phyllis Katz Foundation (New York), an American foundation that prefers to be anonymous, other private sources, and the Forschungsfonds der Fakultät für klinische Medizin Mannheim for steady support.

References

1 Vogelstein B, Kinzler KW: The multistep nature of cancer. Trends Genet 1993;9:138–141.

2 Fujimura J: Crafting Science: A Sociohistory of the Quest for the Genetics of Cancer. Cambridge, Harvard University Press, 1996.

3 Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA: Creation of human tumour cells with defined genetic elements. Nature 1999;400:464–468.

4 Li R, Sonik A, Stindl R, Rasnick D, Duesberg P: Aneuploidy versus gene mutation hypothesis of cancer: recent study claims mutation, but is found to support aneuploidy. Proc Natl Acad Sci USA 2000;97:3236–3241.

5 Li R, Rasnick D, Duesberg P: Correspondence re: Zimonjic D, et al: Derivation of human tumor cells in vitro without widespread genomic instability. Cancer Res 2001;61:8838–8844. Cancer Res 2002;62:6345–6348; discussion 6348–6349.

6 Marx J: Debate surges over the origins of genomic defects in cancer. Science 2002;297:544–546.

7 Gibbs WW: Untangling the roots of cancer. Sci Am 2003;289:56–65.
8 Duesberg PH: Are cancers dependent on oncogenes or on aneuploidy? Cancer Genet Cytogenet 2003;143:89–91.
9 Thilly WG: Have environmental mutagens caused oncomutations in people? Nat Genet 2003;34:255–259.
10 Duesberg P, Li R: Multistep carcinogenesis: a chain reaction of aneuploidizations. Cell Cycle 2003;2:202–210.
11 Steinberg D: Appraising aneuploidy as a cancer cause. Scientist 2004;18:26–27.
12 Radford IR: Chromosomal rearrangement as the basis for human tumourigenesis. Int J Radiat Biol 2004;80:543–557.
13 Schneider BL, Kulesz-Martin M: Destructive cycles: the role of genomic instability and adaptation in carcinogenesis. Carcinogenesis 2004;25:2033–2044.
14 Pihan G, Doxsey SJ: Mutations and aneuploidy: co-conspirators in cancer? Cancer Cell 2003;4:89–94.
15 Zimonjic D, Brooks MW, Popescu N, Weinberg RA, Hahn WC: Correspondence re: Zimonjic D, et al: Derivation of human tumor cells in vitro without widespread genomic instability. Cancer Res 2002;62:6348–6349.
16 Miklos GLG: The human cancer genome project – one more misstep in the war on cancer. Nat Biotechnol 2005;23:535–537.
17 Brinkley BR, Goepfert TM: Supernumerary centrosomes and cancer: Boveri's hypothesis resurrected. Cell Motil Cytoskeleton 1998;41:281–288.
18 Dey P: Aneuploidy and malignancy: an unsolved equation. J Clin Pathol 2004;57:1245–1249.
19 Tomlinson IP, Novelli MR, Bodmer WF: The mutation rate and cancer. Proc Natl Acad Sci USA 1996;93:14800–14803.
20 Sieber OM, Heinimann K, Tomlinson IP: Genomic instability – the engine of tumorigenesis? Nat Rev Cancer 2003;3:701–708.
21 Sen S: Aneuploidy and cancer. Curr Opin Oncol 2000;12:82–88.
22 Duesberg P, Li R, Rasnick D: Aneuploidy approaching a perfect score in predicting and preventing cancer: highlights from a conference held in Oakland in January 2004. Cell Cycle 2004;3:823–828.
23 Soto AM, Sonnenschein C: The somatic mutation theory of cancer: growing problems with the paradigm? BioEssays 2004;26:1097–1107.
24 Harris H: A long view of fashions in cancer research. BioEssays 2005;27:833–838.
25 Duesberg P: Does aneuploidy or mutation start cancer? Science 2005;307:41.
26 Michor F, Iwasa Y, Vogelstein B, Lengauer C, Nowak MA: Can chromosomal instability initiate tumorigenesis? Semin Cancer Biol 2005;15:43–49.
27 Varmus HT: Retroviruses and oncogenes, I – Nobel Lecture, Dec 8, 1989. Biosci Rep 1990;10:413–430.
28 Peltomaki P, Aaltonen LA, Sistonen P, Pylkkanen L, Mecklin J-P, Jarvinen H, Green JS, Jass JR, Weber JL, Leach FS, Petersen GM, Hamilton SR, de la Chapelle A, Vogelstein B: Genetic mapping of a locus predisposing to human colorectal cancer. Science 1993;260:810–812.
29 Bishop JM: Cancer: the rise of the genetic paradigm. Genes Dev 1995;9:1300–1315.
30 Vogelstein B, Kinzler KW: The Genetic Basis of Human Cancer: New York, McGraw-Hill Health Professions Division, 1998.
31 Griffiths AJF, Miller JH, Suzuki DT, Lewontin RC, Gelbart WM: Genetic analysis, ed 7. New York, Freeman, 2000.
32 Knudson AG: Two genetic hits (more or less) to cancer. Nat Rev Cancer 2001;1:157–162.
33 Vogelstein B, Kinzler KW: Cancer genes and the pathways they control. Nat Med 2004;10:789–799.
34 Lodish H, Berk A, Matsudaira P, Kaiser CA, Krieger M, Scott MP, Zipursky SL, Darnell J: Molecular Cell Biology, ed 5. New York, Freeman, 2004.
35 Pierce BA: Genetics, a conceptual approach, ed 2. New York, Freeman, 2005.
36 Nordling CO: A new theory on the cancer-inducing mechanism. Br J Cancer 1953;7:68–72.
37 Armitage P, Doll R: The age distribution of cancer and a multi-stage theory of carcinogenesis. Br J Cancer 1954;8:1–12.
38 Cairns J: Cancer: Science and Society. San Francisco, Freeman, 1978.
39 Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ: Cancer statistics, 2003. CA Cancer J Clin 2003;53:5–26.

40 Pitot HC: Fundamentals of Oncology, ed 4. New York, Marcel Dekker, 2002.
41 Renan MJ: How many mutations are required for tumorigenesis? Implications from human cancer data. Mol Carcinog 1993;7:139–146.
42 Hahn WC, Weinberg RA: Modelling the molecular circuitry of cancer. Nat Rev Cancer 2002;2:331–341.
43 Vogelstein B, Kinzler KW: The Genetic Basis of Human Cancer. New York, McGraw-Hill, 1998.
44 Berenblum I, Shubik P: An experimental study of the initiating stage of carcinogenesis, and a re-examination of the somatic cell mutation theory of cancer. Br J Cancer 1949;3:109–118.
45 Foulds L: Neoplastic Development. London, Academic Press, 1975.
46 Kuroki T, Huh NH: Why are human cells resistant to malignant cell transformation in vitro? Jpn J Cancer Res 1993;84:1091–1100.
47 Holliday R: Neoplastic transformation: the contrasting stability of human and mouse cells; in Tooze J (eds): Genetic Instability in Cancer. Plainview, Cold Spring Harbor Laboratory Press, 1996, vol 28, pp 103–115.
48 Rhim JS, Dritschilo A: Neoplastic transformation in human cell culture; in Dritschilo A (ed): Mechanism of Carcinogenesis. Totowa, Humana Press, 1991.
49 Boice JD Jr, Monson RR: Breast cancer in women after repeated fluoroscopic examinations of the chest. J Natl Cancer Inst 1977;59:823–835.
50 Doi M, Yukutake M, Tamura K, Watanabe K, Kondo K, Isobe T, Awaya T, Shigenobu T, Oda Y, Yanmakido K, Koyama K, Kohno N: A retrospective cohort study on respiratory tract cancers in the workers of the Japanese army poison-gas-factory operated from 1929 to 1945. Proc Am Soc Clin Oncol 2002;21:439a.
51 Caspersson T: Chemical variability in tumor cell populations. Acta Unio Int Contra Cancrum 1964;20:1275–1279.
52 Lewin B: Genes VI. Oxford, Oxford University Press, 1997.
53 Li R, Hehlmann R, Sachs R, Duesberg P: Aneuploidy, the primary cause of the high rates and wide ranges of drug resistance in cancer cells. Cancer Genet Cytogenet 2005;in press.
54 Duesberg P, Stindl R, Hehlmann R: Explaining the high mutation rates of cancer cells to drug and multidrug resistance by chromosome reassortments that are catalyzed by aneuploidy. Proc Natl Acad Sci USA 2000;97:14295–14300.
55 Fabarius A, Hehlmann R, Duesberg PH: Instability of chromosome structure in cancer cells increases exponentially with degrees of aneuploidy. Cancer Genet Cytogenet 2003;143:59–72.
56 Harris H: The Cells of the Body: A History of Somatic Cell Genetics. Plainview, Cold Spring Harbor Lab Press, 1995.
57 Tlsty TD: Genomic instability and its role in neoplasia; in Kastan MB (ed): Genetic Instability and Tumorigenesis. Berlin, Springer, 1997, vol 221, pp 37–46.
58 Duesberg P, Stindl R, Hehlmann R: Origin of multidrug resistance in cells with and without multidrug resistance genes: chromosome reassortments catalyzed by aneuploidy. Proc Natl Acad Sci USA 2001;98:11283–11288.
59 Harris JF, Chambers AF, Hill RP, Ling V: Metastatic variants are generated spontaneously at a high rate in mouse KHT tumor. Proc Natl Acad Sci USA 1982;79:5547–5551.
60 Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF: Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 2003;100:3983–3988.
61 Heim S, Mitelman F: Cancer Cytogenetics, ed 2. New York, Wiley-Liss, 1995.
62 Koller PC: The Role of Chromosomes in Cancer Biology. New York, Springer, 1972.
63 Hauschka TS: The chromosomes in ontogeny and oncogeny. Cancer Res 1961;21:957–981.
64 Nowell PC: The clonal evolution of tumor cell populations. Science 1976;194:23–28.
65 Sandberg AA: The Chromosomes in Human Cancer and Leukemia, ed 2. New York, Elsevier, 1990.
66 Lengauer C, Kinzler KW, Vogelstein B: Genetic instabilities in human cancers. Nature 1998;396:643–649.
67 Little JB: Radiation carcinogenesis. Carcinogenesis 2000;21:397–404.
68 Vogel F, Motulsky AG: Human Genetics: Problems and Approaches. Berlin, Springer, 1986.
69 Tlsty TD: Normal diploid human and rodent cells lack a detectable frequency of gene amplification. Proc Natl Acad Sci USA 1990;87:3132–3136.

70 Oshimura M, Barrett JC: Chemically induced aneuploidy in mammalian cells: mechanisms and biological significance in cancer. Environ Mutagen 1986;8:129–159.
71 Strauss BS: The origin of point mutations in human tumor cells. Cancer Res 1992;52:249–253.
72 Harris CC: Chemical and physical carcinogenesis: advances and perspective for the 1990s. Cancer Res 1991;51(18 suppl):5023s–5044s.
73 Duesberg P, Fabarius A, Hehlmann R: Aneuploidy, the primary cause of the multilateral genomic instability of neoplastic and preneoplastic cells. IUBMB Life 2004;56:65–81.
74 Wang TL, Rago C, Silliman N, Ptak J, Markowitz S, Willson JK, Parmigiani G, Kinzler KW, Vogelstein B, Velculescu VE: Prevalence of somatic alterations in the colorectal cancer cell genome. Proc Natl Acad Sci USA 2002;99:3076–3080.
75 Tomlinson I, Bodmer W: Selection, the mutation rate and cancer: insuring that the tale does not wag the dog. Nat Med 1999;5:11–12.
76 Zang KD, Singer H: Chromosomal constitution of meningiomas. Nature 1967;216:84–85.
77 Zang KD: Cytological and cytogenetical studies on human meningioma. Cancer Genet Cytogenet 1982;6:249–274.
78 Atkin NB, Baker MC: Chromosome abnormalities as primary events in human malignant disease: evidence from marker chromosomes. J Natl Cancer Inst 1966;36:539–557.
79 Yamamoto T, Rabinowitz Z, Sachs L: Identification of the chromosomes that control malignancy. Nat New Biol 1973;243:247–250.
80 Oshimura M, Hesterberg TW, Barrett JC: An early, nonrandom karyotypic change in immortal Syrian hamster cell lines transformed by asbestos: trisomy of chromosome 11. Cancer Genet Cytogenet 1986;22:225–237.
81 Balaban GB, Herlyn M, Clark WH Jr, Nowell PC: Karyotypic evolution in human malignant melanoma. Cancer Genet Cytogenet 1986;19:113–122.
82 Atkin NB: Chromosome 1 aberrations in cancer. Cancer Genet Cytogenet 1986;21:279–285.
83 Atkin NB: Non-random chromosomal changes in human neoplasia; in Obe G (ed): Eukaryotic Chromosomes: Structural and Functional Aspects. New Dehli, Narosa, 1991, pp 153–164.
84 Fabarius A, Willer A, Yerganian G, Hehlmann R, Duesberg P: Specific aneusomies in Chinese hamster cells at different stages of neoplastic transformation, initiated by nitrosomethylurea. Proc Natl Acad Sci USA 2002;99:6778–6783.
85 Pejonic T, Heim S, Oerndal C, Jin Y, Mandahl N, Willen H, Mitelman F: Simple numerical chromosome aberrations in well-defined malignant epithelial tumors. Cancer Genet Cytogenet 1990; 49:95–101.
86 Johansson B, Bardi G, Pandis N, Gorunova L, Backman PL, Mandahl N, Dawiskiba S, Andren-Sandberg A, Heim S, Mitelman F: Karyotypic pattern of pancreatic adenomas correlates with survival and tumor grade. Int J Cancer 1994;58:8–13.
87 Virtaneva K, Wright FA, Tanner SM, Yuan B, Lemon WJ, Caligiuri MA, Bloomfield CD, de La Chapelle A, Krahe R: Expression profiling reveals fundamental biological differences in acute myeloid leukemia with isolated trisomy 8 and normal cytogenetics. Proc Natl Acad Sci USA 2001;98:1124–1129.
88 Petersen I, Langreck H, Wolf G, Schwendel A, Psille R, Vogt P, Reichel MB, Ried T, Dietel M: Small-cell lung cancer is characterized by a high incidence of deletions on chromosomes 3p, 4q, 5q, 10q, 13q and 17p. Br J Cancer 1997;75:79–86.
89 Ried T, Heselmeyer-Haddad K, Blegen H, Schrock E, Auer G: Genomic changes defining the genesis, progression, and malignancy potential in solid human tumors: a phenotype/genotype correlation. Genes Chromosomes Cancer 1999;25:195–204.
90 Jiang F, Richter J, Schraml P, Bubendorf L, Gasser T, Sauter G, Mihatsch MJ, Moch H: Chromosomal imbalances in papillary renal cell carcinoma: genetic differences between histological subtypes. Am J Pathol 1998;153:1467–1473.
91 Richter J, Beffa L, Wagner U, Schraml P, Gasser TC, Moch H, Mihatsch MJ, Sauter G: Patterns of chromosomal imbalances in advanced urinary bladder cancer detected by comparative genomic hybridization. Am J Pathol 1998;153:1615–1621.
92 Nupponen NN, Kakkola L, Koivisto P, Visakorpi T: Genetic alterations in hormone-refractory recurrent prostate carcinomas. Am J Pathol 1998;153:141–148.

93 Weber RG, Scheer M, Born IA, Joos S, Cobbers JM, Hofele C, Reifenberger G, Zoller JE, Lichter P: Recurrent chromosomal imbalances detected in biopsy material from oral premalignant and malignant lesions by combined tissue microdissection, universal DNA amplification, and comparative genomic hybridization. Am J Pathol 1998;153:295–303.
94 Gebhart E, Liehr T: Patterns of genomic imbalances in human solid tumors. Int J Oncol 2000;16:383–399.
95 Dellas A, Torhorst J, Jiang F, Proffitt J, Schultheiss E, Holzgreve W, Sauter G, Mihatsch MJ, Moch H: Prognostic value of genomic alterations in invasive cervical squamous cell carcinoma of clinical stage IB detected by comparative genomic hybridization. Cancer Res 1999;59: 3475–3479.
96 Patel AS, Hawkins AL, Griffin CA: Cytogenetics and cancer. Curr Opin Oncol 2000;12:62–67.
97 Wilkens L, Flemming P, Gebel M, Bleck J, Terkamp C, Wingen L, Kreipe H, Schlegelberger B: Induction of aneuploidy by increasing chromosomal instability during dedifferentiation of hepatocellular carcinoma. Proc Natl Acad Sci USA 2004;101:1309–1314.
98 Hoglund M, Sall T, Heim S, Mitelman F, Mandahl N, Fadl-Elmula I: Identification of cytogenetic subgroups and karyotypic pathways in transitional cell carcinoma. Cancer Res 2001;61:8241–8246.
99 Heselmeyer-Haddad K, Sommerfeld K, White NM, Chaudhri N, Morrison LE, Palanisamy N, Wang ZY, Auer G, Steinberg W, Ried T: Genomic amplification of the human telomerase gene (TERC) in pap smears predicts the development of cervical cancer. Am J Pathol 2005;166:1229–1238.
100 Meijer GA, Hermsen MA, Baak JP, van Diest PJ, Meuwissen SG, Belien JA, Hoovers JM, Joenje H, Snijders PJ, Walboomers JM: Progression from colorectal adenoma to carcinoma is associated with non-random chromosomal gains as detected by comparative genomic hybridisation. J Clin Pathol 1998;51:901–909.
101 Aragane H, Sakakura C, Nakanishi M, Yasuoka R, Fujita Y, Taniguchi H, Hagiwara A, Yamaguchi T, Abe T, Inazawa J, Yamagishi H: Chromosomal aberrations in colorectal cancers and liver metastases analyzed by comparative genomic hybridization. Int J Cancer 2001;94:623–629.
102 Nakao K, Shibusawa M, Ishihara A, Yoshizawa H, Tsunoda A, Kusano M, Kurose A, Makita T, Sasaki K: Genetic changes in colorectal carcinoma tumors with liver metastases analyzed by comparative genomic hybridization and DNA ploidy. Cancer 2001;91:721–726.
103 Al-Mulla F, Keith WN, Pickford IR, Going JJ, Birnie GD: Comparative genomic hybridization analysis of primary colorectal carcinomas and their synchronous metastases. Genes Chromosomes Cancer 1999;24:306–314.
104 Hermsen M, Postma C, Baak J, Weiss M, Rapallo A, Sciutto A, Roemen G, Arends JW, Williams R, Giaretti W, De Goeij A, Meijer G: Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability. Gastroenterology 2002;123:1109–1119.
105 Knosel T, Schluns K, Stein U, Schwabe H, Schlag PM, Dietel M, Petersen I: Chromosomal alterations during lymphatic and liver metastasis formation of colorectal cancer. Neoplasia 2004;6:23–28.
106 Petersen I, Petersen S: Towards a genetic-based classification of human lung cancer. Anal Cell Pathol 2001;22:111–121.
107 Schimke RT: Gene amplification, drug resistance, and cancer. Cancer Res 1984;44:1735–1742.
108 Hauschka TS, Levan A: Inverse relationship between chromosome ploidy and host-specificity of sixteen transplantable tumors. Exp Cell Res 1953;4:457–467.
109 Vogt M: A study of the relationship between karyotype and phenotype in cloned lines of strain HeLa. Genetics 1959;44:1257–1270.
110 Hauschka T, Levan A: Cytologic and functional characterization of single cell clones isolated from the Krebs-2 and Ehrlich ascites tumors. J Natl Cancer Inst 1958;21:77–111.
111 Reeves RH: Recounting a genetic story. Nature 2000;405:283–284.
112 Mao R, Zielke CL, Zielke HR, Pevsner J: Global up-regulation of chromosome 21 gene expression in the developing Down syndrome brain. Genomics 2003;81:457–467.
113 Shapiro BL: Down syndrome: a disruption of homeostasis. Am J Med Genet 1983;14:241–269.
114 Epstein C: The Consequences of Chromosome Imbalance: Principles, Mechanisms, and Models. Cambridge, Cambridge University Press, 1986.
115 Schoenlein PV: Molecular cytogenetics of multiple drug resistance. Cytotechnology 1993;12:63–89.
116 Pollack JR, Sorlie T, Perou CM, Rees CA, Jeffrey SS, Lonning PE, Tibshirani R, Botstein D, Borresen-Dale AL, Brown PO: Microarray analysis reveals a major direct role of DNA copy number

alteration in the transcriptional program of human breast tumors. Proc Natl Acad Sci USA 2002;99:12963–12968.
117 Furge KA, Lucas KA, Takahashi M, Sugimura J, Kort EJ, Kanayama HO, Kagawa S, Hoekstra P, Curry J, Yang XJ, Teh BT: Robust classification of renal cell carcinoma based on gene expression data and predicted cytogenetic profiles. Cancer Res 2004;64:4117–4121.
118 Aggarwal A, Leong SH, Lee C, Kon OL, Tan P: Wavelet transformations of tumor expression profiles reveals a pervasive genome-wide imprinting of aneuploidy on the cancer transcriptome. Cancer Res 2005;65:186–194.
119 Caspersson T, Foley GE, Killander D, Lomakka G: Cytochemical differences between mammalian cell lines of normal and neoplastic origins: correlation with heterotransplantability in Syrian hamsters. Exp Cell Res 1963;32:553–565.
120 Pihan GA, Purohit A, Wallace J, Knecht H, Woda B, Quesenberry P, Doxsey SJ: Centrosome defects and genetic instability in malignant tumors. Cancer Res 1998;58:3974–3985.
121 Lingle WL, Barrett SL, Negron VC, D'Assoro AB, Boeneman K, Liu W, Whitehead CM, Reynolds C, Salisbury JL: Centrosome amplification drives chromosomal instability in breast tumor development. Proc Natl Acad Sci USA 2002;99:1978–1983.
122 Pihan GA, Wallace J, Zhou Y, Doxsey SJ: Centrosome abnormalities and chromosome instability occur together in pre-invasive carcinomas. Cancer Res 2003;63:1398–1404.
123 Ghadimi BM, Sackett DL, Difilippantonio MJ, Schrock E, Neumann T, Jauho A, Auer G, Ried T: Centrosome amplification and instability occurs exclusively in aneuploid, but not in diploid colorectal cancer cell lines, and correlates with numerical chromosomal aberrations. Genes Chromosomes Cancer 2000;27:183–190.
124 Lingle WL, Lutz WH, Ingle JN, Maihle NJ, Salisbury JL: Centrosome hypertrophy in human breast tumors: implications for genomic stability and cell polarity. Proc Natl Acad Sci USA 1998;95:2950–2955.
125 Doxsey S, Zimmerman W, Mikule K: Centrosome control of the cell cycle. Trends Cell Biol 2005;15:303–311.
126 Bernards R, Weinberg RA: A progression puzzle. Nature 2002;418:823.
127 Goldie JH: Drug resistance in cancer: a perspective. Cancer Metastasis Rev 2001;20:63–68.
128 Doubre H, Cesari D, Mairovitz A, Benac C, Chantot-Bastaraud S, Dagnon K, Antoine M, Danel C, Bernaudin JF, Fleury-Feith J: Multidrug resistance-associated protein (MRP1) is overexpressed in DNA aneuploid carcinomatous cells in non-small cell lung cancer (NSCLC). Int J Cancer 2005;113:568–574.
129 Hayflick L, Moorhead PS: The serial cultivation of human diploid cell strains. Exp Cell Res 1961;25:585–621.
130 Bishop JM: Enemies within: genesis of retrovirus oncogenes. Cell 1981;23:5–6.
131 Tabin CJ, Bradley SM, Bargmann CI, Weinberg RA, Papageorge AG, Scolnick EM, Dhar R, Lowy DR, Chang EH: Mechanism of activation of a human oncogene. Nature 1982;300:143–149.
132 Bishop JM: The molecular genetics of cancer. Science 1987;235:305–311.
133 Varmus HE: The molecular genetics of cellular oncogenes. Annu Rev Genet 1984;18:533–612.
134 Boland CR, Ricciardello L: How many mutations does it take to make a tumor? Proc Natl Acad Sci USA 1999;96:14675–14677.
135 Kallioniemi OP, Kallioniemi A, Kurisu W, Thor A, Chen LC, Smith HS, Waldman FM, Pinkel D, Gray JW: ERBB2 amplification in breast cancer analyzed by fluorescence in situ hybridization. Proc Natl Acad Sci USA 1992;89:5321–5325.
136 Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, Vogelstein B, Kinzler KW: Gene expression profiles in normal and cancer cells. Science 1997;276:1268–1272.
137 Rasnick D, Duesberg P: How aneuploidy affects metabolic control and causes cancer. Biochem J 1999;340:621–630.
138 Akagi T, Sasai K, Hanafusa H: Refractory nature of normal human diploid fibroblasts with respect to oncogene-mediated transformation. Proc Natl Acad Sci USA 2003;100:13567–13572.
139 Atkin NB, Baker MC: Are human cancers ever diploid – or often trisomic? Conflicting evidence from direct preparations and cultures. Cytogenet Cell Genet 1990;53:58–60.
140 Duesberg P, Rausch C, Rasnick D, Hehlmann R: Genetic instability of cancer cells is proportional to their degree of aneuploidy. Proc Natl Acad Sci USA 1998;95:13692–13697.

141 Hansemann D: Ueber asymmetrische Zelltheilung in Epithelkrebsen und deren biologische Bedeutung. Virchows Arch Pathol Anat 1890;119:299–326.
142 Boveri T: On multipolar mitosis as a means of analysis of the cell nucleus; in Willier BH, Oppenheimer JM (eds): Foundations of Experimental Embryology. New York, Prentice Hall, 1902/1964, pp 74–97.
143 Boveri T: Zur Frage der Entstehung maligner Tumoren. Jena, Fischer, 1914.
144 Darlington CD: Plasmgene theory and cancer genesis; in Genetics and Cancer; A collection of papers presented at the thirteenth annual symposium on fundamental cancer research, 1959. Austin, University of Texas MD Anderson Hospital and Tumor Institute, 1959, pp 9–24.
145 Rous P: Surmise and fact on the nature of cancer. Nature 1959;183:1357–1361.
146 Johansson B, Mertens F, Mitelman F: Primary vs. secondary neoplasia-associated chromosomal abnormalities – balanced rearrangements vs. genomic imbalances? Genes Chromosomes Cancer 1996;16:155–163.
147 Levan A, Levan G, Mitelman F: Chromosomes and cancer. Hereditas 1977;86:15–30.
148 Pennisi E: Trigger for centrosome replication found. Science 1999;238:770–771.
149 Grimm D: Genetics. Disease backs cancer origin theory. Science 2004;306:389.
150 Lengauer C, Wang Z: From spindle checkpoint to cancer. Nat Genet 2004;36:1144–1145.
151 Rajagopalan H, Lengauer C: Aneuploidy and cancer. Nature 2004;432:338–341.
152 Hede K: Which came first? Studies clarify role of aneuploidy in cancer. J Natl Cancer Inst 2005;97:87–89.
153 Rajagopalan H, Jallepalli PV, Rago C, Velculescu VE, Kinzler KW, Vogelstein B, Lengauer C: Inactivation of hCDC4 can cause chromosomal instability. Nature 2004;428:77–81.
154 O'Brien S, Menotti-Raymond M, Murphy W, Nash W, Wirnberg J, Stanyon R, Copeland N, Jenkins N, Womack J, Marshall Graves J: The promise of comparative genomics in mammals. Science 1999;286:458–481.
155 Lindsley DL, Sandler L, Baker BS, Carpenter ATC, Denell RE, Hall JC, Jacobs PA, Gabor Miklos GL, Davis BK, Gethmann RC, Hardy RW, Hessler A, Miller SM, Nozawa H, Parry DM, Gould-Somero M: Segmental aneuploidy and the genetic gross structure of the *Drosophila* genome. Genetics 1972;71:157–184.
156 Matzke MA, Mittelsten-Scheid O, Matzke AJM: Rapid structural and epigenetic changes in polyploid and aneuploid genomes. BioEssays 1999;21:761–767.
157 Hernandez D, Fisher EM: Mouse autosomal trisomy: two's company, three's a crowd. Trends Genet 1999;15:241–247.
158 Duesberg P, Rasnick D: Aneuploidy, the somatic mutation that makes cancer a species of its own. Cell Motil Cytoskeleton 2000;47:81–107.
159 Sachidanandam R, Weissman D, et al: A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature 2001;409:928–933.
160 Kinzler KW, Vogelstein B: Gatekeepers and caretakers. Nature 1997;386:761–762.
161 Kacser H, Burns JA: Molecular democracy: who shares the controls? Biochem Soc Trans 1979;7:1149–1160.
162 Hartman JL, Garvik B, Hartwell L: Principles for the buffering of genetic variation. Science 2001;291:1001–1004.
163 Cornish-Bowden A: Metabolic control analysis in biotechnology and medicine. Nature Biotechnology 1999;17:641–643.
164 Ames B, Durston WE, Yamaski E, Lee FD: Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci USA 1973;70:2281–2285.
165 Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD: Molecular Biology of the Cell. New York, Garland, 1994.
166 Burdette WJ: The significance of mutation in relation to origin of tumors. Cancer Res 1955;15:201–226.
167 Conti JC, Aldaz CM, O'Connell J, Klen-Szanto AJ-P, Slaga TJ: Aneuploidy, an early event in mouse skin tumor development. Carcinogenesis 1986;7:1845–1848.
168 Lijinsky W: A view of the relation between carcinogenesis and mutagenesis. Environ Mol Mutagen 1989;14(suppl 16):78–84.

169 Li R, Yerganian G, Duesberg P, Kraemer A, Willer A, Rausch C, Hehlmann R: Aneuploidy correlated 100% with chemical transformation of Chinese hamster cells. Proc Natl Acad Sci USA 1997;94:14506–14511.
170 Duesberg P, Li R, Rasnick D, Rausch C, Willer A, Kraemer A, Yerganian G, Hehlmann R: Aneuploidy precedes and segregates with chemical carcinogenesis. Cancer Genet Cytogenet 2000;119:83–93.
171 Kadhim MA, Macdonald DA, Goodhead DT, Lorimore SA, Marsden SJ, Wright EG: Transmission of chromosomal instability after plutonium α-particle irradiation. Nature 1992;355:738–740.
172 Holmberg K, Falt S, Johansson A, Lambert B: Clonal chromosome aberrations and genomic instability in X-irradiated human T-lymphocyte cultures. Mutat Res 1993;286:321–330.
173 Trott DA, Cuthbert AP, Overell RW, Russo I, Newbold RF: Mechanisms involved in the immortalization of mammalian cells by inonizing radiation and chemical carcinogens. Carcinogenesis 1995;16:193–204.
174 Wright EG: Inherited and inducible chromosomal instability: a fragile bridge between genome integrity mechanisms and tumourigenesis. J Pathol 1999;187:19–27.
175 Roschke AV, Tonon G, Gehlhaus KS, McTyre N, Bussey KJ, Lababidi S, Scudiero DA, Weinstein JN, Kirsch IR: Karyotypic complexity of the NCI-60 drug-screening panel. Cancer Res 2003;63:8634–8647.
176 Kost-Alimova M, Fedorova L, Yang Y, Klein G, Imreh S: Microcell-mediated chromosome transfer provides evidence that polysomy promotes structural instability in tumor cell chromosomes through asynchronous replication and breakage within late-replicating regions. Genes Chromosomes Cancer 2004;40:316–324.
177 Camps J, Ponsa I, Ribas M, Prat E, Egozcue J, Peinado MA, Miro R: Comprehensive measurement of chromosomal instability in cancer cells: combination of fluorescence in situ hybridization and cytokinesis-block micronucleus assay. FASEB J 2005;19:828–830.
178 Lengauer C, Kinzler KW, Vogelstein B: Genetic instability in colorectal cancers. Nature 1997;386: 623–627.
179 Das SK, Kunkel TA, Loeb LA: Effects of altered nucleotide concentrations on the fidelity of DNA replication. Basic Life Sci 1985;31:117–126.
180 Knudson AG: Chasing the cancer demon. Annu Rev Genet 2000;34:1–19.
181 Hoeijmakers JH: Genome maintenance mechanisms for preventing cancer. Nature 2001;411: 366–374.
182 German J: Bloom's syndrome. II. The prototype of genetic disorders predisposing to chromosome instability and cancer; in German J (ed): Chromosomes and Cancer. New York, Wiley, 1974, pp 601–617.
183 Hanks S, Coleman K, Reid S, Plaja A, Firth H, Fitzpatrick D, Kidd A, Mehes K, Nash R, Robin N, Shannon N, Tolmie J, Swansbury J, Irrthum A, Douglas J, Rahman N: Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. Nat Genet 2004;36: 1159–1161.
184 Kajii T, Kawai T, Takumi T, Misu H, Mabuchi O, Takahashi Y, Tachino M, Nihei F, Ikeuchi T: Mosaic variegated aneuploidy with multiple congenital abnormalities: homozygosity for total premature chromatid separation trait. Am J Med Genet 1998;78:245–249.
185 Lanza A, Lagomarsini P, Casati A, Ghetti P, Stefanini M: Chromosomal fragility in the cancer-prone disease xeroderma pigmentosum preferentially involves bands relevant for cutaneous carcinogenesis. Int J Cancer 1997;74:654–663.
186 Benedict WF, Banerjee A, Mark C, Murphree AL: Non-random retinoblastoma gene is a recessive cancer gene. Cancer Genet Cytogenet 1983;10:311–333.
187 Gardener HA, Gallie BL, Knight LA, Phillips RA: Multiple karyotypic changes in retinoblastoma tumor cells: presence of normal chromosome No. 13 in most tumors. Cancer Genet Cytogenet 1982;6:201–211.
188 Hamel PA, Phillips RA, Muncaster M, Gallie BL: Speculations on the roles of RB1 in tissue-specific differentiation, tumor initiation, and tumor progression. FASEB J 1993;7:846–854.
189 Amare Kadam PS, Ghule P, Jose J, Bamne M, Kurkure P, Banavali S, Sarin R, Advani S: Constitutional genomic instability, chromosome aberrations in tumor cells and retinoblastoma. Cancer Genet Cytogenet 2004;150:33–43.

190 Worsham MJ, Carey TE, Benninger MS, Gasser KM, Kelker W, Zarbo RJ, Van Dyke DL: Clonal cytogenetic evolution in a squamous cell carcinoma of the skin from a xeroderma pigmentosum patient. Genes Chromosomes Cancer 1993;7:158–164.
191 Hassold TJ: Chromosome abnormalities in human reproductive wastage. Trends Genet 1986;2:105–110.
192 Bauer KH: Das Krebsproblem, ed 2. Berlin, Springer, 1963.
193 Fusenig NE, Boukamp P: Multiple stages and genetic alterations in immortalization, malignant transformation, and tumor progression of human skin keratinocytes. Mol Carcinog 1998;23:144–158.
194 Urano K, Katakai Y, Tokuda Y, Ueyama Y, Nomura T, Yamamoto S: Failure of genotoxic carcinogens to produce tumors in human skin xenografts transplanted to SCID mice. Carcinogenesis 1995;16:2223–2226.
195 Soballe PW, Montone KT, Satyamoorthy K, Nesbit M, Herlyn M: Carcinogenesis in human skin grafted to SCID mice. Cancer Res 1996;56:757–764.
196 Roschke AV, Stover K, Tonon G, Schaffer AA, Kirsch IR: Stable karyotypes in epithelial cancer cell lines despite high rates of ongoing structural and numerical chromosomal instability. Neoplasia 2002;4:19–31.
197 Atkin NB: Nuclear size in premalignant conditions of the cervix uteri. Nature 1964;202:201.
198 Winge O: Zytologische Untersuchungen ueber die Natur maligner Tumoren. II. Teerkarzinome bei Maeusen. Z Zellforsch Mikrosk Anat 1930;10:683–735.
199 Spriggs AI: Cytogenetics of cancer and precancerous states of the cervix uteri; in German J (ed): Chromosomes and Cancer. New York, Wiley, 1974, p 423.
200 Frankfurt OS, Chin JL, Englander LS, Greco WR, Pontes JE, Rustum YM: Relationship between DNA ploidy, glandular differentiation, and tumor spread in human prostate cancer. Cancer Res 1985;45:1418–1423.
201 Bocking A, Chatelain R: Diagnostic and prognostic value of DNA cytometry in gynecologic cytology. Anal Quant Cytol Histol 1989;11:177–186.
202 Choma D, Daures JP, Quantin X, Pujol JL: Aneuploidy and prognosis of non-small-cell lung cancer: a meta-analysis of published data. Br J Cancer 2001;85:14–22.
203 Schoch C, Haferlach T, Haase D, Fonatsch C, Loffler H, Schlegelberger B, Staib P, Sauerland MC, Heinecke A, Buchner T, Hiddemann W: Patients with de novo acute myeloid leukaemia and complex karyotype aberrations show a poor prognosis despite intensive treatment: a study of 90 patients. Br J Haematol 2001;112:118–126.
204 Grimwade D, Walker H, Harrison G, Oliver F, Chatters S, Harrison CJ, Wheatley K, Burnett AK, Goldstone AH: The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood 2001;98:1312–1320.
205 Giaretti W: A model of DNA aneuploidization and evolution in colorectal cancer. Lab Invest 1994;71:904–910.
206 Shackney SE, Berg G, Simon SR, Cohen J, Amina S, Pommersheim W, Yakulis R, Wang S, Uhl M, Smith CA, Pollice A, Hartsock R: Origins and clinical implications of aneuploidy in early bladder cancer. Cytometry 1995;22:307–316.
207 Kim SH, Roth KA, Moser AR, Gordon JI: Transgenic mouse models that explore the multistep hypothesis of intestinal neoplasia. J Cell Biol 1993;123:877–893.
208 Smits R, Kielman MF, Breukel C, Zurcher C, Neufeld K, Jagmohan-Changur S, Hofland N, van Dijk J, White R, Edelmann W, et al.: Apc1638T: a mouse model delineating critical domains of the adenomatous polyposis coli protein involved in tumorigenesis and development. Genes Dev 1999;13:1309–1321.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 45–65

Adult Stem Cell Theory of the Multi-Stage, Multi-Mechanism Theory of Carcinogenesis: Role of Inflammation on the Promotion of Initiated Stem Cells

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Abstract

Inflammation, induced by microbial agents, radiation, endogenous or exogenous chemicals, has been associated with chronic diseases, including cancer. Since carcinogenesis has been characterized as consisting of the 'initiation', 'promotion' and 'progression' phases, the inflammatory process could affect any or all three phases. The stem cell theory of carcinogenesis has been given a revival, in that isolated human adult stem cells have been isolated and shown to be 'targets' for neoplastic transformation. Oct4, a transcription factor, has been associated with adult stem cells, as well as their immortalized and tumorigenic derivatives, but not with the normal differentiated daughters. These data are consistent with the stem cell theory of carcinogenesis. In addition, Gap Junctional Intercellular Communication (GJIC) seems to play a major role in cell growth. Inhibition of GJIC by non-genotoxic chemicals or various oncogenes seems to be the mechanism for the tumor promotion and progression phases of carcinogenesis. Many of the toxins, synthetic non-genotoxicants, and endogenous inflammatory factors have been shown to inhibit GJIC and act as tumor promoters. The inhibition of GJIC might be the mechanism by which the inflammatory process affects cancer and that to intervene during tumor promotion with anti-inflammatory factors might be the most efficacious anti-cancer strategy.

There exist two opposing hypotheses concerning the origin of all cancers, namely, the 'stem cell hypothesis' [1–8] and the 'de-differentiation' hypothesis [9]. The following brief review of these very old ideas will be put in the context

of the overall goal of this book, namely the role of infection and inflammation in carcinogenesis. To attempt such an integration, several assumptions will be made, namely that (a) carcinogenesis is a multi-step, multi-mechanism process, consisting of an 'initiation', 'promotion' and 'progression' step [10, 11]; (b) while a tumor consists of many genotypes and phenotypes, suggesting 'genomic instability' [12], the evidence points to a monoclonal origin of these tumor cells [4, 5]; (c) chronic inflammatory process affects the promotion/progression phases of carcinogenesis, not the initiation phase [13]; and (d) while the evidence, which can be used to disprove either the stem cell or de-differentiation hypotheses is still not unequivocal, using 'Ockham's razor', it seems that newer studies seem to be consistent with the stem cell hypothesis.

'Initiation', 'Promotion' and 'Progression' Concept of Carcinogenesis

The experimental carcinogenesis studies in rodent skin and liver have, operationally, demonstrated that after a single exposure to a 'subthreshold' dose/concentration of a physical or chemical carcinogen, which does not lead to an induction of cancers during the lifetime of the animal, an irreversible change had occurred in a single cell, since subsequent chronic and regular exposures to a non-carcinogen, led to the appearance of skin and liver cancers [14, 15]. While the 'irreversible' event in a single normal cell has been assumed to be the result of a mutation caused by DNA damage and error-prone repair of that damage, stable epigenetic events might also contribute to initiating the carcinogenic process, such as in the case of teratocarcinomas. Known physical and suspected chemical mutagens seem to be able to 'initiate' animals, irreversible epigenetic events or the selection of pre-existing, spontaneously mutated cells, caused by ubiquitous mutagens, endogenous oxidative damage or error-prone replication, might also explain the 'initiation' event. In the case of teratocarcinoma induction, where cells from the teratocarcinoma could be placed back into a normal blastocyst to give rise to a normal mouse, suggests that an epigenetic event that could be reversed by placing this abnormal tumorigenic cell into a normal micro-environment might explain this form of cancer [16].

Promotion, operationally, is that process that brings about a clonal expansion of the single 'initiated' cell, such that, after many cell divisions, additional changes could occur in at least one of the initiated cells to bring about all those phenotypes associated with a malignant cell, the so-called 'hallmarks of cancers' [17]. The increase of the initiated cell by the promotion process can and probably occurs because of both an increase in the birth of new initiated cells and a decrease in their death. In other words, promotion involves both the

selective mitogenesis, and not mutagenesis, and decreased apoptosis of the initiated cells. Promotion could occur then by agents and conditions that could stimulate cell proliferation, such as endogenous mitogenic growth factors, hormones, cytokines, occurring during normal growth cycles, wound healing after burns, surgery, organ cytotoxicity or, in the context of this review, chronic inflammatory processes leading to hyperplasia [18]. In addition, exogenous exposure to agents that either or both stimulate cell proliferation or inhibit apoptosis. If a mutagen, such as UV light, causes not only mutations in skin cells but also cell death, it could be both an initiator, as well as an indirect promoter, by inducing compensatory hyperplasia of any surviving UV-induced initiated cell. Nonmutagenic chemicals, such as alcohol or carbon tetrachloride or certain viruses that kill cells by necrosis, could act as an indirect promoter by inducing compensatory hyperplasia. Inhaled solid particles, such as asbestos, would induce chronic inflammatory process that, then, could release secreted chemicals, e.g. interleukin-1, interleukin-6, prostaglandin E2, and tumor necrosis factor that might act as either or both mitogens and apoptosis inhibitors to any initiated cell in the proximity of the irritant.

Promoters also have other characteristics that distinguishes them from 'initiators', in that there seems to be a requirement that (a) there be a 'threshold' amount of the promoter; (b) the promoter be applied in a regular, sustained fashion; (c) the promoter be given in the absence of an antipromoter; and (d) the promotion process can be interrupted, if not reversed [18, 19]. A wide variety of agents and conditions seem to be able to promote tumors [20], suggesting multi-molecular/biochemical mechanisms that can lead to a common cellular basis for mitogenesis and to the inhibition of apoptosis of the initiated cell. Promoters are, also, species, organ and cell-type specific.

Progression, on the other hand, seems to be a late step in carcinogenesis that confers on a single cell in the mass of a promoted clone of initiated cells the property of being autonomous of an endogenous or exogenous promoter, and thereby able to acquire addition 'hallmarks' of cancer that enables it to invade surrounding tissue, to metastasize and to induce angiogenesis to supply needed nutrients to the tumor.

What Is that 'Initiated' Cell?

If that tumor, consisting of billions of genotypic/phenotypic heterogeneous metastatic cells, originated from a single normal cell, the question remains is that single 'target' cell any cell of the body or is that a 'special' cell, such as an adult stem cell? Obviously, if that cell is a terminally differentiated cell, such as a red blood cell, or a lens cell, which has lost its genome during the differentiation

process, it can not de-differentiate and then give rise to a tumor cell with abnormal chromosomes and genes. However, if the cell is a progenitor cell that has been derived from an adult stem cell of a given organ, been committed to differentiate to an organ-specific manner and has lost telomerase activity and lost significant telomeres, it seems destined to senesce, unless it can reactivate its telomerase and restore its telomeres. If, however, the progenitor cell has not lost telomerase activity or significant amount of its telomeres, might it have the ability to de-differentiate from its young differentiated or committed lineage of that organ?

First, it seems appropriate to define what might be an initiated cell. The initiation process must prevent a single cell to terminally different or senesce. Second, initiated cells seem also to resist apoptosis. Functionally, an initiated cell must be able to have unlimited proliferative potential, and be able to resist apoptosis, two of the 'hallmarks of cancer'.

Initiation: Is It the Induction of 'Immortalization' of a Normal, 'Mortal' Cell or the Inhibition of 'Mortalization' of a Normal 'Immortal' Adult Stem Cell?

One of the major paradigms, driving current thinking in the field of cancer research, is the hypothesis that the initial/ 'initiating' step of the carcinogenesis process must involve the 'immortalization' of a normal cell [21, 22], so that it can survive long enough to accrue all of the other 'hallmarks' associated with an invasive, metastatic cancer cell. For decades, investigators have tried to 'immortalize' human fibroblast and epithelial cells with all sorts of carcinogens, only to fail most of the times. Although rodent cells seem to 'immortalize' fairly easily with carcinogens and transfected oncogenes, such as 'myc', only by the use of 'immortalizing' viruses or their genes and by transfection with the human telomerase reverse transcriptase (hTERT) gene, could human cells be 'immortalized' [23–25].

However, there is a challenge to this paradigm. In that, with the isolation of normal human adult stem cells, one can now view normal adult stem cells, found in most, if not all, organs, as being normally 'immortal' until they are induced to differentiate or 'mortalize' [26, 27]. If the adult stem cell is exposed to an initiating agent which prevents the stem cell from terminally differentiating, it would maintain many stem cell characteristics through the end of the carcinogenic process. In other words, the metastatic cell would phenotypically resemble the stem cell more than the normal differentiated cells of that lineage. This will have consequences to the current trend to use sophisticated DNA micro-array and differential gene expression studies on normal tissues and the

cancers found in those tissues. The reason being that in the extracts of normal tissues, not only contain normal cells at various stages of the cell cycle, various differentiated stages and differentiated cells of the common organ-specific adult stem cell, apoptotic cells and stressed cells, but also, the very few adult stem cells that might be the 'target' cell for the cancer [27]. Moreover, in the tumor itself, because of genomic instability, differential oxygenation of the tumor, cell cycle differences of the dividing tumor cells, as well as invasive inflammatory cells, and more recently, the identification of 'cancer stem' cells [28–36], the mixture of genes expressed in the normal tissue would mask the genes expressed in the few adult stem cells which might be the parent cell of the 'cancer stem' cell. In other words, these micro-array profiles, using the current methodology, would not reveal the underlying mechanisms leading to the differences that are seen in these studies.

Finally, if this interpretation is correct, then the 'immortalizing' viruses used to isolate these 'initiated' or 'immortalized' cells really do not 'immortalize' an already normally immortal adult stem cell, but they prevent the 'mortalization' of an already 'immortal' adult stem cell. When one re-examines the studies on 'immortalizing' human epithelial cells, one notes that the frequency of obtaining an 'immortalized' cell approaches a normal mutation frequency. If one thinks about the population of a normal primary culture, one would imagine only a few stem cells would be found in the population. If the virus (simian virus (SV) 40, papilloma virus) infects all cells, those, that were already committed to senesce, would senesce or go through 'crises'. On the other hand, if the population of primary cells had but a few stem cells, and these cells are prevented from 'mortalizing' by the SV40 of E6, E7 genes, they would survive this 'crises' phenomenon seen in this type of experiment.

Characteristics of Adult Stem Cells: Clues to the Stem Cell Hypothesis of Carcinogenesis

While it appears clear that with the explosion of studies on both embryonic and adult stem cells, many of the past definitions and concepts of stem cell biology are being called into question. However, it seems that one commonly-agreed on definition of a stem cell is that it is a cell that has the ability to divide symmetrically to produce two daughters with stem cell capacity in order to expand the stem cell pool. On the other hand, these same stem cells appear to be able to divide asymmetrically to produce one daughter that maintains stemness, while the other daughter cell is now committed to differentiate into the lineage of the particular organ it finds itself. Without addressing current controversies related to 'trans-differentiation' of adult stem cells' [37], it appears that, obviously,

an embryonic stem cell can be considered as 'toti-potent', in that it is capable of giving rise to all cell types within the multi-cellular organism. On the other hand, an adult stem cell, while it maintains several characteristics of the embryonic stem cell such as symmetrical and asymmetrical proliferation ability, it has been specifically committed to exhibit some organ specific genes.

Are There Adult Stem Cell Markers?

In order to test the stem cell hypothesis, one must first identify stem cells. Aside from testing individual cells for the inability to exhibit functions, such as symmetrical and asymmetrical cell divisions, their capacity for unlimited proliferative potential, and their ability to differentiate upon exposure to appropriate stimuli into cell lineages found in the organ in which they were isolated, can one find molecular markers that might be associated with these functions?

In trying to test the stem cell hypothesis, Chang et al. [38] assumed adult tissues must have stem cells if the stem cell is the 'target' cell for initiating the carcinogenic process. Having no direct evidence of any molecular marker or function, it was assumed that, from the field of gap junction biology, if the toti-potent stem cell, the fertilized egg, had no expressed gap junction genes (connexins) or functional gap junctional intercellular communication (GJIC), then stem cells, both embryonic and adult, must be devoid of GJIC to remain undifferentiated in their 'niche' [39]. Once these undifferentiated stem cells are induced to express their connexins and have functional GJIC, they can then start to differentiate [40]. Using an in vitro strategy based on this assumption, cells were isolated in a 'kiss of death' method, by which dis-associated cells of normal tissue, which contains a few stem cells, many GJIC positive progenitor cells and terminally-differentiated cell, were placed on a confluent mat of lethally radiated GJIC positive cells. Those cells of the biopsy that had functional GJIC coupled with the dying irradiated cells of the mat and died. The terminally differentiated cells did not proliferate on the mat. However, the few cells, derived from the dis-associated tissue that did not have expressed connexins or functional GJIC, landed on the mat of lethally irradiated cells and formed colonies within days. Upon further characterization, these cells were shown to be deficient in GJIC.

Assuming all the previous studies on the role of GJIC in cell proliferation and differentiation [41], these human kidney cells were assumed to represent adult human stem cells. To put these studies into perspective, it was known at that time that cancer cells, which do not have growth control, do not terminally differentiate, are immortal, and have abnormal apoptotic behavior, lacked either functional homologous or heterologous GJIC [42]. Later, other adult cells,

which did not express connexins or have functional GJIC, have been isolated [43–50] and shown to have the ability to divide both symmetrically and asymmetrically, as well as being able to differentiate and be relatively easily blocked from 'mortalization' and subsequently neoplasically transformed [49, 51].

Later, after it was shown that embryonic stem cells seemed to be characterized by the expression of the Oct-4 transcription factor gene [52–54], as well in the cancer cells that were tested, but not in normal adult tissue [55–57], our group assumed that adult stem cells might exhibit the Oct-4 gene. Since we had multiple human adult stem cells isolated from the kidney, breast, liver, pancreas, and mesenchymal tissue, as well human cancer cells derived either from the specific human adult stem cell or from the same organ from which the stem cell was isolated, we showed that all these normal adult stem cells expressed Oct-4, whereas their differentiated daughters did not. However, cancer cells, derived from the stem cell or from the organ from which the stem cells were isolated, also expressed Oct-4. In addition, although it had been claimed that in normal adult tissue Oct-4 was not seen, because there are so few stem cells in normal adult tissue, one could not easily see these few Oct-4 expressing cells. We, however, knowing what to look for, did observe a few Oct-4 cells in normal human and dog skin tissues [58]. These observations, together with those showing that adult stem cells to not express connexin genes, suggest that the noncancer, 'initiated' cells and cancerous cell lines were derived from the adult stem cells, thus supporting the 'stem cell' hypothesis of carcinogenesis.

Role of Gap Junctional Intercellular Communication in Normal Growth Control and Its Dysfunction in Carcinogenesis

Building on the previous observations and interpretations, it is now assumed that the adult stem cell, with its expressed Oct-4 transcription gene and its nonexpressed connexin genes and nonfunctional GJIC, is the initial cell that is 'initiated' by some mechanism. However, at this stage of carcinogenesis, this cell is not a cancer cell, nor has it lost growth control. It is only unable to terminally differentiate and resist apoptosis. It must be promoted to clonally expand in number so it can accrue addition changes needed to acquire the other phenotypes classified as 'hallmarks' of cancer.

Since to date, many chemical tumor promoters have been shown to reversibly inhibit GJIC [20] and to inhibit apoptosis [59–61]. GJIC was postulated to play a role in the tumor promotion mechanism [62–64]. This then raises a potential conundrum, because when one examines many cancer cells, one observes something very interesting, namely, some cancer cells do not express

any connexins and have no functional GJIC such as HeLa and MCF-7 cells [65, 66]. On the other hand, other cancer cells express some connexins but have no homologous or heterologous GJIC [67]. In those cells, many have activated oncogenes, such as src, ras, raf, neu, which have been shown to down regulate the function of connexins [42]. One explanation for this is that, while all tumor cells seem to lack functional GJIC, they can do so via either by being derived from a adult stem cell that has its connexin genes transcriptionally repressed or from a early progenitor cell that has expressed its connexin genes, had functional GJIC, started to differentiate but was initiated or blocked from its ability to repress its Oct-4 gene, to repress its telomerase activity so as not to lose its telomeres ('ongogeny as blocked or partially blocked ontogency' [68]; fig. 1). Of course, another possibility is that both types of GJIC negative tumor cells were derived from the adult stem cells and only in some tumors are some of the tumor-derived stem cells capable of partial differentiation, i.e. in which the connexin genes were expressed and the cells started to differentiate because of micro-environmental factors within the tumor.

Cancer Stem Cells: Something New or a Newly Discovered Old Prediction

A number of recent publications have provided experimental evidence that not all the cells of a tumor have the capacity to perpetuate the tumor. However, within the heterologous population of tumor cells are what appears to be the 'cancer stem cells' [28–36]. To many, this constitutes a major conceptual breakthrough in the understanding of carcinogenesis. Yet, insights, generated decades again, suggested that adult cancers were derived from adult stem cells and teratocarcinomas were derived from embryonic stem cells. Cancers, conceived of as a 'disease of differentiation' [1], cancers as a 'stem cell disease' [2], and 'oncogeny as partially blocked ontogeny' [68], as well as insights gained from the monoclonal nature of tumors [4, 5], have been long thought of as having been derived from stem cells [8].

One property of both stem cells and cancer cells seems that to provide some indirect evidence for the cancer having been derived from the stem cell. That property or 'hallmark' is the 'immortality' of both. While it is generally accepted that tumor cells are immortal, it has not been universally accepted that the original target cell from which the cancer cell is derived is immortal. In fact, the prevailing paradigm has been that the normal target cell, that is 'initiated' to become a cancer cell, is 'mortal' and that it must be 'immortalized' in order that it can escape senescence to be able to proliferate indefinitely to accrue all the other 'hallmarks' needed to acquire the ultimate phenotypes of invasiveness,

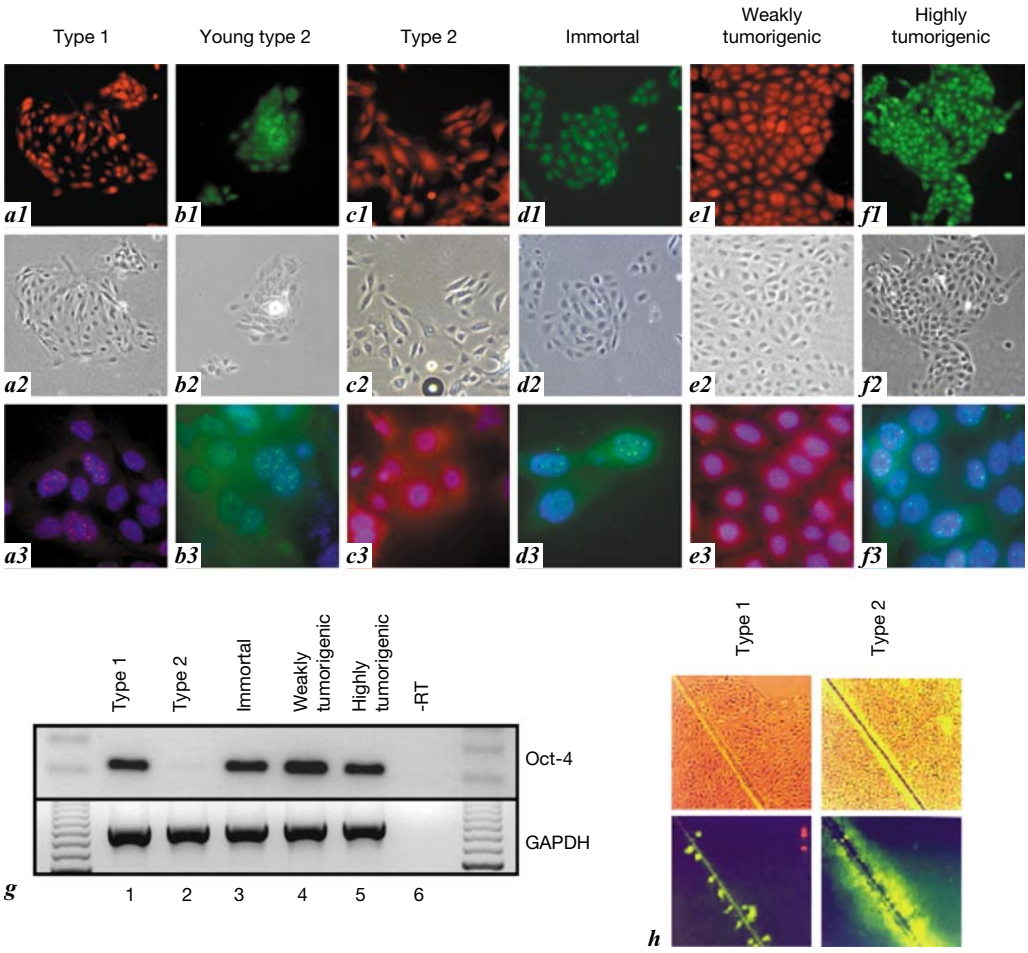

Fig. 1. Oct-4 expression in human breast epithelial cells. The presence or absence of the expression of Oct-4 transcription factor gene in human breast epithelial cells. In all panels: ***1*** = low magnification; ***2*** = phase contrast; ***3*** = high magnification. The term 'type 1' refers to the normal human breast epithelial stem cell, the term 'type 2' refers to the normal differentiated human breast epithelial cell after the type 1 has been induced to differentiate and examined. ***a1–a3*** Type 1 cells. ***b1–b3*** Young type 2 cells. ***c1–c3*** Type 2 cells. ***d1–d3*** SV40 immortalized type 1 cells. ***e1–e3*** Nontumorigenic SV40 immortalized type 1 cells that became weakly tumorigenic after X radiation. ***f1–f3*** Highly tumorigenic type 1 cells derived from the weakly tumorigenic cells that were transfected with the activated neu oncogene. Oct-4 is expressed in normal breast epithelial stem cell, its non-tumorigenic SV40 'immortalized' cells, the weakly and highly tumorigenic immortalized cells, but is not expressed in the normal mature differentiated breast epithelial cells. ***g*** Verification of Oct-4 expression by RT-RCR analyses. ***h*** Type 1 cells are GJIC negative in contrast to type 2 cells. GJIC was measured by the transfer of Lucifer yellow dye via the scrape loading/dye transfer technique.

metastasis and angiogenesis [69]. Many reports, as mentioned above, have shown that 'immortalizing viruses' could produce 'immortal' human cells that could be subsequently neoplastically transformed by other carcinogenic agents [70–72].

However, if one considers that a normal adult stem cell in a developing organism can invade tissues, 'metastasize' to a distal tissue and even induce angiogenesis, one should conclude these are phenotypes not unique to only cancer cells. Moreover, conceptually, a stem cell should be viewed as being normally 'immortal' until it is induced to terminally differentiate or to 'mortalize'. Again, the cancer cell is not only characterized as 'immortal' but unable to terminally differentiate under usual micro-environment conditions.

This, then, leads to the recent observations that within a tumor there appears to be cancer stem cells, as well as tumor cells that have acquired other phenotypes that suggest 'partial differentiation' or 'mortalization' has occurred. It seems obvious that the micro-environment within the tumor is very different, particularly with factors such as proximity to surround normal cells (stromal-epithelial interactions [74, 75]) and conditions of oxygenation/nutrient supply. If the cancer stem cells are derivatives of a normal adult stem cell, they probably have the capacity to differentiate, given the right micro-environment stimuli. This is clearly seen within a teratocarcinoma, derived probably from embryonic or less committed adult stem cells, where highly differentiated tissues are seen such as bone, hair, teeth, etc. In adult tumors, lack of factors from the stromal-epithelial interactions and from nutrient/oxygenation supply might be the inducing triggers for some of the cancer stem cells to 'differentiate' or 'mortalize' by apoptosis or terminal differentiation.

Stem Cells, Oncogenic Viruses, and Cancer

In the cancer field, the 'virus theory' of carcinogenesis has a long history. It should be clear, today, that if viruses do play a role in carcinogenesis, they probably must conform to the multistage, multimechanism of carcinogenesis. That is, the viral role would be in either or both the initiation or promotion phases of carcinogenesis. As indicated above, the SV40 and human papilloma viruses can 'immortalize' human cells so that these cells can have unlimited proliferative capacity to accrue all the other cancer phenotypes. However, as interpreted by this review, these viruses might have blocked the ability of the few adult stem cells to terminally differentiate. These viruses might be able to prevent the Oct-4 gene transcription factor from suppressing other genes needed for differentiation, such as the connexin genes. We have shown that normal adult human breast and pancreatic stem cells, when transfected with the SV40

and E6-E7 papilloma viral genes, can be 'immortalized', but not directly neoplastically transformed. These cells do not express their connexin genes or have functional gap junctional communication [40, 43]. However, if these cells are exposed to agents that seem to trigger the expression of the connexin genes, induce GJIC and suppress the Oct-4 gene, they differentiate. In these cases, the role of the virus is to be an 'initiator' of cancer. These viral-initiated cells could then be 'promoted' in the similar fashion as any other physical or chemical mutagen.

Clearly, if viral infection leads to massive tissue cell death, the viral infection might be viewed as an 'indirect tumor promoter', in that the death of cells might stimulate the proliferation of any surviving initiated cell in that tissue. Lastly, one cannot rule out, without rigorous experimental evidence, that viral 'insertional mutagenesis' of critical genes that contribute to the carcinogenic process.

Lastly, viral-derived 'oncogenes', such as src, ras, raf, neu, mos, have been shown to modify, posttranslationally, the connexin protein, thereby, acting as a stable, endogenously supplied tumor promoter by inhibiting GJIC [42].

Inflammation, Tumor Promotion and Carcinogenesis

Evidence seems to be mounting that the evolutionarily adaptive process of acute inflammation, which is, e.g., needed for wound-healing or eliminating infections, can be maladaptive if it is sustained chronically. Cancer, cataracts, arthritis, chronic bowel disease, diabetes, atherosclerosis and Alzheimer's disease have been associated with chronic inflammation [75–92]. In addition, several anti-inflammatory chemicals and drugs have been associated with either the prevention or treatment of many of these diseases [93–96].

Physical agents such as asbestos or small air particulates [97–99], bacterial infections [100–102], viral infections [103–107], fungal contamination [108–111], as well as parasitic infections [112] have been linked to inflammation, cancers, other acute and chronic diseases. Many of these studies suggest that the inflammatory related effects on carcinogenesis seem to involve the tumor promotion phase, rather than the initiation phase [111].

Oxidative stress, induced by physical, chemical or microbial agents, seems to be the triggering event in cells that initiate the inflammatory process [113–116]. Secreted factors, such as arachidonic acid, cytokines, and other secretogues, could, in principle, either damage macromolecules (e.g. DNA, proteins, membrane components) or trigger signaling molecules in surrounding cells.

While the prevailing paradigm, again, seems to view agents inducing oxidative stress as inducing DNA damage, mutating nuclear genes, and causing

'initiation', an alternative view is that oxidative stress is that which contributes to the promotion phase of carcinogenesis [117, 118]. Supporting that interpretation is the oxidative-stress induced inflammatory process must be chronic and sustained. Second, many non-DNA inducing, but oxidative-stress inducing chemicals are not mutagens, can trigger signal transduction, block GJIC and act as tumor promoters but not tumor initiators. Several excellent examples would be that of phorbol esters [119], perfluorated compounds [120], pentachlorophenol [121], and small-molecular-weight polynuclear aromatic hydrocarbons (PAHs) [122]. All have been shown to induce oxidative stress, inhibit GJIC, and act as tumor promoters [123–125].

With the recent demonstration that antioxidants seem to be correlated with the reduced risks to chronic diseases, as well as the use of cyclooxygenase-2 (COX-2) inhibitors, some support for the role of the chronic inflammatory process in chronic diseases [126]. However, application of a strategy to use intervention with pure anti-oxidants or anti-inflammatory drugs to reduce risk to various chronic diseases has led to several unexpected serious side effects [127]. Yet, upon reflection, 'anti-oxidants' can be 'pro-oxidants' under different sets of conditions [128]. In addition, where anti-oxidants or nutrient supplements might be positively effective is in individuals that might be deficient in these critical factors. However, to expose individuals that are constitutively 'proficient' in these factors, these anti-oxidants and nutrient supplements might actually be in-effective or even negatively effective.

Implications of the Stem Cell Theory for Cancer Chemoprevention and Chemotherapy: Cancer as a 'Treatable Chronic Disease'

The current paradigm of cancer treatment has led to a 'slash and burn' approach to deal with a tumor. The idea was to kill the cancer cells without killing the patients. Newer approaches have been to induce either terminally differentiation or apoptosis of the cancer cells. Others included 'targeted' immunotherapy to kill the cancer cells, or in case of viral carcinogenesis, to prevent viral 'initiation' of cancer by vaccination against the viral protein [129].

Starting with the two major observations, namely, that there exists cancer stem cells and cancer-non-stem cells within tumors [28–36] and that two kinds of tumor cells exist, one without any expressed connexin genes (HeLa, MCF-7 cells [65, 66]) and the other with expressed connexins but non-functional GJIC [27] (fig. 2), it should be clear that prevention and treatment of these two types tumor cells will be very different. Indeed, the very idea that the multi-stage, multi-mechanism process of carcinogenesis directly implies that each step of

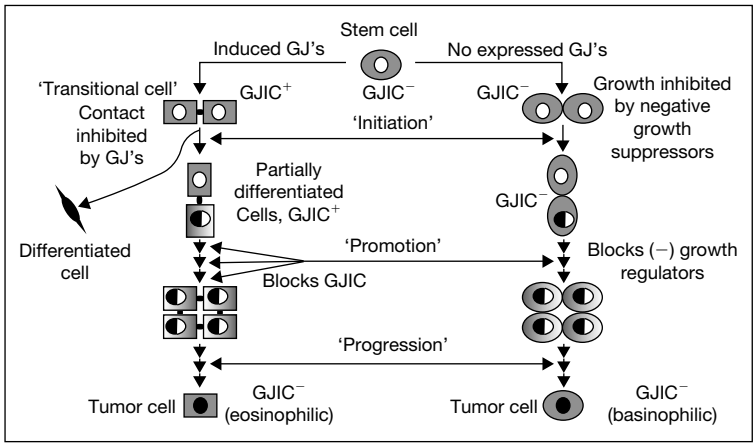

Fig. 2. The stem cell theory of carcinogenesis. This diagram illustrates how two types of cancer cells could arise from either pluri-potent stem cells (lacking expressed connexin genes and having no GJIC) or from very early transit cells, which express connexin genes and have functional GJIC after exposure to an initiator. Initiation is that process which would prevent the stem or transit cell from terminal differentiation. These initiated stem or initiated transit cells would be growth suppressed either by secreted negative growth regulators or by gap junction-dependent 'contact inhibition', respectively. If these initiated stem or initiated transit cells are exposed, chronically, to agents that either inhibit the secreted negative growth regulator or down regulate gap junctional intercellular communication, these initiated cells would proliferate, accumulate and accrue sufficient genetic/epigenetic changes sufficient to become 'promoter independent' and invasive and metastatic. In the end, both tumor types lack function GJIC, one due to the transcriptional suppression of the connexin genes, the other because various mutations, activated oncogenes, deactivated or loss of tumor suppressor genes cause down regulation of the expressed connexins and gap junctions. Strategically and tactically, based on this hypothesis, the approach to chemoprevention and chemotherapy would be very different.

this complex evolutionary acquisition of the 'hallmarks' of cancer means the physiological stage of the cells in the initiated, promoted and progression steps would be different. Therefore, no one chemopreventive/chemotherapeutic treatment will work equally at all stages or on either of the two types of tumor cells.

Starting from the realization that one class of tumor cells, such as the HeLa or MCF-7 types, does not express their connexin genes, does express Oct-4 transcription factor, and does not have functional GJIC, it should be obvious that this class of tumor cells would be very 'stem cell-like' or very primitive. On the other hand, those tumor cells that express their connexin genes but do have defective GJIC, they would be 'partially-differentiated'. Therefore, in the former case, targeting 'HeLa-like' tumor cells with agents that could transcriptionally

activate the connexin genes, so that they might start to contact inhibit and to transcriptionally inactivate the Oct-4 gene in order that they can differentiate or apoptose, would be the theoretical strategy for chemotherapy. Given that the initiated 'HeLa-like' cell is a stem-like cell that is prevented from proliferating because mitogenic suppression by some secreted negative growth regulator, chemoprevention would be with agents that negate or ameliorate the effect of promoters that inhibit the negative secreted growth regulators on the initiated cells with no expressed connexins.

In those tumor cells that have no functional GJIC but do express their connexin genes, targeting those oncogenes – e.g. src, Erb-2/neu, ras, raf, mos – that alter the connexin protein via their coded proteins (tyrosine kinases, G-proteins, etc.) would restore GJIC and cells would then contact inhibit. Clearly, not all oncogene products render the connexin proteins non-functional in the identical fashion. Therefore, specific inhibitors would have to be targeted to the activated oncogene in the specific tumor. Lovastatin, for example, restored growth control and reduced tumorigenicity to Ha-ras-transfected tumor cells, but not the src- or neu-transfected tumor cells [130]. In addition, chemoprevention of initiated pre-malignant cells with expressed connexins and functional GJIC would occur by preventing endogenous or exogenous chemicals from promoting these cells by inhibiting gap junction function. Again, not all tumor promoters work to reversibly down-regulate GJIC exactly the same manner. The tumor promotor 12-O-tetradecanoylphorbol-13-acetate (TPA) activates the protein kinase C (PKC) and subsequently hyper-phosphorylates the connexin proteins to inactivate GJIC [131]. DDT also inhibits GJIC but by a completely different mechanism [132].

In other words, there cannot be a 'universal' chemopreventive or therapeutic strategy for these two classes of tumors found in every organ. What might make treatment of cancers even more complex is the observation that not all the cells within a tumor are genetically/phenotypically identical. While all these diverse phenotypic tumor cells were clonally derived from a single initiated cell, both 'genomic instability' [12] and the micro-environmentally induced phenotypes of tumor cells constitute different 'targets' of sensitivity to any therapeutic strategy. The existence of the 'cancer stem cells' [28–36] could be the source of the 'partially' differentiated tumor cells. These two classes of tumor cells might be due to the micro-environmental conditions such as nutrient and oxygen supply that induce connexin expression, leading to partially differentiation of the tumor cells. These partially differentiated cells might be more sensitive to agents that induce apoptosis or cytotoxicity. The remaining, 'resistant' tumor cells might simply be the few cancer stem cells. In fact, rather than interpreting the induction of drug-resistance by the therapy, the therapy might be just selecting pre-existing 'cancer stem cells' that are naturally resistant to the therapy.

If this explanation is correct, then a strategy would be the use of combined therapy to account for these two classes of tumor cells within a tumor. To kill the 'partially-differentiated', GJIC positive cells with one type of agent that can take advantage of the 'bystander' effect that might be mediated through the gap junctions in these tumor cells. To control the 'cancer stem cells', agents, that might suppress the Oct-4 genes and to induce transcription of connexins, have the potential to restore growth control in these cancer cells. This would constitute another strategy for therapy.

Conclusion

An old hypothesis, namely, the stem cell hypothesis of cancer, has been reanalyzed in view of the 'hallmarks of cancer', including integration with the concepts of the multistage, multimechanism process of carcinogenesis and of the role of intercellular communication via both secreted factors and by gap junctions. Old observations, such as the fact that cancer cells lack functional homologous or heterologous GJIC, either because the connexin genes are not expressed – e.g. HeLa, MCF-7 tumor cells – or because the connexin genes were expressed but the expressed proteins were rendered nonfunctional by some activated oncogene, had to be integrated into a new hypothesis to explain that not all cancer cells are alike. In addition, the relative recent isolation of adult human stem cells and their partial characterization has led to new insights as to a new paradigm of carcinogenesis. This paradigm suggests that the stem cell, a naturally immortal cell that expresses Oct-4 transcription factor and does not express connexin genes, can be blocked from 'mortalization' by the initiation process of carcinogenesis. Promotion of these initiated premalignant cells would involve interfering with the mitotic suppressing effect of endogenous factors. In addition, in order to explain the existence of some tumor cells, which do express connexin genes and are partially differentiated, but which do not have functional GJIC, these might be the result of an early partially differentiated daughter of a stem cell that has not yet repressed its Oct-4 gene or its telomerase gene. These communicating initiated cells would be contact inhibited by functional GJIC, but promoted by chemicals that reversibly inhibit GJIC. When stably inhibited by activated oncogenes, these initiated cells can thus become autonomous in their growth. Strategies for chemoprevention and therapy will have to take into account these two classes of tumors and to their differential sensitivities to both chemopreventive and therapeutic agents being used. In the context of the potential role of infections and inflammatory processes, these two factors probably play important roles within the multistage hypothesis of carcinogenesis, but mainly within the promotion phase.

References

1 Markert C: Neoplasia: a disease of differentiation. Cancer Res 1968;28:1908–1914.
2 Pierce GP: Neoplasms, differentiation and mutations. Am J Pathol 1974;77:103–118.
3 Till JE: Stem cells in differentiation and neoplasia. J Cell Physiol 1982;1:3–11.
4 Nowell PC: The clonal evolution of tumour cell population. Science 1976;194:23–28.
5 Fialkow PJ: Clonal origin of human tumours. Am Rev Med 1979;30:135–176.
6 Greaves MF: Differentiation-linked leukemiogenesis in lymphocytes. Science 1986;234:697–704.
7 Kondo S: Carcinogenesis in relation to the stem cell mutation hypothesis. Differentiation 1983:24:1–8.
8 Trosko JE: Stem cell theory of carcinogenesis. Toxicol Lett 1989;49:283–295.
9 Sell S: Cellular origin of cancer: differentiation or stem cell maturation arrest? Environ Health Perspect 1993;101:15–26.
10 Weinstein IB, Cattoni-Celli S, Kirschmeir P, Lambert M, Hsiao W, Backer J, Jeffrey A: Multistage carcinogenesis involves multiple genes and multiple mechanisms. J Cell Physiol 1984;3:127–137.
11 Pitot HC, Dragan YP: Facts and theories concerning the mechanisms of carcinogenesis. FASEB J 1991;5:2280–2286.
12 Tlsty TD, Briot A, Gualberto A, Hall I, Hess S, Hixon M, Kuppuswamy D, Romanov S, Sage M, White A: Genomic instability and cancer. Mutat Res 1995;337:1–7.
13 Philip M, Rowley DA, Schreiber H: Inflammation as a tumour promoter in cancer induction. Semin Cancer Biol 2004;14:433–439.
14 Boutwell RK, Verma AK, Ashendel CL, Astrup E: Mouse skin: a useful model system for studying the mechanism of chemical carcinogenesis; in Hecker E, Fusenig NE, Kunz W, Marks F, Thielmann HW (eds): Carcinogenesis. New York, Raven Press, 1982, vol 7, pp 1–12.
15 Pitot C, Goldsworthy T, Moran S, Sirica AE, Weeks J: Properties of incomplete carcinogens and promoters in hepatocarcinogenesis; in Hecker E, Fusenig NE, Kunz W, Marks F, Thielmann HW (eds): Carcinogenesis. New York, Raven Press, 1982, vol 7, pp 83–98.
16 Mintz B, Illmensee K: Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc Natl Acad Sci USA 1975;72:3585–3589.
17 Hanahan D, Weinberg RA: The hallmarks of cancer. Cell 2000;100:57–70.
18 Trosko JE, Chang CC, Madhukar BV, Dupont E: Intercellular communication: a paradigm for the interpretation of the initiation/promotion/progression model of carcinogenesis; in Arcos JC (ed): Chemical Induction of Cancer. Boston, Birkhauser, 1995, pp 205–225.
19 Trosko JE: Is the concept of tumour promotion a useful paradigm? Mol Carcinog 2001;30:131–137.
20 Trosko JE, Chang CC: in Hart RW, Hoerger FD (eds): Nongenotoxic Mechanisms in Carcinogenesis: role of Inhibited Intercellular Communication. Cold Spring Harbor, Cold Spring Harbor Laboratory, 1988, pp 139–170.
21 Land H, Parada LF, Weinberg RA: Tumourigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 1983;304:596–602.
22 Newbold RF, Overell RW: Fibroblast immortality is a prerequisite for transformation by EJ c-Ha-ras oncogene. Nature 1983;304:648–651.
23 Rhim JS: Neoplastic transformation of human cells in vitro. Crit Rev Oncogen 1993;4:313–335.
24 Kuroki T, Huh N-H: Why are human cells resistant to malignant cell transformation in vitro? Jpn J Cancer Res 1993;84:1091–1100.
25 Bodnar G, Klapper W, Barthe A, Heidorn K, Jonat W, Krupp G, Parwaresch R: Analysis of telomerase expression and proliferative activity in the different layers of cyclic endometrium. Biochem Biophys Res Commun 1998;253:214–221.
26 Trosko JE, Chang CC, Wilson MR, Upham BL, Hayashi T, Wade M: Gap junctions and the regulation of cellular functions of stem cells during development and differentiation. Methods 2000;20:245–264.
27 Trosko JE: The role of stem cells and gap junctional intercellular communication in carcinogenesis. J Biochem Mol Biol 2003;36:43–48.
28 Al Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF: Prospective identification of tumourigenic breast cancer cells. Proc Natl Acad Sci USA 2003;100:3983–3988.

29 Dick JE: Breast cancer stem cells revealed. Proc Natl Acad Sci USA 2003;100:3547–3549.
30 Singh SK, Clarke ID, Terasaki M, Boon VE, Hawkins C, Squire J, Dirks PB: Identification of a cancer stem cell in human brain tumours. Cancer Res 2003;63:5821–5828.
31 Cozzio A, Passegue E, Ayton PM, Karsunky H, Cleary ML, Weissman IL: Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myloid progenitors. Genes Dev 2003;17:3029–3035.
32 Li Y, Welm B, Podsypanina K, Huang S, Chamorro M, Zhang X, Rowlands T, Egeblad M, Cowin P, Werb Z, Tan LK, Rosen JM, Varmus HE: Evidence that transgenes encoding components of the wnt signaling pathway preferentially induce mammary cancers from progenitor cells. Proc Natl Acad Sci USA 2003;100:15853–15858.
33 Kondo T, Setoguchi T, Taga T: Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Proc Natl Acad Sci USA 2004;101:781–786.
34 Beachy PA, Karhadkar SS, Berman DM: Tissue repair and stem cell renewal in carcinogenesis. Nature 2004;432:324–331.
35 Singh SK, Clark ID, Hide T, Dirks PB: Cancer stem cells in the nervous system tumours. Oncogene 2004;23:7267–7273.
36 Mihich E, Jaenisch R: Stem cells and epigenesis in cancer. Cancer Res 2004;64:8474–8477.
37 Camargo FD, Chambers SM, Goodell MA: Stem cell plasticity: from transdifferentiation to macrophage fusion. Cell Prolif 2004;37:55–65.
38 Chang CC, Trosko JE, El-Fouly MH, Gibson DR, d'Ambrosio SM: Contact insensitivity of a subpopulation of normal human fetal kidney epithelial cells and of human carcinoma cell lines. Cancer Res 1987;47:1634–1645.
39 Fuchs E, Tumbar T, Guasch G: Socialising with the neighbours: stem cells and their niche. Cell 2004;116:769–778.
40 Kao CY, Nomata K, Oakley CS, Welsch CW, Chang CC: Two types of normal human breast epithelial cells derived from reduction mammoplasty: phenotypic characterisation and response to SV40 transfection. Carcinogenesis 1995;16:531–538.
41 El-Sabban ME, Sfeir AJ, Daher MH, Kalaany NY, Basdsam RA, Talhouk RS: ECM-induced gap junctional communication enhances mammary epithelial cell differentiation. J Cell Sci 2003;116: 3531–3541.
42 Trosko JE, Ruch RJ: Cell-cell communication in carcinogenesis. Front Biosci 1998;3:245–264.
43 Tai MH, Madhukar BV, Olson LK, Linning KD, VanCamp L, Tsao MS, Trosko JE: Characterisation of gap junctional intercellular communication in immortalised human ductal epithelial cells with stem cell characteristics. Pancreas 2003;26:e18–e26.
44 Matic M, Evans WH, Brink PR, Simon M: Epidermal cells do not communicate through gap junctions. J Invest Dermatol 2002;118:110–116.
45 Matic M, Petrov IN, Chen S, Wang C, Dimitrijevich SD, Wolosin JM: Stem cells of the corneal epithelium lack connexins and metabolite transfer capacity. Differentiation 1997;61:251–260.
46 Lin TM, Tsai JL, Lin SD, Lai CS, Chang CC: Redox state and calcium modulate proliferation and differentiation of human mesenchymal stem cells derived from adipose tissues. Stem Cell Dev 2004;in press.
47 Sun W, Kang KS, Morita I, Trosko JE, Chang CC: High susceptibility of a human breast epithelial cell type with stem cell characteristics to telomerase activation and immortalisation. Cancer Res 1999;59:6118–6123.
48 Linning KD, Tai M-H, Madhukar BV, Chang CC, Reed DN, Ferber S, Trosko JE: Redox-mediated enrichment of self-renewing adult human pancreatic cells with possess endocrine differentiation potential. Pancreas 2004;29:e64–e76.
49 Chang CC, Sun W, Cruz A, Saitoh M, Tai M-H, Trosko JE: A human breast epithelial cell type with stem cell characteristics as targets for carcinogenesis. Radiat Res 2001;155:201–207.
50 Chen Z, de Paiva CS, Luo L, Kretzer FL, Pflugfelder SC, Li DQ: Characterisation of putative stem cell phenotype in human limbal epithelia. Stem Cells 2004;22:355–366.
51 Trosko JE, Chang CC: Role of stem cells and gap junctional intercellular communication in human carcinogenesis. Radiat Res 2001;155:175–180.
52 Okamoto K, Okazawa H, Okuda A, Sakai M, Muramatsu M, Hamada H: A novel octamer binding transcription factor is differentially expressed in mouse embryonic cells. Cell 1990;60:461–472.

53 Scholer HR, Ruppert S, Suzuki N, Chowdhury K, Gruss P: New type of POU domain in germ line-specific protein Oct-4. Nature 1990;344:435–439.
54 Rosner MH, Vigano MA, Ozato K, Timmons PM, Poirier F, Rigby PW, Staudt LM: A POU-domain transcription factor in early stem cells and germ cells of the mammalial embryo. Nature 1990;345:686–692.
55 Monk M, Holding C: Human embryonic genes re-expressed in cancer cells. Oncogene 2001;20:8085–8091.
56 Gidekel S, Pizov G, Bergman Y, Pikarsky E: Oct3/4 is a dose-dependent oncogenic fate determinant. Cancer Cell 2003;4:361–370.
57 Abate-Shen C: Homeobox genes and cancer. New OCTaves for an old tune. Cancer Cell 2003;4:329–330.
58 Tai M-H, Chang CC, Kiupel M, Webster JD, Olson LK, Trosko JE: Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis. Carcinogenesis 2005;26:495–502.
59 Bursch F, Oberhammer F, Schulte-Hermann R: Cell death by apoptosis and its protective role against disease. Trends Pharmacol Sci 1992;13:245–251.
60 Trosko JE, Goodman JA: Intercellular communication may facilitate apoptosis: implications for tumour promotion. Mol Carcinog 1994:11:8–11.
61 Wilson MR, Close TW, Trosko JE: Cell population dynamics (apoptosis, mitosis, and cell-cell communication) during disruption of homeostasis. Exp Cell Res 2000;254:257–268.
62 Yotti LP, Chang CC, Trosko JE: Elimination of metabolic cooperation in Chinese hamster cells by a tumour promoter. Science 1979;206:1089–1091.
63 Trosko JE, Chang CC, Medcalf A: A mechanism of tumour promotion: potential role of intercellular communication. Cancer Invest 1983;1:511–526.
64 Trosko JE: Is the concept of tumour promotion a useful paradigm? Mol Carcinogen 2001;30: 131–137.
65 King TJ, Fukushima LH, Donlon TA, Hieber AD, Shimabukuro KA, Bertram JS: Correlation between growth control, neoplastic potential and endogenous connexin43 expression in HeLa cell lines: implications for tumour progression. Carcinogenesis 2000;21:311–315.
66 Momiyama M, Omori Y, Ishizaki Y, Nishikawa Y, Tokairin T, Ogawa J, Enomoto K: Connexin26-mediated gap junctional communication reverses the malignant phenotype of MCF-7 breast cancer cells. Cancer Sci 2003;94:501–507.
67 Yamasaki H, Hollstein M, Mesnil M, Aguelon AM: Selective lack of intercellular communication between transformed and nontransformed cells as a common property of chemical and oncogene transformation of BALB/c 3T3. Cancer Res 1987;47:5658–5664.
68 Potter VR: Phenotypic diversity in experimental hepatomas: the concept of partially-blocked ontogeny. Br J Cancer 1978;38:1–23.
69 Hanahan D, Weinberg RA: The hallmarks of cancer. Cell 2000;100:57–70.
70 DiPaolo JA, Popescu NC, Alvarez L, Woodworth CD: Cellular and molecular alterations in human epithelial cells transformed by recombinant papillomavirus DNA. Crit Rev Oncogen 1993;4: 337–360.
71 Rhim JS: Neoplastic transformation of human cells. Crit Rev Oncogen 1993;4:313–335.
72 Barcellos-Hoff MH: Three down and counting: the transformation of human mammary cells from normal to malignant in three steps. Trends Mol Med 2001;7:142–143.
73 Barcellos-Hoff MH: It takes a tissue to make a tumour: epigenetics, cancer and the microenvironment. J Mammary Gland Biol Neoplasia 2001;6:213–221.
74 Bissell MJ, Radisky D: Putting tumours in context. Nat Rev Cancer 2001;1:46–54.
75 Elson CO: Genes, microbes, and T cells – new therapeutic targets in Crohn's disease. N Engl J Med 2002;346:614–616.
76 Arter JL, Chi DS, Girish M, Fitzgerald M, Guha B, Krishnaswamy G: Obstructive sleep apnea, inflammation and cardiopulmonary disease. Front Biosci 2004;9:2892–2900.
77 Johansson U, Olsson A, Gabrielsson S, Nilsson B, Korsgren O: Inflammatory mediators expressed in human islets of Langerhans: implications for islet transplantation. Biochem Biophys Res Commun 2003;308:474–479.
78 Coussens LM, Werb Z: Inflammation and cancer. Nature 2002;420:860–867.

79 Yun AJ, Lee PY: Maladaptation of the link between inflammation and bone turnover may be a key determinant of osteoporosis. Med Hypoth 2004;63:532–537.
80 Stipp D: The secret killer. Fortune 2003;Oct. 27:109–114.
81 Libby P: Inflammation in atherosclerosis. Nature 2002;420:868–874.
82 Roep BO: The role of T-cells in the pathogenesis of type 1 diabetes: from cause to cure. Diabetologia 2003;46:305–321.
83 Koehler DR, Downey GP, Sweezey NB, Tanswell AK, Hu J: Lung inflammation as therapeutic target in cystic fibrosis. Am J Respir Cell Mol Biol 2004;31:377–381.
84 Aller MA, Arias JL, Nava MP, Arias J: Posttraumatic inflammation is a complex response based on the pathological expression of the nervous, immune, and endocrine functional system. Exp Biol Med 2004;229:170–181.
85 Beachy PA, Karhadkar SS, Berman DM: Mending and malignancy. Nature 2004;431:402.
86 Finch CE, Crimmins EM: Inflammatory exposure and historic changes in human life-spans. Science 2004;305:1736–1739.
87 Bruggemann H, Henne A, Hoster F, Liesegang H, Wiezer A, Strittmatter A, Hujer S, Durre P, Gottschalk G: The complete genome sequence of Propionibacterium acnes, a commensal of human skin. Science 2004;305:671–674.
88 Zhu Z, Zheng T, Homer RJ, Kim Y-K, Chen NY, Cohn L, Hamid Q, Elias JA: Acid mammalial chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. Science 2004;304:1678–1682.
89 Willerson JT, Ridker PM: Inflammation as a cardiovascular risk factor. Circulation 2004;109:2–10.
90 Mattson MP: Pathways towards and away from Alzheimer's disease. Nature 2004;430:631–634.
91 Tuppo EE, Arias HR: The role of inflammation in Alzheimers's disease. Int J Biochem Cell Biol 2005;37:289–305.
92 Kielan T, Esen N: Effects of neuroinflammation on glia-glia gap junctional intercellular communication: a perspective. Neurochem Intl 2004;45:429–436.
93 Chu AJ, Chou T-H, Chen BD: Prevention of colorectal cancer using COX-2 inhibitors: basic science and clinical applications. Front Biosci 2004;9:2697–2713.
94 O'Leary KA, dePascual-Tereas S, Needs PW, Bao Y-P, O'brien NM, Williamson G: Effect of flavonoids and vitamin E on cycloxygenase-2 (COX-2) transcription. Mutat Res 2004;551: 245–254.
95 Basu GD, Pathangey LB, Tinder TL, LaGioia M, Gendler SJ, Mukherjee P: COX-2 inhibitor induces apoptosis in breast cancer cells in an in vivo model of spontaneous metastatic breast cancer. Mol Cancer Res 2004;2:1–11.
96 Rhoden CR, Lawrence J, Godleski JJ, Gonzalez-Flecha B: N-Acetylcysteine prevents lung inflammation after short-term inhalation exposure to concentrated ambient particles. Toxicol Sci 2004;79:296–303.
97 Pope CA, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, Thurston GD: Lung cancer, cardiopulmonary mortality and long-term exposure to fine particular air pollution. JAMA 2002;287: 1132–1141.
98 Knaapen AM, Borm C, Albrecht S, Schins RPF: Inhaled particles and lung cancer. A. Mechanisms. Int J Cancer 2004;109:799–809.
99 Shukla A, Ramos-Nino M, Mossman BT: Cell signalling and transcription factor activation by asbestos in lung injury and disease. Int J Biochem Cell Biol 2003;35:1198–1209.
100 Pisani P, Parkin DM, Munoz NB, Ferlay J: Cancer and infection: estimates of the attributable fraction in 1990. Cancer Epidemiol Biomarkers Prev 1997;6:387–400.
101 Littman AJ, White E, Jackson LA, Thornquist MD, Gaydos CA, Goodman GE, Vaughan TL: Chlamydia pneumoniae infection and risk of lung cancer. Cancer Epid Biomarkers Prev 2004;13:1624–1630.
102 Tokudome S, Kuriki K, Suzuki S, Akasaka S, Kosaka H, Ishikawa H, Yoshimura T, Azuma T, Van DD, Khan NC, Sriamporn S, Wiangnon S, Soeripto F, Triningsih E, Moore MA: Helicobacter pylori infection and gastric cancer: facing the enigmas. Int J Cancer 2004;112:166–167.
103 Haverkos HW: Virus, chemicals and co-carcinogenesis. Oncogene 2004;23:6492–6499.
104 Ganem D, Varmus HE: The molecular biology of the hepatitis B viruses. Annu Rev Biochem 1987;56:651–693.
105 zur Hausen H: SV40 in human cancers – an endless tale? Int J Cancer 2003;107:687.

106 Peek PM, Blaser MJ: Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nature Rev 2002;2:28–37.
107 Pagano JS, Blaser M, Buendia M-A, Damania B, Khalili K, Raab-Traub N, Roizman B: Infectious agents and cancer: criteria for a causal relation. Semin Cancer Biol 2004;14:453–471.
108 Gelderblom WC, Abel S, Smuts CM, Marnewick J, Marasas WFO, Lemmer ER, Ramljak D: Fumonisin-induced hepatocarcinogenesis: mechanisms related to cancer initiation and promotion. Environ Health Perspect 2001;109(suppl 2):291–300.
109 Etzel RA: Mycotoxins. JAMA 2002;287:425–427.
110 Howard PC, Eppley PM, Stack ME; Warbritton A, Voss KA, Lorentzen RJ, Kovach PM, Bucci TJ: Fumonisin B1 carcinogenicity in a two-year feeding study using F344 rats and B6C3F1 mice. Environ Health Perspect 2001;109:277–282.
111 Riley J, Mandel HG, Sinha S, Judah DJ, Neal GE: In vitro activation of the human Harvey-ras proto-oncogene by aflatoxin B1. Carcinogenesis 1997;18:905–910.
112 Rosin MR, Anwar WA, Ward AJ: Inflammation, chromosomal instability and cancer: the schistosomiasis model. Cancer Res 1994;54:1929–1933.
113 Rahman I: Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung disease. Biochem Molec Biol 2003;36:95–109.
114 Melillo G: Hif-1: a target for cancer, ischemia and inflammation. Cell Cycle 2004;3:154–155.
115 Closa D, Folch-Puy E: Oxygen free radicals and systemic inflammatory response. IUBMB Life 2004;56:185–191.
116 Balkwill F, Coussenbs LM: An inflammatory link. Nature 2004;431:405–406.
117 Philip M, Rowley DA, Schreiber H: Inflammation as a tumour promoter in cancer induction. Semin Cancer Biol 2004;14:433–439.
118 Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galum E, Ben-Neriah Y: NF-kB functions as a tumour promoter in inflammation-associated cancer. Nature 2004;432:461–463.
119 Cerruti P: Prooxidant states and tumour promotion. Science 1985;227:375–381.
120 Hu W, Jones PD, Upham BL, Trosko JE, Lau C, Giesy JP: Inhibition of gap junctional intercellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines in vitro and Sprague-Dawley rats in vivo. Toxicol Sci 2002;68:429–436.
121 Sai K, Kang KS, Hirose A, Hasegawa R, Trosko JE, Inoue T: Inhibition of apoptosis by pentachlorophenol in V-myc-transfected rat liver epithelial cells: relation to down-regulation of gap junctional intercellular communication. Cancer Lett 2001;173:163–174.
122 Upham BL, Weis LM, Trosko JE: Modulated gap junctional intercellular communication as a biomarker of PAH's epigenetic toxicity in structure/function relationship. Environ Health Persp 1998;106:975–981.
123 Klaunig JE, Kamendulis LM: The role of oxidative stress in carcinogenesis. Ann Rev Pharmacol Toxicol 2004;44:239–267.
124 Chipman JK, Mally A, Edwards GO: Disruption of gap junctions in toxicology and carcinogenesis. Toxicol Sci 2003;71:146–153.
125 Upham BL, Wagner JG: Toxicant-induced oxidative stress in cancer. Toxicol Sci 2001;64:1–3.
126 Couzin J: Withdrawal of Vioxx casts a shadow over COX-2 inhibitors. Science 2004;306:384–385.
127 Chu AJ, Chou T-H, Chen BD: Prevention of colorectal cancer using COX-2 inhibitors: basic science and clinical applications. Front Sci 2004;9:2697–2713.
128 Moini H, Packer L, Saris N-E: Antioxidant and prooxidant activities of alpha-lipoic acid and dihydrolipoic acid. Toxicol Appl Pharmacol 2002;182:84–90.
129 Stevenson FK, Ottensmeier CH, Johnson P, Zhu D, Buchan SL, McCann KJ, Roddick JS, King AT, McNichll F, Savelyeva N, Rice J: DNA vaccines to attach cancer. Proc Natl Acad Sci USA 2004;101:14646–14652.
130 Ruch RJ, Madhukar BV, Trosko JE, Klaunig JE: Reversal of ras-induced inhibition of gap junctional intercellular communication, transformation, and tumourigenesis by lovastatin. Mol Carcinog 1993;7:50–59.

131 Ruch RJ, Trosko JE, Madhukar BV: Inhibition of connexin 43 gap junctional intercellular communication by TPA requires ERK activation. J Cell Biochem 2001;83:163–169.
132 Ruch RJ, Bonney WJ, Sigler K, Guan L, Matesic DF, Schafer LD, Dupont E, Trosko JE: Loss of gap junctions from DDT-treated rat liver epithelial cells. Carcinogenesis 1994;15:301–306.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis. Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 66–80

Helicobacter pylori and Gastric Neoplasia

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Abstract

Helicobacter pylori is present in the stomach of more than half of the world population. Based on compelling epidemiological evidences, it was classified by the World Health Organization as a type I gastric carcinogen. It is generally believed that gastric cancer development is a multi-step progression from chronic gastritis to atrophy, intestinal metaplasia, dysplasia, and cancer. Individuals infected with *H. pylori* have at least a 2-fold increase in risk of gastric cancer development though only a small proportion of infected individuals will ultimately develop this malignancy. The exact mechanisms underlying how *H. pylori* triggers or causes gastric cancer remain elusive. Certain *H. pylori* genotypes like cagA, vacA s1 or babA1 are considered to be of higher virulent potential. Apart from the bacterial factors, the host response to chronic *H. pylori* infection may also attribute to the cancer risk. It was found that individuals who carry pro-inflammatory cytokine gene polymorphism have a substantial increase in risk of cancer development. The combination of bacterial and host genotypes may have a synergistic effect on cancer development. Despite the strong causal link between chronic *H. pylori* infection and gastric cancer, the role of *H. pylori* eradication in preventing gastric cancer remains controversial. More long-term data may be necessary to clarify this controversy.

Helicobacter pylori is a gram-negative organism that lives in the microaerophilic and acidic environment of the human stomach. The rediscovery of *H. pylori* two decades ago has revolutionized the concept and management of gastroduodenal diseases [1]. This organism is casually linked to the pathogenesis of gastric and duodenal ulcer [2, 3]. Eradication of *H. pylori* from the human host leads to long term cure of peptic ulcer diseases [4]. Moreover, *H. pylori* is etiologically linked to the development of adenocarcinoma of the stomach [5, 6] as well as MALT lymphoma of the stomach [7].

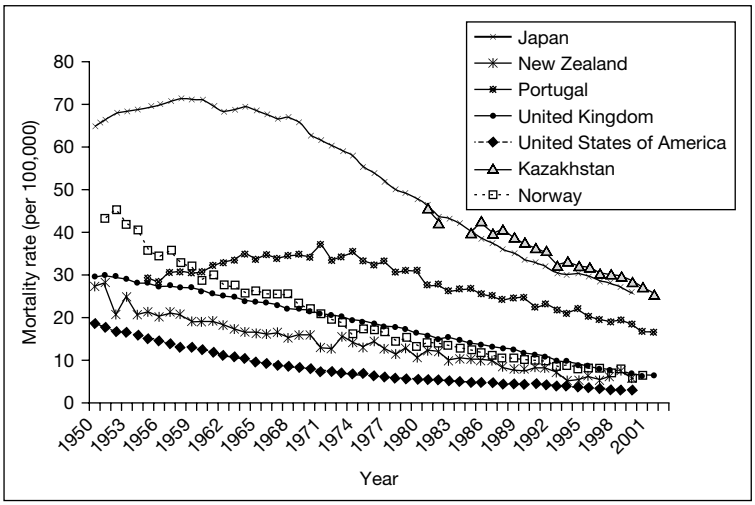

Fig. 1. Trend of gastric cancer mortality in seven different countries (1950–2002). Data derived from World Health Statistical Annual. World Health Organisation (WHO) Databank, Geneva, Switzerland (http://www-depdb.iarc.fr/who/menu.htm).

Whilst virtually all *H. pylori* infected individuals have certain degrees of gastric inflammation, only a subgroup of individuals will develop complications like peptic ulcer or gastric cancer. The precise mechanism underlying this development remains undefined but it is increasingly recognized that the interaction between host and bacterial factors may govern the development of these complications. This review will summarize the current knowledge on the association between *H. pylori* and gastric neoplasia.

Epidemiology of Gastric Carcinoma

Gastric carcinoma is the second commonest cause of cancer related death in the world with a mortality rate of more than 600,000 people each year [8]. Over the past few decades, a global declining trend in the overall gastric cancer incidence is observed (fig. 1). The disease showed marked geographic variations with most diseases occurring in East Asian countries where *H. pylori* is most prevalent.

Interestingly, the gastric carcinoma can be broadly categorized into two types according to the tumor location: cardia or noncardia. The two types differ substantially on the epidemiology and etiology. Noncardia, or distal, cancer is associated with chronic *H. pylori* infection and is more prevalent in Asia and

other developing countries. In contrast, cardia cancer is commonly associated with gastroesophageal reflux disease and is more prevalent in western countries. This type of cancer may be inversely related to the prevalence of *H. pylori* infection. The reduction in gastric cancer incidence over the past few decades largely reflects a decline in cancers of the distal stomach and mostly of the intestinal type. On the other hand, there has been a steady rise in the incidence of adenocarcinoma of the proximal stomach and the gastroesophageal junction in the past three decades [9]. According to the United States National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) database, proximal gastric lesions were increasing at a rate of 3.6–5.6% per year [9].

Epidemiology of *H. pylori* Infection

H. pylori is a chronic bacterial infection of the human stomach. Once infected, most people will remain infected for the rest of the life unless treatment is given. Infections are usually acquired in the first few years of life, and childhood appears to be the 'golden period' for acquisition of this infection [10]. There are overwhelming data suggesting that interpersonal spread of infection within the family is the prime route of transmission of *H. pylori* although the exact details remain obscure [11, 12]. Individuals with infected parents or siblings have a much higher chance of contracting the infection than those with uninfected family members.

The prevalence of a *H. pylori* infection varies considerably between developing countries and developed countries, and according to ethnicity, place of birth and socio-economic factors among people living in the same country. *H. pylori* infection is prevalent in undeveloped or developing regions in which up to 90% of population may be infected [13]. In contrast, the overall prevalence of *H. pylori* is low in developed countries. Notably, intrafamilial spread appears to play a central role on the transmission of *H. pylori* infection in both developing and developed countries.

Based on several large-scale epidemiological cohort studies published in the early 1990s' [5, 6], the International Agency for Research on Cancer (IARC) classified *H. pylori* as a group 1 carcinogen in 1994 [14]. In a recent combined analysis of 12 case control studies nested within prospective cohorts [15], the association with *H. pylori* was found to be restricted to noncardia cancers and was stronger when blood samples for *H. pylori* serology were collected more than 10 years before cancer diagnosis (odds ratio 5.9; 95% CI 3.4–10.3). This study also showed that the magnitude of the association may be underestimated in previous studies when the *H. pylori* status is assessed closely to cancer diagnosis.

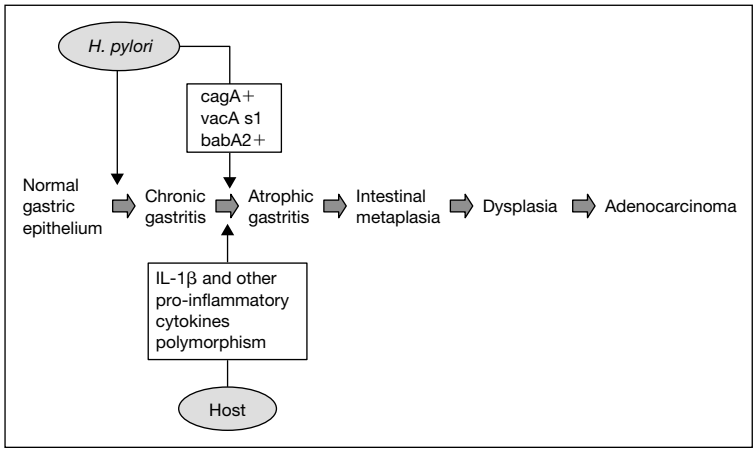

Fig. 2. Role of *H. pylori* and host on the multistep gastric carcinogenesis cascade.

Gastric Carcinogenesis Cascade

Apart from the location of the tumor, gastric adenocarcinoma can be divided into two distinct histological types. Intestinal type adenocarinoma usually progresses through well-defined series of histological changes and is more common in elderly male. Diffuse type adenocarcinoma which consists of neoplastic cells that do not form glandular structures. Although both cancer subtypes are linked to *H. pylori*, the carcinogenic mechanisms may be different.

Even before the identification of *H. pylori*, it was observed that the stomach of gastric cancer patients also harbor premalignant gastric lesions, particularly intestinal metaplasia [16]. Further observational study from high risk population demonstrated a model of progression from gland neck hyperplasia, atrophy with gland loss, and intestinal metaplasia to dysplasia [16]. This is widely quoted as the Correa's model of gastric carcinogenesis and is more applicable to the intestinal-type adenocarcinoma (fig. 2). With the identification of *H. pylori*, this progression is considered to be triggered by chronic *H. pylori* infection [17]. In a prospective study from Japan, it was found that individuals harboring gastric atrophy or intestinal metaplasia have an about 5 to 6 fold increase in risk of gastric cancer development [18].

On the other hand, this multistep progression may be less applicable to the diffuse type gastric carcinoma though epidemiological data showed that both cancer subtypes are associated with chronic *H. pylori* infection [19]. Recently,

germline mutation in the *E-cadherin* gene is found to play an instrumental role on the development of hereditary diffuse type cancer [20].

Molecular Events during Gastric Carcinogenesis

In contrast to the well-defined genetic events that occur during colorectal carcinogenesis, the molecular events associated with the progression of one histological stage to the next in the gastric carcinogenesis cascade are still poorly defined. *H. pylori* infection is known to induce mutation in the stomach. By using the 'Big Blue transgenic mouse model', it was found that the gastric mutant frequency was 4-fold higher in mice 6-month after infection with *H. pylori* [21]. This genotoxicity can be attributed to oxidative DNA damage involving the inflammatory host response. In keeping with this, various molecular changes like p53 mutation [22], overexpression of cyclooxygenase-2 (COX-2) [23], transforming growth factor alpha and epidermal growth factor receptor [24], and cyclin-D2 [25] have been reported in premalignant gastric lesion, particularly intestinal metaplasia. Eradication of *H. pylori* reverses the aberrant expression of cyclin D2 and p27 in intestinal metaplasia [25].

Moreover, we have previously shown that micro-satellite instability is frequently detected in intestinal metaplasia of patients with or without gastric cancer [26]. While micro-satellite instability is a result of inactivation of the DNA mismatch repair (MMR) function, infection of gastric epithelial cells by *H. pylori* leads to a decrease in DNA MMR proteins [27]. These data suggest that *H. pylori* infection might increase the risk of mutation accumulation in gastric mucosa cells and the risk of gastric cancer during chronic *H. pylori* infection. Recently, epigenetic alterations have emerged as an important alternative pathway leading to inactivation of tumor suppressor genes in the absence of alteration of genetic sequences. Epigenetic silencing of tumor associated genes is frequently found in human gastric cancer [28] as well as in gastric intestinal metaplasia [29]. Interestingly, promoter hypermethylation in the E-cadherin gene was detected in the non-neoplastic gastric mucosa of *H. pylori* infected individuals, which may implicate their role in gastric carcinogenesis [30].

Bacterial Factors

Despite the strong etiological link between *H. pylori* infection and gastric cancer, there was no direct evidence demonstrating the tumorigenic effect of *H. pylori* alone till the establishment of the Mongolian gerbil model of gastric

carcinogenesis. In the absence of exogenous mutagens, Japanese investigators demonstrated that Mongolian gerbils infected with *H. pylori* developed severe active chronic gastritis, ulcers, and intestinal metaplasia within 6 months of infection [31]. After 1 year, adenocarcinoma of stomach was detected in one-third of the infected gerbils.

Interestingly, there is considerable genetic heterogeneity of *H. pylori* strains circulating in different regions. Recent studies show that *H. pylori* can be broadly divided into seven populations and subpopulations with distinct geographical distributions which can be traced back to human migrations [32]. More importantly, studies show that certain genotypes are more prevalent in patients with gastric cancer than in control population, and are therefore regarded to be of higher virulence/oncogenetic potential.

cagA

Among various putative virulence factors identified, the *cagA* gene which encodes the CagA protein is being most studied [33]. The *cagA* gene is localized at one end of the 40-kb cag pathogenicity island (PAI) which contains 31 putative genes. Several cag island genes have homology to genes that encode type IV secretion system proteins. The cagA is commonly used as a marker for the entire cag locus.

The CagA protein is a 120- to145-kDa protein with a carboxy-terminal variable region. *H. pylori* strains can then be broadly divided into two main groups according to the cagA status. The *cagA*-positive strains are associated with a higher degree of inflammation and are found to be associated with peptic ulcers as well as gastric carcinoma [34]. In a recent meta-analysis, it was shown that individuals infected with *cagA* positive strains have an additional 2-fold increase in risk of noncardia cancer when compared to individuals infected with *cagA*-negative strains [35].

While the molecular mechanisms by which *H. pylori* triggers the gastric carcinogenesis process remain largely unknown, it was found that the CagA protein is actively delivered into gastric epithelial cells by the bacterial type IV secretion system [36–39]. This is followed by tyrosine phosphorylation by kinases of the SRC family. In particular, the phosphorylated CagA binds and activates the SHP2 oncoprotein, which may promote gastric carcinogenesis [40]. One of the phenotypic characteristics of this is the induction of cell spreading, elongation, and cytoskeletal rearrangements, the so-called the ‘hummingbird phenotype’.

Tyrosine phosphorylation of CagA occurs at the 5-amino acid carboxy-terminal variable region, or the EPIYA motif. Based on the sequence variation

of these binding sites, *H. pylori* can be subclassified into two types: East-Asian *cagA* and Western *cagA* [41]. The former shows stronger SHP2 binding activity, which may underlie the high incidence of gastric carcinoma in East Asian countries.

vacA

The vacuolating toxin (vacA), a water-soluble, 88-kDa protein that assembles on membranes to form a hexameric anion-selective pore, is another putative virulence factor of *H. pylori*. VacA causes cellular vacuolation in mammalian cells though the exact mechanism remains elusive. Though all *H. pylori* strains possess the vacA gene, only approximately 50% of *H. pylori* strains express the vacA protein, which is related to the sequence variations in *vacA*. Regions of major sequence diversity are localized to secretion-signal sequence (s1a, s1b, s1c, and s2) and the mid-region (m1 or m2) [42]. Notably, there are considerable geographic variations in distribution of vacA subtypes. While 's1a' is the predominant strain in northern and Eastern Europe, 's1b' is the dominant strain in Central and South America. Subtype 's1c' is detected in more than 70% of East Asian strains [43].

It is found that *H. pylori* vacA-secreting strains are more common among patients with distal gastric cancer [44]. Moreover, the presence of *H. pylori vacA* s1, *vacA* m1, *cagA* positive genotypes were significantly associated with a higher *H. pylori* density, higher degrees of lymphocytic and neutrophilic infiltrates, atrophy, the type of intestinal metaplasia, and presence of epithelial damage [45].

babA2

BabA, encoded by *babA2* gene, is a member of a family of highly conserved outer-membrane proteins which binds the Lewis^b histo-blood-group antigen on gastric epithelial cells [46]. The presence of *babA2* is correlated with the presence of *cagA* and *vacA* s1. *H. pylori* strains that possess the *babA2* gene are associated with the gastric adenocarcinoma [47]. Transgenic Lewis^b-expressing mice are more likely to develop severe gastritis, atrophy and anti-parietal cell antibodies after challenge with babA2 positive *H. pylori* strains [48]. In populations where *cagA* positive strains are prevalent, we found that infection by *babA2* positive *H. pylori* strains alone or in combination with *cagA* positive and *vacA* s1 further increase the risk of pre-neoplastic gastric lesions [49].

Host Factors

It is well known that *H. pylori* infection is associated with the development of both gastric cancer as well as gastric and duodenal ulcers. In a large Swedish cohort study, it is found that patients with gastric ulcer have about 2-fold increase in risk of gastric cancer whereas patients with duodenal ulcer have about 2-fold decrease in risk [50]. The paradoxical association between duodenal ulcer and gastric cancer remains enigmatic, but the pattern of gastritis may underlie the divergent outcome to *H. pylori* infection [4].

The duodenal ulcer phenotype is characterized by the antral predominant nonatrophic type of gastritis whereas gastric cancer patients tend to have multifocal or extensive corpus atrophic gastritis. This hypothesis is confirmed by a recent Japanese study which showed that those with pan-gastritis and corpus predominant gastritis have a 16- and 35-fold increase in risk of gastric cancer when compared to those with antral-predominant gastritis [18]. The reason underlying the development of different patterns of gastritis in different individuals has been recently linked to the genetic make up of the host and more precisely, the interaction between the host and the bacteria.

H. pylori infection induces a T-helper (T_H) 1-type cellular response in humans. It is found that concurrent helminth and *H. pylori* infection in mouse shifts the T_H1 responses to a less-damaging T_H2 response [51], which may attenuate the progression of *H. pylori*-associated gastric changes. This finding also helps to explain the unexpectedly low incidence of gastric cancer in African countries which is sometimes called the 'African enigma'.

Interleukin-1β (IL-1β) is a T_H1 cytokine and a potent inhibitor of gastric acid secretion. It has been demonstrated that polymorphisms in the promoter region of IL-1β may underlie the predisposition for the development of hypochlorhydria, gastric atrophy and hence gastric cancer [52]. Subsequent studies from different ethnic groups confirmed this important observation [53–55]. Interestingly, the effect of IL-1β polymorphism is less obvious in areas with high prevalence for gastric cancer since control subjects from the high prevalence region also have a high background frequency of the pro-inflammatory genotype IL-1β-511T/T [55]. Whether this could explain the high geographic variations of gastric cancer incidences in China needs to be verified. In addition to development of gastric cancer, it was also found that carriers of the pro-inflammatory alleles, IL-1β-511T/-31C and IL-1RN*2, had an increased risk for the development of atrophy, intestinal metaplasia and severe inflammation [56].

Apart from IL-1β, pro-inflammatory genotypes in other cytokines also increase the risk of developing gastric cancer. Carriage of certain polymorphism in tumor necrosis factor-α (TNF-α) that is pro-inflammatory is found to have

about 2-fold increase in risk of gastric cancer development [57]. Conversely, polymorphisms that reduce the expression of anti-inflammatory cytokine IL-10 have been associated with an elevated risk of distal gastric cancer [57]. In addition, carriers of the IL-10 GCC haplotype were found to have higher mucosal IL-10 mRNA levels than ATA haplotype carriers, and were associated with colonization by more virulent *cagA*-positive, *vacA* s1, and *babA2*-positive *H. pylori* strains [58].

Notably, the carriage of multiple pro-inflammatory polymorphisms of IL-1β, TNF-α and IL-10 confers an even higher risk of cancer development [57]. Individuals with three or more high-risk genotypes have an about 27-fold increase in cancer risk. In keeping with this, the combination of host genotypes and bacterial virulence factors has a synergistic effect for the development of gastric cancer. Infections with *cagA*-positive, *vacA* s1 or m1 genotypes and the presence of IL-1β-511T pose a substantial risk of gastric cancer [54] as well as severe gastric histological changes [56].

Prevention of Gastric Adenocarcinoma by *H. pylori* Eradication

Despite the strong links between *H. pylori* infection and gastric cancer, the role of *H. pylori* eradication in the prevention of gastric cancer remains controversial. These interventional studies are extremely difficult to perform due to the long lead-time in gastric cancer development. One uncontrolled study showed that eradication of *H. pylori* after endoscopic mucosal resection of early gastric cancer reduced the risk of subsequent cancer recurrence [59]. Other studies attempted to look into changes in pre-neoplastic lesions as a surrogate endpoint. There are conflicting data in the literature due to inconsistencies in interpretation of histological grading, sampling errors, lack of proper control, and different study populations. Many of these results were summarized by Hojo et al. [60] who found that only 5 of the 28 studies reported a significant improvement in intestinal metaplasia after treatment of *H. pylori*.

Results of a few large-scale randomized control studies were published recently. In the Colombian study that involved 976 subjects, study subjects were randomized to receive eight different treatments that included vitamin supplements and anti-*H. pylori* therapy alone or in combination for up to 6 years [61]. Of the 79 subjects that received anti-*H. pylori* therapy, there was a borderline regression of intestinal metaplasia when compared with placebo (15 vs. 6%; relative risk 3.1, 95% confidence interval 1.0 to 9.3). The supplementation of β-carotene or ascorbic acid resulted in a similar degree of improvement in intestinal metaplasia, 20 vs. 19%, respectively. However, the combination of antibiotics and vitamins did not confer any additional benefits.

More importantly, the progression rate of intestinal metaplasia was comparable irrespective of the treatments received. The progression rate was 23% in placebo vs. 17% in *H. pylori*-eradicated patients.

In our previous study, 587 *H. pylori* infected Chinese subjects from a region with high gastric cancer incidence were randomized to receive anti-*H. pylori* therapy vs. placebo [62]. At one year, there was no significant improvement in intestinal metaplasia in those treated with anti-*H. pylori* therapy. On the other hand, subjects with persistent *H. pylori* infection had a significant deterioration of corpus atrophy. In the 5-year follow up, subjects who had a successful eradication of *H. pylori* had a significantly reduced progression of intestinal metaplasia towards those with persistent infection [63]. Gastric atrophy also appeared to regress after eradication of *H. pylori*. Although our results strongly support the eradication of *H. pylori* in the prevention of metaplasia progression, it is imperative to note that substantial proportions (>50%) of individuals in both treatment groups had deterioration of intestinal metaplasia over the 5-year follow-up period. Further analysis showed that persistent *H. pylori* infection, age >45 years, amount of alcohol consumption, and consumption of water from a well were all independent risk factors associated with intestinal metaplasia progression [64]. Conversely, the presence of duodenal ulcer was an independent protective factor against progression of intestinal metaplasia.

Recently, a study using gastric cancer incidence as primary end point also failed to show any significant difference between treatment groups with *H. pylori* eradication and placebo groups after 7.5 years of follow-up [65]. It was only in subgroup analyses that individuals with no precancerous gastric lesions at baseline was found to have a marginal lower risk of gastric cancer development. Whether gastric intestinal metaplasia represents a point of no return in terms of oncogenesis deserves further evaluation.

MALT Lymphoma

The MALT lymphoma was first described in 1983 by Issacson and Wright [66] as a distinct pathological entity. The majority of MALT lymphomas (>90%) are related to chronic *H. pylori* infection [7]. It was also suggested that high grade MALT lymphoma transformation may be more likely to occur following infection by *cagA*-positive strains [67]. Ectopic expression of CagA in B cells inhibited cell proliferation by suppressing the JAK-STAT signaling and impairing p53-dependent apoptosis [68]. On the other hand, there are overwhelming evidences to show that cure of *H. pylori* infection results in long-term cure of low-grade gastric MALT lymphoma in the majority of patients [69, 70].

The overall success of antibiotics in achieving complete remission in stage E1 lymphomas is about 80% [71]. Monoclonal B cells, as detected by polymerase chain reaction, may persist up to several years after cure of *H. pylori* infection and complete histological and endoscopic remission [72, 73]. Patients with disease confined to mucosa and submucosa are more likely to have complete regression of the MALT lymphoma after anti-*H. pylori* therapy [74]. The presence of t(11;18), which results in a chimeric transcript between the API2 and MLT genes, may also predict resistance to antibiotic treatment [75].

References

1 Marshall BJ, Warren JR: Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984;i:1311–1315.
2 Anonymous: NIH Consensus Conference. *Helicobacter pylori* in peptic ulcer disease. NIH consensus development panel on *Helicobacter pylori* in peptic ulcer disease. JAMA 1994;272: 65–69.
3 Anonymous: Current European concepts in the management of *Helicobacter pylori* infection: the Maastricht consensus report. European *Helicobacter pylori* study group. Gut 1997;41:8–13.
4 Chan FK, Leung WK: Peptic ulcer disease. Lancet 2002;360:933–941.
5 Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ: *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 1991;325:1132–1136.
6 Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK: *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127–1131.
7 Parsonnet J, Hansen S, Rodriguez L, Chang Y, Vogelman JH, Orentreich N, Sibley RK: *Helicobacter pylori* infection and gastric lymphoma. N Engl J Med 1994;330:1267–1271.
8 Ferlay J, Bray F, Pisani P, Parkin DM: GLOBOCAN 2002: Cancer incidence, mortality and prevalence worldwide, Version 2.0. IARC CancerBase No 5. Lyon, IARC Press, 2004.
9 Blot WJ, Devesa SS, Kneller RW, Fraumeni JF Jr: Rising incidence of adenocarcinoma of the esophagus and gastric cardia. JAMA 1991;265:1287–1289.
10 Mitchell HM, Li YY, Hu PJ, Liu Q, Chen M, Du GG, Wang ZJ, Lee A, Hazell SL: Epidemiology of *Helicobacter pylori* in southern China: identification of early childhood as the critical period for acquisition. J Infect Dis 1992;166:149–153.
11 Webb PM, Knight T, Greaves S, Wilson A, Newell DG, Elder J, Forman D: Relation between infection with *Helicobacter pylori* and living conditions in childhood: evidence for person to person transmission in early life. BMJ 1994;308:750–753.
12 Tindberg Y, Bengtsson C, Granath F, Blennow M, Nyren O, Granstrom M: Helicobacter pylori infection in Swedish school children: lack of evidence of child-to-child transmission outside the family. Gastroenterology 2001;121:310–316.
13 Perez-Perez GI, Rothenbacher D, Brenner H: Epidemiology of *Helicobacter pylori* infection. Helicobacter 2004;9(suppl 1):1–6.
14 IARC Working Group on the evaluation of carcinogenic risks to humans. Schistosomes, liver flukes and *Helicobacter pylori*. Lyon, 7–14 June 1994. IARC Monogr Eval Carcinog Risks Hum 1994;61:1–241.
15 Helicobacter and Cancer Collaborative Group: Gastric cancer and *Helicobacter pylori*: a combined analysis of 12 case control studies nested within prospective cohorts. Gut 2001;49:347–353.
16 Correa P, Haenszel W, Cuello C, Zavala D, Fontham E, Zarama G, Tannenbaum S, Collazos T, Ruiz B: Gastric precancerous process in a high risk population: cross-sectional studies. Cancer Res 1990;50:4731–4736.

17 Correa P: Human gastric carcinogenesis: a multistep and multifactorial process: first American cancer society award lecture on cancer epidemiology and prevention. Cancer Res 1992;52: 6735–6740.
18 Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ: *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 2001;345:784–789.
19 Huang JQ, Sridhar S, Chen Y, Hunt RH: Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer. Gastroenterology 1998;114:1169–1179.
20 Suriano G, Oliveira C, Ferreira P, Machado JC, Bordin MC, De Wever O, Bruyneel EA, Moguilevsky N, Grehan N, Porter TR, Richards FM, Hruban RH, Roviello F, Huntsman D, Mareel M, Carneiro F, Caldas C, Seruca R: Identification of CDH1 germline missense mutations associated with functional inactivation of the E-cadherin protein in young gastric cancer probands. Hum Mol Genet 2003;12:575–582.
21 Touati E, Michel V, Thiberge JM, Wuscher N, Huerre M, Labigne A: Chronic *Helicobacter pylori* infections induce gastric mutations in mice. Gastroenterology 2003;124:1408–1419.
22 Shiao YH, Rugge M, Correa P, Lehmann HP, Scheer WD: p53 alteration in gastric precancerous lesions. Am J Pathol 1994;144:511–517.
23 Sung JJY, Leung WK, Go MYY, To KF, Cheng AS, Ng EK, Chan FK: Cyclooxygenase-2 expression in *Helicobacter pylori*-associated pre-malignant and malignant gastric lesions. Am J Pathol 2000;157:729–735.
24 Filipe MI, Osborn M, Linehan J, Sanidas E, Brito MJ, Jankowski J: Expression of transforming growth factor alpha, epidermal growth factor receptor and epidermal growth factor in precursor lesions to gastric carcinoma. Br J Cancer 1995;71:30–36.
25 Yu J, Leung WK, Ng EK, To KF, Ebert MP, Go MY, Chan WY, Chan FK, Chung SC, Malfertheiner P, Sung JJ: Effect of *H. pylori* eradication on expression of cyclin D2 and p27 in gastric intestinal metaplasia. Aliment Pharmacol Ther 2001;15:1505–1512.
26 Leung WK, Kim JJ, Kim JG, Graham DY, Sepulveda AR: Microsatellite instability in gastric intestinal metaplasia in patients with and without gastric cancer. Am J Pathol 2000;156:537–543.
27 Kim JJ, Tao H, Carloni E, Leung WK, Graham DY, Sepulveda AR: Helicobacter pylori impairs DNA mismatch repair in gastric epithelial cells. Gastroenterology 2002;123:542–553.
28 Leung WK, Yu J, Ng EK, To KF, Ma PK, Lee TL, Go MY, Chung SC, Sung JJ: Concurrent hypermethylation of multiple tumor-related genes in gastric carcinoma and adjacent normal tissues. Cancer 2001;91:2294–2301.
29 To KF, Leung WK, Lee TL, Yu J, Tong JH, Chan MW, Ng EK, Chung SC, Sung JJ: Promoter hypermethylation of tumor-related genes in gastric intestinal metaplasia of patients with and without gastric cancer. Int J Cancer 2002;102:623–628.
30 Chan AO, Lam SK, Wong BC, Wong WM, Yuen MF, Yeung YH, Hui WM, Rashid A, Kwong YL: Promoter methylation of E-cadherin gene in gastric mucosa associated with *Helicobacter pylori* infection and in gastric cancer. Gut 2003;52:502–506.
31 Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M: Helicobacter pylori infection induces gastric cancer in Mongolian gerbils. Gastroenterology 1998;115:642–648.
32 Falush D, Wirth T, Linz B, Pritchard JK, Stephens M, Kidd M, Blaser MJ, Graham DY, Vacher S, Perez-Perez GI, Yamaoka Y, Megraud F, Otto K, Reichard U, Katzowitsch E, Wang X, Achtman M, Suerbaum S: Traces of human migrations in *Helicobacter pylori* populations. Science 2003;299: 1582–1585.
33 Hatakeyama M: Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. Nature Rev Cancer 2004;4:688–694.
34 Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A: Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995;55:2111–2115.
35 Huang JQ, Zheng GF, Sumanac K, Irvine EJ, Hunt RH: Meta-analysis of the relationship between cagA seropositivity and gastric cancer. Gastroenterology 2003;125:1636–1644.
36 Segal ED, Cha J, Lo J, Falkow S, Tompkins LS: Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. Proc Natl Acad Sci USA 1999;96:14559–14564.

37 Asahi M, Azuma T, Ito S, Ito Y, Suto H, Nagai Y, Tsubokawa M, Tohyama Y, Maeda S, Omata M, Suzuki T, Sasakawa C: *Helicobacter pylori* CagA protein can be tyrosine phosphorylated in gastric epithelial cells. J Exp Med 2000;191:593–602.
38 Stein M, Rappuoli R, Covacci A: Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after cag-driven host cell translocation. Proc Natl Acad Sci USA 2000;97:1263–1268.
39 Odenbreit S, Puls J, Sedlmaier B, Gerland E, Fischer W, Haas R: Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. Science 2000;287:1497–1500.
40 Higashi H, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, Hatakeyama M: SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science 2002;295: 683–686.
41 Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, Hatakeyama M: Biological activity of the *Helicobacter pylori* virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. Proc Natl Acad Sci USA 2002;99:14428–14433.
42 Atherton JC, Cao P, Peek RM Jr, Tummuru MK, Blaser MJ, Cover TL: Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*: association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem 1995;270:17771–17777.
43 Van Doorn LJ, Figueiredo C, Megraud F, Pena S, Midolo P, Queiroz DM, Carneiro F, Vanderborght B, Pegado MD, Sanna R, De Boer W, Schneeberger PM, Correa P, Ng EK, Atherton J, Blaser MJ, Quint WG: Geographic distribution of vacA allelic types of *Helicobacter pylori*. Gastroenterology 1999;116:823–830.
44 Miehlke S, Kirsch C, Agha-Amiri K, Gunther T, Lehn N, Malfertheiner P, Stolte M, Ehninger G, Bayerdorffer E: The *Helicobacter pylori* vacA s1, m1 genotype and cagA is associated with gastric carcinoma in Germany. Int J Cancer 2000;87:322–327.
45 Nogueira C, Figueiredo C, Carneiro F, Gomes AT, Barreira R, Figueira P, Salgado C, Belo L, Peixoto A, Bravo JC, Bravo LE, Realpe JL, Plaisier AP, Quint WG, Ruiz B, Correa P, van Doorn LJ: *Helicobacter pylori* genotypes may determine gastric histopathology. Am J Pathol 2001;158: 647–654.
46 Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Boren T: *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science 1998;279:373–377.
47 Gerhard M, Lehn N, Neumayer N, Boren T, Rad R, Schepp W, Miehlke S, Classen M: Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. Proc Natl Acad Sci USA 1999;96:12778–12783.
48 Guruge JL, Falk PG, Lorenz RG, Dans M, Wirth HP, Blaser MJ, Berg DE, Gordon JI: Epithelial attachment alters the outcome of *Helicobacter pylori* infection. Proc Natl Acad Sci USA 1998;95:3925–3930.
49 Yu J, Leung WK, Go MY, Chan MC, To KF, Ng EK, Chan FK, Ling TK, Chung SC, Sung JJ: Relationship between *Helicobacter pylori* babA2 status with gastric epithelial cell turnover and premalignant gastric lesions. Gut 2002;51:480–484.
50 Hansson LE, Nyren O, Hsing AW, Bergstrom R, Josefsson S, Chow WH, Fraumeni JF Jr, Adami HO: The risk of stomach cancer in patients with gastric or duodenal ulcer disease. N Engl J Med 1996;335:242–249.
51 Fox JG, Beck P, Dangler CA, Whary MT, Wang TC, Shi HN, Nagler-Anderson C: Concurrent enteric helminth infection modulates inflammation and gastric immune responses and reduces helicobacter-induced gastric atrophy. Nat Med 2000;6:536–542.
52 El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF Jr, Rabkin CS: Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2000;404: 398–402.
53 Machado JC, Figueiredo C, Canedo P, Pharoah P, Carvalho R, Nabais S, Castro Alves C, Campos ML, Van Doorn LJ, Caldas C, Seruca R, Carneiro F, Sobrinho-Simoes M: A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. Gastroenterology 2003;125:364–371.
54 Figueiredo C, Machado JC, Pharoah P, Seruca R, Sousa S, Carvalho R, Capelinha AF, Quint W, Caldas C, van Doorn LJ, Carneiro F, Sobrinho-Simoes M: *Helicobacter pylori* and interleukin-1

genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. J Natl Cancer Inst 2002;94:1680–1687.
55 Zeng ZR, Hu PJ, Hu S, Pang RP, Chen MH, Ng M, Sung JJ: Association of interleukin 1B gene polymorphism and gastric cancers in high and low prevalence regions in China. Gut 2003;52:1684–1689.
56 Rad R, Prinz C, Neu B, Neuhofer M, Zeitner M, Voland P, Becker I, Schepp W, Gerhard M: Synergistic effect of *Helicobacter pylori* virulence factors and interleukin-1 polymorphisms for the development of severe histological changes in the gastric mucosa. J Infect Dis 2003;188:272–281.
57 El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni JF Jr, Chow WH: Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. Gastroenterology 2003;124:1193–1201.
58 Rad R, Dossumbekova A, Neu B, Lang R, Bauer S, Saur D, Gerhard M, Prinz C: Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during *Helicobacter pylori* infection. Gut 2004;53:1082–1089.
59 Uemura N, Mukai T, Okamoto S, Yamaguchi S, Mashiba H, Taniyama K, Sasaki N, Haruma K, Sumii K, Kajiyama G: Effect of *Helicobacter pylori* eradication on subsequent development of cancer after endoscopic resection of early gastric cancer. Cancer Epidemiol Biomarkers Prev 1997;6:639–642.
60 Hojo M, Miwa H, Ohkusa T, Ohkura R, Kurosawa A, Sato N: Alteration of histological gastritis after cure of *Helicobacter pylori* infection. Aliment Pharmacol Ther 2002;16:1923–1932.
61 Correa P, Fontham ET, Bravo JC, Bravo LE, Ruiz B, Zarama G, Realpe JL, Malcom GT, Li D, Johnson WD, Mera R: Chemoprevention of gastric dysplasia: randomised trial of antioxidant supplements and anti-*Helicobacter* therapy. J Natl Cancer Inst 2000;92:1881–1888.
62 Sung JJY, Lin SR, Ching JYL, Zhou LY, To KF, Wang RT, Leung WK, Ng EK, Lau JY, Lee YT, Yeung CK, Chao W, Chung SC: Atrophy and intestinal metaplasia one year after cure of *H. pylori* infection: a prospective, randomised study. Gastroenterology 2000;119:7–14.
63 Zhou L, Sung JJ, Lin S, Jin Z, Ding S, Huang X, Xia Z, Guo H, Liu J, Chao W: A five-year follow-up study on the pathological changes of gastric mucosa after *H. pylori* eradication. Chin Med J 2003;116:11–14.
64 Leung WK, Lin SR, Ching JY, To KF, Ng EK, Chan FK, Lau JY, Sung JJ: Factors predicting progression of gastric intestinal metaplasia: results of a randomised trial on *Helicobacter pylori* eradication. Gut 2004;53:1244–1249.
65 Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK, Chen JS: China gastric cancer study group: *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomised controlled trial. JAMA 2004;291:187–194.
66 Issacson P, Wright DH: Malignant lymphoma of mucosa-associated lymphoid tissue: a distinctive type of B cell lymphoma. Cancer 1983;52:1410–1416.
67 Peng H, Ranaldi R, Diss TC, Isaacson PG, Bearzi I, Pan L: High frequency of CagA+ *Helicobacter pylori* infection in high-grade gastric MALT B-cell lymphomas. J Pathol 1998;185:409–412.
68 Umehara S, Higashi H, Ohnishi N, Asaka M, Hatakeyama M: Effects of *Helicobacter pylori* CagA protein on the growth and survival of B lymphocytes, the origin of MALT lymphoma. Oncogene 2003;22:8337–8342.
69 Fischbach W, Dragosics B, Kolve-Goebeler ME, Ohmann C, Greiner A, Yang Q, Bohm S, Verreet P, Horstmann O, Busch M, Duhmke E, Muller-Hermelink HK, Wilms K, Allinger S, Bauer P, Bauer S, Bender A, Brandstatter G, Chott A, Dittrich C, Erhart K, Eysselt D, Ellersdorfer H, Ferlitsch A, Fridrik MA, Gartner A, Hausmaninger M, Hinterberger W, Hugel K, Ilsinger P, Jonaus K, Judmaier G, Karner J, Kerstan E, Knoflach P, Lenz K, Kandutsch A, Lobmeyer M, Michlmeier H, Mach H, Marosi C, Ohlinger W, Oprean H, Pointer H, Pont J, Salabon H, Samec HJ, Ulsperger A, Wimmer A, Wewalka F: Primary gastric B-cell lymphoma: results of a prospective multicenter study: the German-Austrian gastrointestinal lymphoma study group. Gastroenterology 2000;119:1191–1202.

70 Steinbach G, Ford R, Glober G, Sample D, Hagemeister FB, Lynch PM, McLaughlin PW, Rodriguez MA, Romaguera JE, Sarris AH, Younes A, Luthra R, Manning JT, Johnson CM, Lahoti S, Shen Y, Lee JE, Winn RJ, Genta RM, Graham DY, Cabanillas FF: Antibiotic treatment of gastric lymphoma of mucosa-associated lymphoid tissue: an uncontrolled trial. Ann Intern Med 1999;131:88–95.
71 Stolte M, Bayerdorffer E, Morgner A, Alpen B, Wundisch T, Thiede C, Neubauer A: Helicobacter and gastric MALT lymphoma. Gut 2002;50(suppl 3):III19–24.
72 Thiede C, Wundisch T, Alpen B, Neubauer B, Morgner A, Schmitz M, Ehninger G, Stolte M, Bayerdorffer E, Neubauer A: German MALT lymphoma study group. Long-term persistence of monoclonal B cells after cure of *Helicobacter pylo*ri infection and complete histological remission in gastric mucosa-associated lymphoid tissue B-cell lymphoma. J Clin Oncol 2001;19:1600–1609.
73 Fischbach W, Goebeler-Kolve M, Starostik P, Greiner A, Muller-Hermelink HK: Minimal residual low-grade gastric MALT-type lymphoma after eradication of *Helicobacter pylori*. Lancet 2002;360:547–548.
74 Nakamura S, Matsumoto T, Suekane H, Takeshita M, Hizawa K, Kawasaki M, Yao T, Tsuneyoshi M, Iida M, Fujishima M: Predictive value of endoscopic ultrasonography for regression of gastric low grade and high grade MALT lymphoma after eradication of *Helicobacter pylori*. Gut 2001;48:454–460.
75 Liu H, Ruskon-Fourmestraux A, Lavergne-Slove A, Ye H, Molina T, Bouhnik Y, Hamoudi RA, Diss TC, Dogan A, Megraud F, Rambaud JC, Du MQ, Isaacson PG: Resistance of t(11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to Helicobacter pylori eradication therapy. Lancet 2001;357:39–40.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 81–100

Schistosomiasis and Neoplasia

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Abstract

Schistosomiasis is endemic in at least 75 tropical and subtropical countries where 600 million people are at risk of which over 200 million are infected. Three species, *S. hematobium*, *S. mansoni* and *S. japonicum*, account for the majority of human infections. There is sufficient evidence that *S. hematobium*, the predominant etiologic agent for urinary schistosomiasis, is carcinogenic to humans leading to squamous cell carcinoma of the urinary bladder, a relatively uncommon vesical cancer in nonendemic areas. There is limited evidence suggesting that *S. japonicum* is possibly carcinogenic to humans leading to colorectal cancer and is a risk factor for hepatocellular carcinoma formation. There is inadequate evidence for the carcinogenicity of *S. mansoni* in humans. *S. mansoni* may still be linked to hepatocellular carcinoma through potentiating the effects of hepatitis B virus and hepatitis C virus on the liver. In this article, the relationship between schistosomiasis and neoplasia will be reviewed.

Life Cycle of *Schistosoma*

Schistosomes are bisexual trematodes that live in the blood stream of human beings and animals. The life cycle of this trematode is characterized by 2 stages: a sexual stage in humans and an asexual stage in an intermediate host, the snail, which differs according to the different species. Schistosomal eggs excreted in urine or stools of infected humans into static or slow moving fresh water bodies, at temperatures 20–30°C, will hatch releasing miracidia. The released ciliated miracidia will swim reaching for the specific snail intermediate host: *Biompholaria* in *S. mansoni*, *Bulinus* in *S. hematobuim* and *Oncomelania* in *S. japonicum*. The miracidia will die within 12–18 h unless they find the specific snail. After penetrating the snail, the miracidium loses its ciliated glycocalyx metamorphose into two generations of sporocysts that

migrate to the digestive gland of the snail to mature into hundreds of fork-tailed cercariae. This phase of asexual reproduction usually takes about 3–5 weeks. The cercariae exit the snail and actively swim searching for the human or animal host. The cercariae live on their glycogen stores and will die within 48–72 h unless they find a susceptible host [1].

The cercaiae attach to the skin of the host by means of an oral sucker and then penetrate it after losing their forked tails and glycocalyx and acquiring a bilayered tegument that protects them from the host's immune response. This transformation results in worm-like creatures called schistosomula which migrate thorough the venous circulation to the heart and then the lungs, where they reside and continue to mature for 2–3 weeks.

The developing worms eventually reach the left side of the heart, from where the arterial blood carries them to the small vessels of the portal hepatic circulation where female and male worms reach maturity and mating occurs when the female occupies the gynecophoric canal of the male. The adult worms remain in a state of continuous copulation within the vessels and eventually migrate against the portal flow to the mesenteric venous plexus in *S. mansoni* and *S. japonicum* and to the perivesical plexus in *S. hematobum*, where the female, after leaving the male, deposits ova. The life span of adult worms is 3–5 years although survival of up to 30 years has been reported [2].

Ova deposition begins 4–6 weeks after penetration of the skin by cercariae. The deposited ova take about 10 days to mature into a shell containing a fully developed embryo called the miracidium. The morphology of the egg and the location of the spine on the outside of the shell can be used to identify the schistosome species.

The ova deposited intravascularly either migrate through the vascular wall into the tissues of the affected organ such as large and small intestine in *S. mansoni*, *S. japonicum* and *S. intercalatum*, and urinary bladder and ureter in *S. hematobium* or are washed up by the blood to reach distant organs, mainly the liver and the lungs and rarely other organs as the brain and spinal cord.

A local CD4 helper lymphocyte mediated inflammatory response facilitates the passage of eggs into the lumen of the affected organ, and in this case the ova will succeed to get out to the external world via the urine and stools of the host. The daily output of eggs of the female *S. hematobium*, *S. mansoni* and *S. japonicum* is 20–200, 100–300, and 500–3,500 respectively.

Epidemiology of Schistosomiasis

Schistosomiasis is endemic in at least 75 countries in tropical and subtropical areas of Africa, Asia, South America and the Caribbean. The distribution of

infection corresponds to the distribution of the snail hosts [3]. Within endemic areas, transmission may be focal as a result of variations in the prevalence of the intermediate host snail species, patterns of water exposure and sociocultural factors. Epidemiological surveys rely on the fecal and urine egg counts for identification and quantification of infection [4]. Prevalence and intensity of infection are usually correlated in endemic areas. Infection with schistosomiasis begins in childhood, as early as 6 months of age, with peak incidence usually between 10 and 14 years of age in areas with a high disease prevalence [5–7]. The higher prevalence and intensity of infection in males have been linked to their higher exposure to infection. Infection with *Schistosoma* is not synonymous with clinical disease, and many infections are asymptomatic. The outcome of infection is influenced by the intensity of infection, genetic factors, the immune response of the host, the nutritional status, and concomitant infections (e.g. viral hepatitis). Clinical disease is a sequel of heavy infection [8, 9].

Diagnosis of Schistosomiasis

The demonstration of parasite eggs in stool and urine specimens remains the gold standard for the diagnosis of schistosomiasis. The schistosome species can be differentiated based on the characteristic morphology of their eggs. All infections can be quantified by egg counts in urine (*S. hematobium*) and feces (*S. mansoni* and *S. japonicum*) and this is used in epidemiologic and clinical studies for estimating the severity of infection and assessing the parasite burden [10, 11]. Biopsy of the rectal mucosa is more sensitive than fecal egg detection for the diagnosis of schistosomiasis [12]. Highly specific immunodiagnostic assays have been developed to detect specific antibodies to *Schistosoma* adult worm antigens but they do not distinguish active from past resolved infections [13, 14].

Detection of schistosomal antigens in the sera and/or urine of actively infected patients shows good correlation with worm burden and can be used to assess the intensity of infection and monitor response to therapy. Enzyme-linked immunosorbent assays (ELISA) have been developed for the detection of two proteoglycan antigens associated with the gut of adult worms, the circulating cathodic antigen (CCA) and the circulating anodic antigen (CAA) [15].

The determination of circulating soluble egg antigens (CSEA) are potentially useful markers of intensity of infection and successful chemotherapy [16].

Abdominal ultrasonography is a useful diagnostic tool which allows accurate measurements of the liver and spleen size, grading of hepatic fibrosis and, diagnosis of portal hypertension, and the assessment of urinary tract morbidity [17, 18].

Schistosomiasis and Bladder Cancer

There is epidemiological, experimental and histopathological evidence associating schistosomiasis and bladder cancer (BC).

Epidemiological Evidence

Squamous cell carcinoma (SCC) of the bladder is over-represented in Egypt, Mozambique, Zimbabwe, Zambia, Iraq and Kuwait where *S. hematobium* is endemic. In Egypt, bladder cancer accounts for 30.8% of the total cancer incidence and ranks first among all types of cancer recorded in males and second only to breast cancer in females [19, 20]. An age-adjusted mortality rate for bladder cancer of 10.8 per 100,000 males places Egypt at the top of the list of the 54 countries providing data for the 1987 WHO database [21]. In countries where schistosomiasis is not endemic, bladder cancer ranks from the 5th to the 7th most common cancer in men and from the 7th to the 14th in women [22–25].

In countries where *S. hematobium* is endemic the peak incidence of bladder cancer is in the 5th decade of life [19, 26–31], while in nonendemic countries the peak incidence is in the 6th or 7th decades of life. The male to female ratio is reported to be 5:1 (range 4:1 to 5.9:1), which is higher than the 3:1 ratio reported in nonendemic countries [32, 33]. In endemic areas the prevalence of *S. hematobuim* infection is higher in males and this probably explains the higher prevalence of bladder cancer in this gender [34].

Experimental Evidence

The carcinogenic effects of *S. hematobium* infection have been studied in experimental animals mostly through the evaluation of the pathological changes that occur in the bladder mucosa. Infection with *S. hematobium* resulted in epithelial proliferation, squamous cell metaplasia and transitional cell carcinoma of the urinary bladder in a talapoin monkey (*Cercopithecus talapoin*), a capuchin monkey (*Cebus apella*), gibbons (*Hybbates lar*), and opossums (*Didelphy marsupialis*) [35–38]. *S. hematobium* ova, lyophilized worms and urine from schistosomal patients were not found to be carcinogenic to mice [39, 40].

Experimental studies were also performed on schistosome-infected animals treated with urinary bladder carcinogens. Epithelial hyperplasia and metaplasia were found in the urinary bladder of mice infected with *S. hematobium* after pretreatment with an aromatic amine such as acetyl aminofluorene [41]. Similar

changes were observed in *S. hematobium* infected mice that have been infected with *Escherichia coli* and treated with 2-napthylamine [42]. Four of 10 schistosome-infected bladders developed extensive cancer of the urinary bladder after being treated with N-butyl-N-(4-hydroxybutyl)nitrosamine [43]. *S. hematobium* ova deposited in the mucosa and submucosa of the urinary bladder induce chronic inflammatory lesions that promote proliferation of the urothelium. In some situations the proliferating cells may become neoplastic particularly with prolonged irritation and concomitant exposure to low (subcarcinogenic) doses of carcinogens like N-nitroso compounds [43, 44].

Histopathological Evidence

Bladder cancer associated with *S. hematobium* has several distinct features which differ from those of bladder cancer in countries where schistosomiasis is not endemic. In western countries, bladder cancer frequently arises in the trigone, while in countries where *S. hematobium* is endemic it usually involves the anterior and posterior walls. The scanty or absent submucosal tissue of the trigone discourages significant deposition of *S. hematobium* ova explaining this difference. In countries where *S. hematobium* is endemic squamous cell carcinoma of the bladder dominates while in western countries transitional cell carcinoma (TCC) prevails [45, 46]. Even within the same country, squamous cell carcinoma of the bladder is over-represented only in areas where *S. hematobium* is endemic [47, 48]. Moreover, the intensity of *S. hematobium* infection appears to play a role since squamous cell carcinoma is over-represented in areas of moderate and high worm burdens while transitional cell carcinoma occurs more commonly in areas with low intensity of infection [49]. The predominance of squamous cell carcinoma in patients with *S. hematobium* can be explained by the continuous exposure of the bladder mucosa to carcinogens, which are detected in larger quantities in the urine of these patients [43, 50–52].

Pathogenesis of Bladder Cancer in *S. hematobium* Chronic Inflammation

The *S. hematobium* ova deposited in the submucosa of the urinary bladder will lead to the formation of granulomas, nodules, polyps ulcerations and sandy patches. The granuloma is not a precancerous lesion, but the inflammatory cells such as macrophages and neutrophils are important sources of endogenous oxygen radicals which are implicated in the formation of carcinogenic N-nitrosamines [53], and the activation of procarcinogens to their carcinogenic

metabolites [54]. Inflammatory cells can also induce mutations [55], sister chromatid exchanges [56] and DNA strand breaks [57] through the release of hydroxyl radicals. The chronic inflammation in bilharzial cystitis can lead to squamous metaplasia which is a precancerous lesion. Aberrations of chromosome 9 in the urothelium may be a predictor of incipient carcinoma in patients with schistosomal cystitis [58].

Urinary Tract Infection

In Egypt 39–66% of hospitalized patients with schistosomiasis were found to have bacteriuria [59, 60]. Community-based studies show that the prevalence of bacteriuria is 10% in Tanzania [61], 1–3.2% in Nigeria [62] and 6.6% in Gambia [63] among persons infected with schistosoma. Although the prevalence of bacteriuria in persons infected with schistosomiasis may vary from one country to another and even in different reports from the same country, yet the prevalence is much higher than that reported from nonendemic areas [64]. This high prevalence of bacteriuria in schistosomal patients may be due to complications of schistosomal infection like obstructive lesions due to fibrosis of the neck of the bladder, vesical calculi and ulcers. There could also be a relationship between the schistosome worms and the bacteria, in which the bacteria become fixed on the cutaneous surface of the worms [65] or colonise the cecum of the parasite [66]. In vitro and in vivo studies show that co-cultivation or dual infection of schistosome worms with *Salmonella paratyphi* yielded more bacterial growth than the absence of the worms [67].

Bacterial infection of the urinary tract has been reported to increase the risk of bladder cancer in patients with *S. hematobium* infection [68] probably due to the increased urinary excretion of nitrite and N-nitroso compounds [69]. Infection with *S. hematobium* increases significantly the ability of the bacterial flora of the urinary bladder to reduce nitrates to the nitrite precursors of N-nitroso compounds [70]. Urinary tract infection in schistosomal patients is associated with increased chromosomal damage in the urothelium and this is significantly reduced after antihelminthic treatment [71].

Altered Carcinogen Metabolism

Environmental chemicals play a significant role in bladder cancer initiation. Carcinogens derived from occupational exposures, cigarette smoking, and inflammatory conditions associated with schistosomiasis are important factors in the initiation of bladder cancer. Bladder cancer susceptibility depends on the

expression profiles of the enzymes responsible for the activation and detoxification of carcinogens [72].

Disturbed Carcinogen Activation in Schistosomiasis

The cytochrome P-450 system participates in the bioactivation of polycyclic aromatic hydrocarbons and other carcinogens to their reactive intermediates [51, 73–76]. The expression of cytochrome P-450 proteins 1A, 2C, 3A was found in 68, 28 and 68% of human transitional-cell bladder cancers, and the expression of Cyp1A correlated with tumor grade [77].

It has been demonstrated that *S. mansoni* infection increased the activity of drug-metabolizing enzymes in the liver including P-450, cytochrome b5, and NADPH-cytochrome C reductase at earlier stages (30 days) of schistosomal infection; at later stages of infection (75 days), these activities subsided again [78]. The decrease in the activity of drug-metabolizing enzymes in the liver of humans and experimental animals in the later stages of the disease might be related to the development of liver fibrosis or to toxic metabolites produced either by adult *S. mansoni* worms or their deposited ova [79, 80]. This reduction in enzyme activity might therefore increase the exposure of other organs to the toxic, reactive, carcinogenic intermediates.

N-nitrosamines (NNA) are an important class of environmental carcinogens. The levels of NNA in urine are higher in Egyptian schistosomal patients than in controls [51, 52, 81]. Demethylases act on NNA leading ultimately to the formation of carbonium ion which can demethylate DNA [82]. The mutagenicity of NNA is therefore dependent on P-450 activities [83, 84] especially in early stages of infection when the demethylases are more active [85].

Occupational exposure to aromatic amines in the manufacture of dyestuffs and tires is a risk factor for bladder cancer [86, 87]. Most aromatic amines are initially activated by N-hydroxylation, mainly in the liver via a cytochrome P-450 catalyzed reaction [88, 89] which is influenced by Schistosomiasis [78]. Thus the effect of Schistosomiasis on the metabolic activation of amines might be similar to those of polycyclic aromatic hydrocarbons. Studies also show that the human urinary bladder contains acetyltransferases, which could serve as a further bioactivation step to form the highly reactive electrophilic N-acetoxy derivative [90, 91].

Disturbed Carcinogen Inactivation in Schistosomiasis

Schistosome infection in mice is associated with a marked increase in hepatic B-glucuronidase and sulfotransferase enzyme activities [80, 92], probably

due to the accumulation of lysosome-rich macrophages at the site of egg deposition in the liver. Sulfation of certain chemical carcinogens could lead to more toxic conjugates, which can cause liver cell necrosis [92]. Peritoneal macrophages are stimulated during murine *S. mansoni* infection. The increased nitrosamine formation and increased hydrolase activities found in infected livers might be due to these activated peritoneal macrophages [93, 94].

A major fraction of the N-hydroxy derivatives of aromatic amines is converted to the glucuronide, which is then excreted in the bile and urine [95]. However, the glucoronide may be hydrolyzed to release the free N-hydroxy arylamine which is a potent electrophil [96].

In TCC of the urinary bladder, the μ and TT forms of glutathione S-transferase were expressed in 56, 72 and 52% of tumors, respectively. These enzymes are important for the detoxification of carcinogens and may influence the response of bladder cancer to chemotherapy [77].

Molecular Mechanisms

Tumor suppressor genes and oncogenes have been implicated in a variety of human cancers. The activation of H-ras [97], inactivation of p53 [98] and inactivation of retinoblastoma gene [99] have been implicated in the progression, and possibly the development of schistosomal bladder cancer.

Tumor Suppressor Genes

The p53 Tumor Suppressor Gene

The p53 gene encodes a 53-kDa transcription factor with a critical role in DNA repair and apoptosis. Mutated p53 protein has a much longer half-life than wild type p53, thus allowing its detection by immunohistochemistry. Approximately 50% of muscle-invasive TCCs show nuclear overexpression of p53 indicating the presence of a mutated protein. This is associated with increased stage and grade [100]. Even in superficially invasive BC (T1), p53 mutant expression is associated with poorer outcome and a higher rate of disease progression. In muscle invasive TCC, an altered p53 status has been associated with a doubling of the risk of death, and is a predictor of decreased survival [101].

In 7 Egyptian patients with schistosomiasis-associated BC, 6 patients had p53 mutations in exons 5, 6, 7, 8 and 10, and in a Japanese group of 61 patients the mutation frequency increased with tumor grade. Habitual smoking in the Japanese group did not increase the frequency of p53 mutations, but an unusual AT: GC mutation was observed [102]. In 30 of 90 patients with

schistosomiasis-associated BC there were mutations in exons 5 through 8 of the p53 gene. Nitric oxide, produced by the inflammatory response to schistosome eggs, may cause such mutations directly by deamination of 5-methylcytosine or indirectly via its capacity to act as a nitrosating agent, leading to the formation of endogenous N-nitroso compounds which cause DNA alkylation and hence mutation in the p53 gene [103].

Retinoblastoma Tumor Suppressor Gene

The retinoblastoma (Rb) gene encodes a nuclear phosphoprotein (pRb) that functions as a cell cycle regulator. Normal cells express the Rb protein while mutations or gene deletions, which often result in lack of protein expression, can be identified by the lack of Rb protein expression. Rb gene mutations are seen in approximately 30% of BC [104]. Inability to detect pRb immunohistochemically is associated with increased tumor grade and stage, especially muscle invasion [105]. Inactivation of the Rb gene has also been observed in schistosomal BC [99].

Chromosome 9

Deletions on chromosome 9 not only appear to occur in greater than 60% of BC across all grades and stages, but also are likely an initiating event [106]. Cytogenetic and molecular evidence has shown that it is often the only chromosomal aberration in early disease. Evidence points to the CDKN2 or p16 locus as the tumor suppressor gene since it encodes a cyclin-dependent kinase inhibitor that prevents the phosphorylation of Rb, thereby maintaining an active Rb and blocking the exit from the G1 phase of the cell cycle. Loss of function of p16, by permitting Rb phosphorylation, results in unregulated cell growth as the cell is able to escape in the S phase [107].

Tamini et al. [108] found that 25 of 47 schistosomal BC patients showed p16 gene alterations (23 deletions and 2 mutations). In another study, deletions in chromosome 9p, where the CDKN2 gene is located, were found in 92% of SCC in Egyptian and Swedish patients compared to only 10% of TCC from a literature-based sample [109].

Microsatellite Instability

Within the human genome are repetitive sequences of DNA – usually 1–4 bases long – that are lost in many types of cancers, including BC. This

phenomenon is called microsatellite instability. Microsatellite DNA sequences vary from individual to individual but, being inherited, are identical in all of an individual's cells. However, within cancer cells, there are often variations in many of the sequences caused by errors in DNA replication. Since microsatellite DNA repeats are almost exclusively found within introns, it is unclear how these DNA replicative errors generate mutations that provide survival advantage resulting in clonal expansion. What may be more important is that microsatellite instability represents DNA replication errors that also occur in exons which go unrecognized because of the paucity of microsatellite repeats in exons, and these are expressed as mutated tumor suppressor genes or oncogenes leading to tumor growth and progression.

Microsatellite instability has been advocated as a means to detect BC. Mao et al. [110] identified microsatellite instability in urine sediments from 19 of 20 patients who were diagnosed with BC. Steiner et al. [111] correctly diagnosed 20 of 21 patients being followed for BC recurrence using microsatellite analysis with 20 markers in a blinded fashion. Mowah et al. [112] reported similar results by detecting microsatellite instability in 10 of 12 patients with BC.

Oncogenes

H-ras

H-ras, which codes for a protein anchored to the cytoplasmic side of the cell membrane that is involved in signal transduction, may play a role in BC genesis. H-ras mutations have been found in up to 36% of bladder tumors [113], and these mutations were similar for BC associated with schistosomiasis and those associated with other causes [114].

Bcl-2 Gene

The Bcl-2 gene was discovered in chromosomal translocations identified in B cell leukemias and follicular lymphomas. Expression of this gene results in extended viability of cells by overriding apoptosis thus increasing the risk of acquiring genetic changes that may result in malignant transformation. The Bcl-2 gene is overexpressed in some schistosomiasis-associated BC [115]. Bcl-2 is overexpressed in SCC and adenocarcinoma but not significantly expressed in TCC. The high level of Bcl-2 expression in malignant cells, but not in precancerous cells, suggests that the gene may be upregulated in the later stages of tumor progression.

Schistosomiasis and Colorectal Cancer

Intestinal schistosomiasis is usually caused by *S. mansoni* and *S. japonicum*. Lesions are mostly present in the large intestine especially the rectum and sigmoid colon. The lesions are due to deposition of ova in the submucosa producing a granulomatous reaction. In severe cases, mainly in Egypt, exaggerated reaction in the submucosa may lead to polyp formation. These polyps may be sessile or pedunculated and may show a cauliflower appearance. Histologically, the polyps are inflammatory lesions with glandular proliferation and destruction but with no adenomatous changes [116]. In Egypt, the data available tend to deny any association of *S. mansoni* and cancer colon [116]. In Asia, *S. japonicum* infection is considered a significant risk factor for colonic cancer. The considerably greater number of eggs deposited by *S. japonicum* worms could cause more pathological problems and explain this discrepancy [117].

In one report from an endemic area in china, 48% of colectomy specimens for colorectal carcinoma obtained from 1951 to 1964 were associated with *S. japonicum* infection. The mean age of the patients was 36.9 years and 10 had multicentric carcinoma [118]. The same group later reported a pathological study of 454 colectomy specimens for colorectal carcinoma; 63.6% were associated with *S. japonicum*. 92% of cancers were well differentiated, compared to 69% in the group without schistosomiasis. Patients with colorectal cancer and *S. japonicum* infestation were, on the average, 6 years younger than those without *S. japonicum* infestation. Specimens from patients with schistosomiasis showed associated inflammatory changes, pseudopolyps, and transitional mucosal changes of schistosomal granulomatous disease progressing to mucosal atypia and to carcinoma were reminiscent of colorectal carcinoma in patients with ulcerative colitis. A common feature in schistosoma-associated cases was the widespread colonic infection and the long history of colitic symptoms [119]. An ecologic study of 49 Chinese rural counties indicates that both schistosomal infestation and dietary factors contribute to the remarkable geographic variation of colorectal cancer in China [120].

Schistosomiasis and Liver Cancer

Schistosome eggs deposited into the mesenteric venous plexus may be carried by the blood flow into the portal circulation where they lodge in the small portal vein tributaries where they incite granuloma formation leading to pylephlebitis, peripylephlebitis, and periportal fibrosis. Despite the intense periportal fibrosis, the lobular architecture of the hepatic parenchyma is preserved. The resulting periportal fibrosis can lead to portal hypertension that can

be complicated by splenomegaly, esophageal varices, hematemesis and death [121, 122].

Epidemiological and clinical studies in China and Japan support a role of *S. japonicum* infection as one of the risk factors in hepatocellular carcinoma (HCC) formation. However, additional risk factors for the development of HCC, including viral infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) and alcohol abuse, are usually present [123, 124]. Experimental studies have shown that liver cancer appears early and in larger numbers in animals experimentally infected with *S. japonicum* and given a known carcinogen. The mechanism of schistosome mediated enhancement of carcinogenesis is not clear, but it has been observed that in *S. japonicum*-infected mice the carcinogen metabolizing activity including P-450 was decreased. Thus, an administered carcinogen persisted for a longer period than uninfected mice [123].

The link between *S. mansoni* and HCC appears to be an indirect one. Patients with *S. mansoni* infection have higher rates of hepatitis B surface antigen (HBsAg) carriage and hepatitis C seropositivity than do noninfected controls [125, 126]. The higher exposure of patients infected with schistosomiasis to HBV and HCV could be explained, at least in part, by transmission of these viruses during blood transfusion and parenteral therapy for schistosomiasis using contaminated needles [127, 128]. Furthermore, studies have shown that the cell-mediated immune response is depressed even in simple active intestinal schistosomiasis and this suppression increases with advancement of the disease and the development of hepatosplenomegaly [129, 130]. Thus, patients with schistosomiasis tend to retain HBV and HCV for longer periods and attain a carrier state with a higher risk of developing chronic hepatitis, cirrhosis and even HCC [131, 132].

Patients infected with schistosomiasis have an increased risk of chronic infection with HBV and HCV after an episode of acute viral hepatitis [127, 133]. Patients with hepatosplenic schistosomiasis who are coinfected with HBV or HCV are at a higher risk of earlier deterioration of liver function, the development of cirrhosis, and more rapid progression toward end-stage liver disease and even HCC [131, 132, 134, 135].

Schistosomiasis and Prostate Cancer

Schistosomiasis of the prostate is poorly documented in the medical literature. Among 190 consecutive autopsies of patients who died of varying causes at the university hospital in Cairo, the prostates from patients with *S. hematobium* and *S. mansoni* showed a mean of approximately 8,000 eggs and 11 eggs per

gram prostatic tissue, respectively. There were 3 cases of prostatic carcinoma: 2 in the schistosomiasis and 1 in the control group; however, it was not specified whether the prostates had concomitant schistosomal infections [136]. In Zambia, a consecutive autopsy study of 50 patients who died of varying causes found that 62% of bladders, 58% of seminal vesicles, and 50% of prostates were infected with *S. hematobium* eggs. No major structural prostatic derangements were described [137]. There are 6 reported cases of prostatic adenocarcinoma (PAC) and schistosomiasis. One case of simultaneous PAC and *S. mansoni* gland infection was diagnosed in a 49-year-old Puerto Rican-born patient living in the United States for 25 years [138]. Cohen et al. [139] described 3 patients, 27 to 29 years old, seen within a 7-month period in a rural endemic area in South Africa who had elevated prostate specific antigen (PSA), advanced PAC and simultaneous florid *S. hematobium* of the gland. There is also a Canadian case report of a 55-year-old Ghanaian residing in Canada for 19 years who had PAC and simultaneous *S. hematobium* of the seminal vesicles, but not in the prostate [140]. Another 68-year-old Brazilian man with PAC and concomitant *S. mansoni* infection of the gland was also reported [141]. Since there are no strong epidemiological data to suggest a true cause and effect relationship between *S. hematobium* and PAC, these isolated cases probably represent coexistence of two common unrelated disorders.

Schistosomiasis and Cancer of Other Sites

In Egyptian hospital material, the male-to-female breast cancer ratio is substantially greater than in the West. If corroborated by incidence studies, this observation would be a valuable epidemiologic observation worthy of further investigation. Hyperestrogenism secondary to bilharzial liver fibrosis has been invoked as one possible cause. Eight cases of solitary follicular lymphoma of the spleen were found among 863 spleens removed from patients with hepatosplenic schistosomiasis. The rarity of an isolated tumor at this site and of this type suggests a causal link, possibly mediated by cycles of follicular hyperplasia and involution occurring in the spleen in the course of advanced schistosomiasis [142]. In a Nigerian series, lymphoreticular tumours were over-represented in infected individuals (16%) as compared with uninfected ones [143].

Egyptian cases indicate no relationship between bilharziasis and cancer of the lungs, pancreas, prostate, seminal vesicles, urethra, vulva, vagina, cervix uteri, body of the uterus, or ovaries [21]. As would be expected, surgical or autopsy material in countries with high schistosomal endemicity from time to time shows the presence of *Schistosoma* ova in various tissues, including cancerous ones. The literature contains a number of isolated reports of such coincidences.

References

1 Strickland GT, Abdel-Warib MF: Schistosomiasis; in Strickland GT (ed): Hunter's Tropical Medicine, ed 7. Philadelphia, Saunders, 1991, pp 781–802.
2 Goddard MJ, Jordan P: On the longevity of *Schistosoma* mansoni in man on St. Lucia, West Indies. Trans R Soc Trop Med Hyg 1980;74:185.
3 Anonymous: The control of schistosomiasis: second report of the WHO Expert Committee. WHO Tech Rep Ser 1993;830:1–86.
4 Chan MS, Guyatt HL, Bundy DAP, et al: Dynamic models of schistosomiasis morbidity. Am J Trop Med Hyg 1996;55:52.
5 Marguerite M, Gallissot MC, Diagne M, et al: Cellular immune responses of a Senegalese community recently exposed to *Schistosoma* mansoni: correlations of infection level with age and inflammatory cytokine production by soluble egg antigen-specific cells. Trop Med Int Health 1999;4:530.
6 Wiest PM, Wu G, Zhong S, et al: Morbidity due to schistosomiasis japonica in the People's Republic of China. Trans R Soc Trop Med Hyg 1992;86:47.
7 Butterworth AE, Dunne DW, Fulford AIC, Ouma JH, Sturrock RE: Immunity and morbidity in *Schistosoma* mansoni infection: quantitative aspects. Am J Trop Med Hyg 1996;55:109–115.
8 Butterworth AE, Curry AJ, Dunne DW, et al: Immunity and morbidity in human schistosomiasis mansoni. Trop Geogr Med 1994;46:197.
9 Fulford AJC, Mbugua GG, Ouma JH, et al: Differences in the rate of hepatosplenomegaly due to *Schistosoma mansoni* infection in Machakos District, Kenya. Trans R Soc Trop Med Hyg 1991;85:481.
10 Katz N, Chaves A, Pellegrino J: A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo 1972;14:397.
11 Schutte CHJ, Pienaar R, Becker PJ, et al: Observations on the techniques used in the qualitative and quantitative diagnosis of schistosomiasis. Ann Trop Med Parasitol 1994;88:305.
12 Abdel-Hafez MA, Bolbol AH: Fibre-optic sigmoidoscopy compared with the Kato technique in diagnosis and evaluation of the intensity of *Schistsoma mansoni* infection. Trans R Soc Trop Med Hyg 1992;86:641.
13 Tsang VCW, Wilkins PP: Immunodiagnosis of schistosomiasis: screen with FAST-ELISA and confirm with immunoblot. Clin Lab Med Parasitol 1992;86:257.
14 Bergquist NR: Present aspects of immunodiagnosis of schistosomiasis (review). Mem Inst Oswaldo Cruz 1992;87(suppl 4):29.
15 Deelder AM, Qian ZL, Kremsner PG, et al: Quantitative diagnosis of *Schistosoma* infections by measurement of circulating antigens in serum and urine. Trop Geogr Med 1994;46:233.
16 Nibbeling HAM, Van lieshout L, Deelder AM: Levels of circulating soluble egg antigen in urine of individuals infected with *Schistosoma* mansoni before and after treatment with praziquantel. Trans R Soc Trop Med Hyg 1998;92:675.
17 Abdel-Wahab MF , Esmat G, Farrag A, et al: Grading of hepatic schistosomiasis by the use of ultrasonography. Am J Trop Med Hyg 1992;46:403.
18 Abdel-Wahab MF, Esmat G, Ramzy I, et al: *Schistosoma haematobium* infection in Egyptian schoolchildren: demonstration of both hepatic and urinary tract morbidity by ultrasonography. Trans R Soc Trop Med Hyg 1992;86:406.
19 Ibrahim AS: Site distribution of cancer in Egypt: twelve years experience (1970–1981); in Khogali M, Omar YT, Gjorgov A, Ismail AS (eds): Cancer Prevention in Developing Countries. Pergamon Press, Oxford, 1986, pp 45–50.
20 Kahan E, Ibrahim AS, El Najjar K, Ron E, Al-Agha H, Polliak A, El-Bolkasiny NM: Cancer patterns in the Middle East. Special report from the Midle East Cancer Society. Act Oncol 1997;36:631–636.
21 Bedwani R, El-Khwsky F, La Vecchia C, et al: Descriptive epidemiology of bladder cancer in Egypt. Int J Cancer 1993;55:351.
22 Becker N, Beyme RF, Wagner G: Atlas of cancer mortality in the Federal Republic of Germany. Berlin, Springer, 1984.

23 Devesa SS, Silverman DT, Young JL: Cancer incidence and mortality trends among whites in the United States, 1947–84. JNCI 1987;79:701–770.
24 Office of Population Censuses and Surveys: Cancer statistics registrations: England and Wales. Government Statistical Services. London, HMSO, 1983.
25 Bilir N: Turkey: National histopathological survey. IARC Sci Publ 1977;75:303–307.
26 Al-Adnani MS, Saleh KM: Schistosomiasis and bladder cancer in southern Iraq. J Trop Med Hyg 1983;86:93–97.
27 El-Bolkainy MN, Mokhtar M, Ghonim MA, Hussein MH: The impact of schistosomiasis on the pathology of bladder carcinoma. Cancer 1981;48:2643–2648.
28 Elem B, Purohit R: Carcinoma of urinary bladder in Zambia: a quantitative estimate of *Schistosoma haematobium* infection. Br J Urol 1983;55:275–278.
29 Gelfand M, Weinberg RW, Castle WM: Relation between carcinoma of the bladder and infestation with *Schistosoma haematobium*. Lancet 1967;i:1249–1251.
30 Lucas SB: Squamous cell carcinoma of the bladder and schistosomiasis. East Afr Med J 1982;59:345–351.
31 Malik MO, Veress B, Daoud EH, El-Hassan M: Pattern of bladder cancer in the Sudan and its relation to schistosomiasis: a study of 255 vesical carcinomas. J Trop Med Hyg 1975;78: 219–233.
32 El-Sebai I: Cancer of the bilharzial bladder. Urol Res 1978;6:233–236.
33 Isha, KG, Le Golvan OC, El-Sebai I: Malignant bladder tumours associated with bilharziasis, a gross and microscopic study; in Mostofi FK (ed): Bilharziasis. New York, Springer, 1967, p 67.
34 Aboul-Nasr AL, Boutrous SG, Hussien MH: Egypt: Cairo Metropolitan Cancer Registry, 1978–1979. IARC Sci Publ 1986;75:37–41.
35 Kuntz RE, Cheever AW, Myers BJ: Proliferative epithelial lesions of the urinary bladder of nonhuman primates infested with *Schistosoma* haematobium. J Natl Cancer Inst 1972;48:223.
36 Kuntz RE, Cheever AW, Myers BJ: *Schistosoma haematobium* infection in the opossums (*Didelphis marsupialis*): involvement of urogenital system. Bull WHO 1971;45:21–25.
37 Kuntz RE, Cheever AW, Myers BJ: Proliferative lesions of the urinary bladder of non-human primates infected with *Schistosoma haematobium*. J Natl Cancer Inst 1972;48:223–235.
38 Kuntz RE, Cheever AW, Myers BJ: Calcification of the bladder and papillary tumours of the bladder and ureters in gibbons (*Hylobateslar*) infected with *Schistosoma haematobium*. Trans R Soc Trop Med Hyg1975;69:494–502.
39 El-Ghaffar YA: Failure to induce bladder cancer in mice: bladder implantation with paraffin wax pellets of lyophilised urine from bilharzial patients. Cancer 1966;19:1225.
40 Shimkin MB, Mustacchi PO, Cram EB, Wright WH: Lack of carcinogenicity of lyophilised *Schistosoma* in mice. J Natl Cancer Inst 1955;16:47.
41 Hashem M, Boutros K: The influence of bilharzial infection on the carcinogenesis of the bladder: an experimental study. J Egypt Med Assoc 1961;44:598–606.
42 Al-Aaser AA, Merzabani MM, Higgy NA, Abdel-Kader MM: A study on the etiological factors of bilharzial bladder cancer in Egypt. 3. Urinary b-glucuronidase. Eur J Cancer 1978;15:573–583.
43 Hicks RM, James C, Webbe G: Effect of schistosomiasis haematobium and N-butyl-N-(4-hydroxybutyl)nitrosamine on the development of urothelial neoplasia in baboon. Br J Cancer 1980;42:730–755.
44 Hicks RM: The canopic worm: role of bilharziasis in the aetiology of human bladder cancer. J R Soc Med 1983;76:16.
45 Halawani A, Tomani A: Preliminary report of the cytological diagnosis and incidences of the bilharzial cancer of the bladder in Egypt. J Egypt Med Assoc 1955;38:455–465.
46 Higginson J, Oettle AG: Cancer of the bladder in the South African Bantu. Acta Unio Inter Contra Cancrum 1962;18:580.
47 Kitinya JN, Lauren PA, Eshleman LJ: The incidence of squamous and transitional cell carcinomas of the urinary bladder in northern Tanzania in areas of high and low levels of endemic *Schistosoma haematobium* infection. Trans R Soc Trop Med Hyg 1986;80:935.
48 Thomas JE, Nassett MT, Sigola LB, Taylor P: Relationship between bladder cancer incidence, *Schistosoma haematobium* infection, and geographical region in Zimbabwe. Trans R Soc Trop Med Hyg 1990;84:551.

49 Koraitim NM, Metwalli NE, Atta MA, El-Sadr AA: Changing age incidence and pathological types of *Schistosoma*-associated bladder carcinoma. J Urol 1995;154:1714–1716.
50 Hicks RM: Nitrosamines as possible etiological agents in bilharzial bladder cancer. Banbury Rep 1982;12:455–471.
51 Mostafa MH, Helmi S, Badawi AF, Tricker AR, Spiegelhalder B, Preussman R: Nitrate, nitrite and volatile N-nitroso compounds in the urine of *Schistosoma mansoni*-infected patients. Carcinogenesis 1994;15:619–625.
52 Tricker AR, Mostafa MH, Spiegelhalder B, Preussmann R: Urinary excretion of nitrate, nitrite and N-nitroso compounds in schistosomiasis and bilharzial bladder cancer patients. Carcinogenesis 1989;10:547–552.
53 Marletta MA: Mammalian synthesis of nitrite, nitrate, nitric oxide and N-nitrosating agents. Chem Res Toxicol 1988;1:249–257.
54 O'Brien PJ: Radical formation during the peroxidase-catalised metabolism of carcinogens and xenobiotics: the reactivity of these radicals with GSH, DNA and unsaturated fatty lipid. Free Radical Biol Med 1988;4:216–226.
55 Weitzman SA, Stossel PT: Mutation caused by human phagocytes. Science 1981;212:546–547.
56 Weitberg AB: Effect of combinations of antioxidant on phagocyte induced sister chromatid exchange. Mutat Res1989;224:1–4.
57 Shaeter E, Beecham EJ, Covey JM, Kohn K, Potter M: Activated neutrophils induce prolonged DNA damage in neighbouring cells. Carcinogenesis 1988;9:2297–2304.
58 Ghaleb AH, Pizzolo JG, Melamed MR: Abberrations of chromosomes 9 and 17 in bilharzial bladder cancer as detected by fluorescence in situ hybridization. Am J Clin Pathol 1996; 106:234.
59 El-Hawey A, Massoud A, Badr D, Waheeb A, Abdel-Hamid S: Bacterial flora in hepatic encephalopathy in bilharzial and non-bilharzial patients. J Egypt Soc Parasitol 1989;19: 797–804.
60 Lehman JS, Farid Z, Smith Z, Bassily JH, El-Masry NA: Urinary schistosomiasis in Egypt: clinical, radiological, bacteriological and parasitological correlations. Trans R Soc Trop Med Hyg1973;67:384–399.
61 Forsyth DM, Bradley DJ: The consequences of bilharziasis; medical and public health importance in north-west Tanzania. Bull WHO 1966;34:715–735.
62 Kassim O: Proteinuria and haematuria as predictors of schistosomiasis in children. Ann Trop Paediatr 1989;9:156–160.
63 Wilkins HA: *Schistosoma haematobium* in a Gambian community. III. The prevalence of bacteriuria and hypertension. Ann Trop Med Parasitol 1977;71:179–186.
64 Laughlin LW, Farid Z, Mansour N, Edman DC, Higashi GI: Bacteriuria in urinary schistosomiasis in Egypt: a prevalence survey. Am J Trop Med Hyg 1978;27:916–920.
65 Penaud A, Nourrit J, Chapoy P, Alessandrini P, Louchet E, Nicoli RM: Bacterio-parasite interactions: enterobacteria and schistosomes. Med Trop 1983;43:331–340.
66 Ottens H, Dickerson C: Studies on the effects of bacteria on experimental schistosome infections in animals. Trans R Soc Trop Med Hyg 1972;66:85–107.
67 Mikhail IA, Higashi GI, Edman SH: Interaction of *Salmonella paratyphi* A and S. mansoni in hamster. Am J Trop Med Hyg 1982;31:828–834.
68 Hicks RM, Walters CL, El-Sebai I, El-Merzabani M, Gough TA: Determination of nitrosamines in human urine: preliminary observations as the possible etiology for bladder cancer in association with chronic tract infections. Proc R Soc Med 1976;70:413–416.
69 Tricker AR, Mostafa HH, Spiegelhalder P, Preussman P: Urinary nitrite and nitroso compounds in bladder cancer patients with schistosomiasis (bilharziasis) IARC Sci Publ 1991;105:178.
70 Hicks RM, Ismail MM, Walters CL, et al: Association of bacteriuria and urinary nitrosamine formation with *Schistosoma haematobium* infection in the Qalyub area of Egypt. Trans R Soc Trop Med Hyg 1982;76:519.
71 Anwar WA, Rosin MP: Reduction in chromosomal damage in schistosomiasis patients after treatment with praziquantel. Mutat Res 1993;298:179.
72 Jung I, Messing E: Molecular mechanisms and pathways in bladder cancer development and progression. Cancer Control 2000;7:325–334.

73 Guengerich FP: Reactions and significance of cytochrome P450. J Biol Chem 1991;226: 10019–10022.
74 Mostafa MH, Weisburger EK: Effect of chloropromazine hydrochloride on carcinogen metabolising enzymes: liver microsomal dimethylnitrosamine demethylase, 4-aminoazobenzene reductase and arylhydrocarbon hydroxylase. JNCI 1980;64:924–929.
75 Mostafa MH, Ruchirawat M, Weisburger EK: Comparative studies on the effects of various microsomal enzyme inducers of the N-demethylation of dimethylnitrosamine. Biochem Pharmacol 1981;30:2007–2011.
76 Sheweita SA, Mostafa MH: N-nitroso compounds induce changes in carcinogen-metabolising enzymes. Cancer Lett 1996;106:243–249.
77 Murray GI, Taylor V, McKay JA, Weaver RJ, Ewen SW, Melvin WT, Burke MD: Expression of xenobiotic metabolising enzymes in tumours of the urinary bladder. Int J Exp Pathol 1995;76: 272–276.
78 Sheweita SA, Mostafa MH: Recovery of the hepatic carcinogen-metabolising capacity in schistosome-infected mice after treatment with the antischistosomal praziquantel. Oncol Rep 1995:155–159.
79 Badawi AF, Mostafa MH: Possible mechanisms of alteration in the capacities of carcinogen metabolising enzymes during schistosomiasis and their role in bladder cancer induction. J Intern Med Res 1993;21:281–305.
80 El-Mouelhi M, Black M, Phillips SM: Hepatic cytochrome P450 system in experimental hepatosplenic schistosomiasis: presence of an artifact in spectrophotometric analysis. Biochem Pharmacol 1987;36:2621–2626.
81 Hicks RM, Walters CL, El-Sebai I, Al-Aaser A, El-Merzabani M, Gough AT: Demonstration of N-nitrosamines in human urine. Proc R Soc Med 1977;70:413–417.
82 Saffhill R, Margison GP, O'Connor PJ: Mechanisms of carcinogenesis induced by alkylating agents. Biochim Biophys Acta 1985;823:111–145.
83 Czygan P, Greim H, Garro AJ, Hutter F, Schaffner F, Popper H, Rosenthal O, Cooper DY: Microsomal metabolism of dimethylnitrosamine and the cytochrome P-450 dependency of its activation to a mutagen. Cancer Res 1973;33:2983–2986.
84 Evarts RP, Mostafa MH: Effects of indole and tryptophan on cytochrome P450, dimethylnitrosamine demethylase and arylhydrocarbon hydroxylase activities. Biochem Pharmacol 1981;30:517–522.
85 Mostafa MH, El-Bassiouni EA, El-Sewedy SM, Akhnouk S, Tawific T, Abel-Rafee A: Hepatic microsomal enzymes in *Schistosoma mansoni*-infected mice. I. Effect of duration of infection and lindane administration on dimethylnitrosamine demethylase. Environ Res 1984;35:154–159.
86 Case RA, Hosker ME, McDonald DB, Rearson JT: Tumours of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry. Part I. Br J Ind Med 1954;11:75–104.
87 Lamm DL, Torti FM: Bladder cancer. CA Cancer J Clin 1996;49:103–112.
88 Kadlubar FF: DNA adducts of carcinogenic aromatic amines. IARC Sci Publ 1994;125:199–216.
89 Kadlubar FF, Butler MA, Kaderlik KR, Chou HC, Lang NP: Polymorphisms of aromatic amine metabolism in humans: relevance for human carcinogenesis. Environ Health Perspect 1992;98: 69–74.
90 Badawi AF, Hirvonen A, Bell DA, Lang NP, Kadlubar FF: Role of aromatic amine acetyltransferases NAT1 and NAT2 in increasing carcinogen-DNA adduct formation in the human urinary bladder. Cancer Res 1995;55:5230–5237.
91 Wild D, Feser W, Michel S, Lord HL, Josephy PD: Metabolic activation of heterocyclic aromatic amines by human arylamine N-acetyltransferase isoenzymes (NAT1 and NAT2) expressed in *Salmonella typhimurium*. Carcinogenesis 1995;16:643–648.
92 El-Mouelhi M, Mansour MM: Hepatic drug conjugation and deconjugation systems in hepatosplenic schistosomiasis. Biochem Pharmacol 1990;40:1923–1925.
93 Miwa M, Stueher DJ, Marletta MA, Wishnok JS, Tannenbaum S: N-nitrosamine formation by macrophages. IARC Sci Publ 1987;84:340–344.
94 Higuchi M, Ito K, Fukuyama K, Epstein W: Biochemical characterisation of arylsulfatases detected in granulomatous livers. Exp Mol Pathol 1984;40:70–80.

95 Irving CC: Conjugates of *N*-hydroxy compounds; in Fishman WH (ed): Metabolic Conjugation and Metabolic Hydrolysis. New York, Academic Press, 1973, vol 1, pp 53–119.
96 Kadlubar FF, Unruh LE, Flammang TJ, Sparks D, Mitchum RK, Mulder GJ: Alteration of the urinary levels of the carcinogen N-hydroxy-2-naphthylamine and its N-glucuronide in the rat by control of urinary pH. Chem Biol Interact 1981;33:129–147.
97 Knowles MA, Williamson M: Mutation of H-*ras* is infrequent in bladder cancer: confirmation by single-strand-conformation-polymorphism analysis, designed restriction-fragment-length polymorphisms, and direct sequencing. Cancer Res 1993;53:133–139.
98 Sidransky D, Von Eschenbach A, Tsai YC, Jones P, Summerhayes I, Marshall F, Pual M, Green P, Vogelstein B: Identification of the *p*53 gene mutations in bladder cancers and urine samples. Science 1991;252:706–709.
99 Ishikawa J, Xu HJ, Hu XS, Yandell DW, Maeda S, Kamidono S, Benedict WF, Takahashi R: Inactivation of the retinoblastoma gene in human bladder and renal-cell carcinomas. Cancer Res 1991;51:5736–5743.
100 Habuchi T, Ogawa O, Kakeni Y, et al: Allelic loss of chromosome 17p in urothelial cancer: strong association with invasive phenotype. J Urol 1992;148:1595–1599.
101 Esrig D, Elmanjian D, Groshen S, et al: Accumulation of nuclear p53 and tumour progression in bladder cancer. N Engl J Med 1994;331:1259–1264.
102 Habuchi T, Takahashi R, Yamada H, Kakehi O, Ourga K, Hamazaki S, Toguchida J, Ishizaki K, Yoshida O: Influence of cigarette smoking and schistosomiasis on *p*53 gene mutation in urothelial cancer. Cancer Res 1993;53:3795–3799.
103 Warren W, Biggs PJ, El-Baz M, Ghoneim MA, Stratton MR, Venitt S: Mutations in the *p*53 gene in schistosomal bladder cancer: a study of 92 tumours from Egyptian patients and a comparison between mutational spectra from schistosomal and non-schistosomal urothelial tumours. Carcinogenesis 1995;16:1181–1189.
104 Cordon-Cardo C, Wartinger D, Petrylak D, et al: Altered expression of the retinoblastoma gene product: prognostic indicator in bladder cancer. J Natl Cancer Inst 1992;84:1251–1256.
105 Xu HJ, Cairns P, Hu SX, et al: Loss of RB protein expression in primary bladder cancer correlates with loss of heterozygosity at the RB locus and tumour progression. Int J Cancer 1993;53: 781–784.
106 Tsai YC, Nichols PW, Hiti AL, et al: Allelic losses of chromosome 9, 11 and 17 in human bladder cancer. Cancer Res 1990;50:44–47.
107 Kamb A, Gruis NA,Weaver-Feldhaus J, et al: A cell cycle regulator potentially involved in the genesis of many tumour types. Science 1994;264:436–440.
108 Tamimi Y, Bringuier PP, Smit F, Bokhoven A, Abbas A, Debruyne FM, Schalken JA: Homozygous deletions of p16INK4 occur frequency in bilharziasis-associated bladder cancer. Int J Cancer 1996;68:183–187.
109 Gonzalez-Zulueta, M, Shibata A, Ohneseit PF, Spruck CH, Busch C, Shamaa M, Elbaz M, Nichols PW, Gonzalgo ML, Malstrom P-U, Jones PA: High frequency of chromosome 9p allelic loss and CDKN2 tumour suppressor gene alterations in squamous cell carcinoma of the bladder. J Natl Cancer Inst 1995;7:1383–1392.
110 Mao L, Schoenberg MP, Scicchitano M, et al: Molecular detection of primary bladder cancer by microsatellite analysis. Science 1996:271;659–662.
111 Steiner G, Schoenberg MP, Linn JF, et al: Detection of bladder cancer recurrence by microsatellite analysis of urine. Nat Med 1997:3:621–624.
112 Mourah S, Cussenot O, Vimont V, et al: Assessment of microsatellite instability in urine in the detection of transitional-cell carcinoma of the bladder. Int J Cancer 1998;79:629–633.
113 Czerniak B, Deitch D, Simmons H, et al: Ha-ras gene codon 12 mutation and DNA ploidy in urinary bladder carcinoma. Br J Cancer 1990;62:762–763.
114 Badawi AF: Molecular and genetic events in schistosomiasis-associated human bladder cancer: role of oncogenes and tumour suppressor genes. Cancer Lett 1996;105:123–138.
115 Chaudhary KS, Lu QL, Abel PD, Khandan N, Shoma AM, El-baz M, Stamp GW, Lalani EN: Expression of bcl-2 and *p*53 oncoproteins in schistosomiasis-associated transitional and squamous cell carcinoma of urinary bladder. Br J Urol 1997;79:78–84.

116 Cheever AW, Kamel IA, Elwi AM, Mosimann JE, Danner R, Sippel JE: *Schistosoma mansoni* and *Schistosoma hematobium* infection in Egypt. III. Extrahepatic pathology. Am J Trop Med Hyg 1978;27:55–75.
117 Ishii A, Matsuoka H, Aji T, et al: Parasite infection and cancer with special emphasis on *Schistosoma japonicum* infections (Trematoda): a review. Mutat Res 1994;305:273.
118 Ming-Chai C, Jen-Chun H, Pei-Yu C, et al: Pathogenesis of carcinoma of the colon and rectum in schistosomiasis japonica. Chin Med J 1965;84:513–525.
119 Ming-Chai C, Chi-Yuan C, Pei-Yu C, Jen-Chun H: Evolution of colorectal cancer in schistosomiasis. Cancer 1980;46:1661–1675.
120 Guo W, Zheng W, Li JY, et al: Correlation of colon cancer mortality with dietary factors, serum markers and schistosomiasis in China. Nutr Cancer 1993;20:13.
121 El-Garem AA: Schistosomaisis. Digestion 1998;59:589–605.
122 Andrade ZA, Peixoto E, Guerret S: Hepatic connective tissue changes in hepatosplenic schistosomaisis. Hum Pathol 1992;23:566–602.
123 Ishii A, Matsuoka H, Aji T, Ohta N, Arimoto S, Wataya Y, Hayatsu H: Parasite infection and cancer: with special emphasis on *Schistosoma japonicum* infections (Trematoda): a review. Mutat Res 1994;305:273–281.
124 Iida F, Lida R, Kamijo H, Takaso K, Miyazaki Y, Funabashi W, Tsuchiya K, Matsumoto Y: Chronic Japanese schistosomaisis and hepatocellular carcinoma: ten years of follow-up in Yamanashi Prefecture, Japan. Bull WHO 1999;77:573–581.
125 Halim AB, Garry RF, Dash S: Effect of schistosomaisis and hepatitis on liver disease. Am J Trop Med Hyg 1999;60:915–919.
126 Lyra LG, Reboucas G, Andrade ZA: Hepatitis B surface antigen carrier state in hepatosplenic schistosomaisis. Gastroenterology 1976;71:641–646.
127 Darwish MA, Raouf TA, Rushdy P: Risk factors associated with high prevalence of hepatitis C virus infection in Egyptian blood donor. Am J Trop Med Hyg 1993;49:440–449.
128 Frank C, Mohamed MK, Strickland GT, Lavanshy D, Arthur RR, Magder LS, El-Khoby T, Abdelwahab Y, Ohn EA, Anwar W, Sallam I: The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 2000;355:887–891.
129 Zakaria S, Mabrouk MA, Elsherif N, Zakaria E, Said El-Din SM, Kamel MA, Elgarem A: T helper and T suppressor cells in schistosomal and nonschistosomal chronic liver disease. Egypt J Bilh 1993;15:17–27.
130 Wahib AA, Masoud AA, Halem AA, Haseeb AN, Hassan AR, Darwish MA, Hegazi M, Abdalla KF: Cell mediated immune response in chronic liver diseases: *Schistosoma*l, viral and neoplastic. J Egypt Soc Parasitol 1998;28:929–939.
131 Bassily S, Farid Z, Higashi GI: Chronic hepatitis B antigenemia in patients with hepatosplenic schistosomiasis. J Trop Med Hyg 1979;82:248–257.
132 Bassily S, Hyams KC, El-Masry NA: Hepatitis C virus infection and hepatosplenic schistosomaisis. Scand J Infect Dis 1992;24:687–696.
133 Ghafar YA, Fattah SA, Kamal M: The impact of endemic schistosomaisis on acute viral hepatitis. Am J Trop Med Hyg 1991;45:743–751.
134 Badawi AF, Michael MS: Risk factors for hepatocellular carcinoma in Egypt: the role of hepatitis B viral infection and schistosomiasis. Anticancer Res 1999;191:4565–4569.
135 Mabrouk GM: Prevalence of hepatitis C infection and schistosomiasis in Egyptian patients with hepatocellular carcinoma. Dis Markers 1997;13:177–182.
136 Smith JH, Kamel IA, Elwi A, Von Lichtenberg F: A quantitative post mortem analysis of urinary schistosomiasis in Egypt. Am J Trop Med Hyg 1974;23:1054–1071.
137 Patil PS, Elem B: Schistosomiasis of the prostate and the seminal vesicles: observations in Zambia. J Trop Med Hyg 1988;91:245–248.
138 Godec CJ, Grunberger I, Carr GA: Simultaneous presence of schistosomiasis and advanced cancer in prostate. Urology 1992;39:547–549.
139 Cohen RJ, Edgar SG, Cooper K: Schistosomiasis and prostate cancer. Pathology 1995;27:115–116.
140 Ma TKF, Srigley JR: Adenocarcinoma of prostate and schistosomiasis: a rare association. Histopathology 1995;27:187–189.

141 Brasilio-de-Oliveira CA, Aquino A, Simon EF, Eyer-Silva WA: Concomitant prostatic schistosomiasis and adenocarcinoma: case report and review. BJID 2002;6:45–49.
142 Andrade ZA, Abreeu WN: Follicular lymphoma of the spleen with hepatosplenic schistosomiasis mansoni. Am J Trop Med Hyg 1971;20:237.
143 Edington GM, von Lichtenberg F, Nwabuebo I, et al: Pathologic effects of schistosomiasis in Ibadan, western state of Nigeria. I. Incidence and intensity of infection; distribution and severity of lesions. Am J Trop Med Hyg 1970;19:982.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 101–117

Relevant Oncogenic Viruses in Veterinary Medicine: Original Pathogens and Animal Models for Human Disease

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Abstract

Oncogenic viruses are important pathogens in farm and companion animals. These original pathogens are classified in various virus families, such as *Retroviridae, Papillomaviridae*, and *Herpesviridae*. Besides a role as pathogens for its original host, animal viruses serve as valuable models for viruses affecting humans, such as hepatitis B virus, and issues of immunity, therapy, but also basic pathophysiological mechanisms, can often only be addressed in those animal systems.

Oncogenic viruses are widespread among animals and can cause economic losses in animal husbandry. They are, however, of significantly lower importance in veterinary medicine compared to human medicine, because the intervention strategies in veterinary medicine are more stringent. In farm animals, oncogenic viruses – or at least diseases caused by those agents – are eliminated by vaccination such as bovine papillomavirus-induced warts in cattle, by genetic selection of resistant host strains, e.g. Marek's Disease in chicken, or preferably by eliminating infected animals, e.g. enzootic bovine leukosis or populations, e.g. avian leukosis. In companion animals, the situation is different. Besides the development of efficient vaccines in those virus systems in which virus-specific immunization is possible, such as feline leukemia virus,

Table 1. Oncogenic viruses of veterinary importance

Virus family/ subfamily	Virus genus	Virus	Host
Retroviridae/ orthoretrovirinae	α-retrovirus	avian leukosis virus (ALV)	poultry
		rous sarcoma virus (RSV)	poultry
		avian sarcoma virus (ASV)	poultry
	β-retrovirus	ovine pulmonary adenocarcinoma (OPA)	sheep
		jaagziekte sheep retrovirus (JSRV)	multi spec
		mouse mammary tumor virus	mouse
	γ-retrovirus	feline leukemia virus (FLV)	cat
		murine leukemia virus (MLV)	mouse
		viper retrovirus	reptile
	δ-retrovirus	bovine leukemia virus (BLV)	cattle
		human T cell lymphotropic viruses-1/-2 (HTLV-1/-2)	human
	ε-retrovirus	walleye dermal sarcoma virus (WDSV)	fish
	lentivirus	feline immunodeficiency virus (FIV)	cat
		human immunodeficiency viruses-1/-2 (HIV-1/-2)	human
Papillomaviridae	papillomavirus	bovine papillomaviruses-1–4 (BPV)	cattle, horse*
		canine oral papillomavirus (COPV)	dog
Herpesviridae/ α-herpesviridae	mardivirus	gallid herpes virus-2 (GHV-2)	poultry
		gallid herpes virus-3 (GHV-3)	poultry
Adenoviridae	mastadenovirus	canine adenovirus-1 (CAdV-1)	dog, hamster*

*Abortive infection, no infectious progeny virus produced.

papillomaviruses or Marek's disease virus, first attempts towards development of antiviral therapy are being developed and applied.

Besides the role of original veterinary pathogens, which will be discussed in some detail here, some oncogenic viruses and their natural animal hosts are very important as animal models for studying viruses of human relevance and their pathophysiological equivalents. Examples will be discussed in this review.

Viral Pathogens of Veterinary Importance

An overview of the oncogenic viruses of veterinary importance is provided in this section and categorized according to virus/host in table 1.

Retroviruses

Numerous retroviruses are known to infect a variety of animals. Some of them cause tumors, others interfere with the immune system and cause substantial diseases by, e.g. immunosuppressive effects. Most of the exogenous retroviruses are replication competent, but also replication incompetent viruses that acquired cellular oncogenes and lost some of their viral genes have been described and are known to cause disease. Their replication requires the action of gene products of helper viruses.

Poultry

Numerous benign and malignant neoplasms of chicken belong to the leukosis/sarcoma (L/S) group of diseases and are caused by the avian leukosis virus (ALV), classified among the α-retroviruses. These viruses are widespread in the chicken population worldwide. They cause significant economic losses either by (1) direct tumor-related mortality; (2) depressive effects on egg production, or (3) general performance due to subclinical infections or immunosuppression [1]. Mortality rates due to tumors can be as high as 20%, but are usually 1–2%. Eradication of these viruses on flock basis is possible, and an increasing number of flocks are ALV free.

ALVs are divided in the subgroups A, B, C, D, E and J based on their envelope genes. Subgroups F, G, H, and I represent endogenous ALVs of wild fowl-like birds. The most severe ALV-induced disease is lymphatic leukosis. Erythroblastosis/erythroid leukosis is less often seen. Lymphoid leukosis is a clonal malignancy of the bursal-dependent lymphoid system. The development of lymphoid leucosis can be enhanced by co-infection with Marek's disease virus serotype 2 (vaccine virus). Besides leukosis and erythroblastosis, various other tumors are associated with ALV infection, such as hemangioma, nephroblastoma, tumors of the connective tissue, or sarcoma [2].

Transmission of exogenous ALVs occurs both horizontally and vertically. At sexual maturity most chicken are infected. Chicken that were infected vertically are usually persistent virus carriers. Horizontal transmission requires direct contact of infected and non-infected birds. It occurs predominantly at hatch, when non-infected chicken encounter high amounts of virus, for example in the feces of persistently infected birds. Both routes of transmission are important to maintain the viruses in the flock.

The oncogenic mechanisms of ALVs are well understood, and retrovirus replication and transformation strategies have first been studied at the molecular level in Rous sarcoma virus by Temin and Rubin [3] in 1958. Acutely transforming ALVs carry viral oncongenes that were taken up from the host cell genome by genetic recombination. These oncogenes are derived from transcription

factors (jun, fos, myc, myb, ets, etc.), receptors (erbA, erbB, fms, src, etc.), are involved in cellular signal transduction (src, abl, ras, raf, etc.), or correspond to the platelet-derived growth factor (sis).

In oncogene-transducing viruses, tumor formation is fast and the chickens die within days. Birds infected with slowly transforming strains, which do not possess a viral oncogen, develop disease several weeks after infection, and tumorgenesis is based on the activation of cellular oncogens by long-terminal repeat (LTR) insertion.

Sheep

A β-retrovirus of sheep has a so-far unique oncogenic strategy. The Jaagsiekte (Africaans for 'hunting disease') virus (JSRV) induces tumors by expression of the viral structural Env protein [4, 5]. The disease occurs worldwide, and only Australia and New Zealand are considered to be free of JSRV. Eradication of the virus has been achieved in Iceland, at the cost of destroying the whole sheep population [6]. Sheep and goats are considered the only susceptible hosts, but JSRV protein and nucleic acid have been found in various species including humans [7, 8].

Natural transmission occurs through aerosols from infected animals. Incubation periods are reported to be 9 months to several years. Full-blown disease is generally seen after 1–3 years. Infection occurs predominantly in suckling lambs. The disease is characterized by neoplasia in the lung, particularly in the small bronchioli. So-called 'Clara cells' and pneumocytes type 2 are predominantly affected and proliferate. This causes obstructions of the bronchioli and alveoles along with an overproduction of surfactant factor [9]. Transformation is mediated by the Env protein and requires in sheep cells the binding to the hyaluronidase 2 virus receptor [4, 10, 11]. After transfection of various mammalian and avian cell lines, transformation occurred via the phosphatidylinositol 3-kinase/Akt and Akt/mTOR pathways [12]. In mice, infection with a recombinant adeno-associated virus AAV6 expressing the Env protein resulted in tumors in immunosuppressed mice. Immunocompetent mice, in contrast, were infected, but did not develop tumors. In this system, binding of the Env protein to the cellular receptor was not a prerequisite for transformation [13]. As transformation is affected by the host response, tumor development can be prevented by immune mechanisms. In sheep, however, the relative abundance of endogenous JSRV renders this species immunotolerant to exogenous JSRV – with fatal consequences.

Cat

The infection of cats with the feline leukemia virus (FeLV), a γ-retrovirus, is widely distributed in the cat populations worldwide. It causes numerous

disease complexes with lymphosarcoma being the most frequently observed. Besides that manifestation, immunosuppression ('feline AIDS'), a particular anemia is observed [14]. This viral disease is well studied. Between 1% and 10% of cats in various populations are infected with FeLV. One intriguing fact after natural FeLV infection is the high percentage of cats that will be able to eliminate the virus after a transient viremia [14]. This natural immunity can also be induced by vaccination with a variety of vaccines, either based on inactivated whole-virus preparations, subunit vaccines (p45 derived as the nonglycosylated form of gp70 after bacterial expression), or recombinant poxviruses carrying the core protein p24 and the envelope protein gp70 [15]. Cats that fail to eliminate the virus will succumb to disease within 3–4 years.

Three FeLV subgroups have been described whereby the FeLV subgroup A virus is the most important and most often isolated one. FeLV subgroup B appears to be produced by recombination with the *env* gene of endogenous FeLVs, whereas FeLV subgroup C is generated form FeLV subgroup A viruses by mutations in the env gene. The subgroup viruses are defined by interference tests in tissue cultures; there is no specific antigenic difference between the individual subgroups. High amounts of p25 protein are released into the blood of persistently infected animals and diagnosis of FeLV is possible by detection of this antigen in blood using commercially available tests.

The oncogenic potential of FeLV is based on its ability to establish replication-defective viruses, so-called 'feline sarcoma viruses', which are recombinants of defective FeLVs and cellular genes. Various oncogens have been found in those viruses, namely c-myc, c-abl, c-fes, c-fgr, c-fms, c-kit, and c-sis.

Natural transmission between cats is via saliva and, most importantly, by vertical transmission to the kittens from a viremic queen. Elimination of the virus in domestic cat populations is easily achieved by regular testing of young cats, vaccination of noninfected cats and isolation of infected cats.

Cattle

Another mechanism of tumor induction is used by the δ-retrovirus enzootic bovine leukosis virus (EBL; also named bovine leukosis virus (BLV)). This virus is also distributed worldwide. Depending on the country and particularly on the herd in question, infection rates can be as high as 100%. It is estimated that, for example in the USA, about 30% of all dairy cattle is infected [16]. EBL/BLV has been eradicated in some countries by applying strict control measures. Those include the regular screening of the cattle populations and removal of virus-positive animals from the herd. EBL/BLV predominantly infects cattle but experimental infection of sheep has also been described. Transmission occurs mainly by transmitting virus-infected lymphocytes by biting insects, but most importantly also iatrogenically by using contaminated

needles. Vertical transmission is possible, but of less importance. The hallmark of disease is B cell leukemia and the formation of lymphoma in various tissues. Approximately 30% of infected animals will develop lymphocytosis and less than 5% tumors [17]. The incubation period can be up to several years. However, due to the strong immunogenicity, serological diagnosis can already be made a few weeks after infection.

The pathogenesis of EBL/BLV infection is only partially understood. The EBL/BLV genome encodes a regulatory protein called Tax [18, 19] within the so-called X region. Tax increases the rate of viral transcription by activating the promoter in the LTR sequence. Detection of only the X region of the viral genome in some neoplastic B cells suggests that tax gene expression may be the only viral genetic component required to cause transformation. Persistent lymphocytosis is due to increased cell proliferation rather than delayed apoptosis of infected B lymphocytes [20]. The role of Tax or other viral gene products in cell proliferation or oncogenicity is poorly defined.

Fish

Although not strictly of veterinary importance, a retrovirus infection of fish is discussed here. Walleye dermal sarcoma, a tumorous disease found in the freshwater perch *Stizostedion vitreum* in North America, is caused by the walleye dermal sarcoma virus (WDSV), an ε-retrovirus [21]. The tumors develop during the cold season but in most cases, regression of tumors is observed during spring and summer period [22]. Most likely, an antiviral immunity is induced, as fish that experienced tumor regression do not appear to develop tumors again. Natural WDSV infection occurs in the spring via direct contact at spawning when high numbers of walleyes, many of them tumor-positive, congregate on shoals or in streams.

Experimentally, walleyes can be infected by the topical, oral and intramuscular route [22]. WDSV is a complex retrovirus with three open reading frames (ORF A-C) encoding auxillary proteins. ORF C downstream the 5′-LTR codes for a protein that can induce apoptosis, and may therefore contribute to tumor regression [23]. ORFs A and B appear to be gene duplicates immediately downstream of the 3′-LTR and encode a cyclin homolog, rv-cyclin [24]. This protein interacts with cellular cyclin-dependent kinases (CDKs) and may cause cell proliferation by enhanced expression of some cellular genes [25]. WDSV cyclin has been shown to interact with CDK8, and therefore the mechanism of transformation may be similar to that of Kaposi sarcoma herpesvirus (KSHV/HHV-8) in Kaposi sarcoma [26]. Cyclin-induced oncogenesis is another retroviral transforming strategy that is, so far, unique to ε-retroviruses.

Herpesviruses

Herpesviruses can induce tumors in humans, such as KSHV/HHV-8, or as by Epstein-Barr virus (EBV). Only one oncogenic herpesvirus, Marek's disease virus of poultry, is of veterinary importance. The gallid herpesvirus 2 (GHV-2) or Marek's disease virus (MDV) has recently been classified in the Mardivirus genus of the α-herpesviridae subfamily. Other members of this group are MDV-2, which is now named GHV-3, and the herpesvirus of turkeys (HVT). Only GHV-2, but not GHV-3 or HVT, can cause Marek's disease [27]. GHV-2 induces T cell lymphoma by an yet unknown mechanism. Recently, by use of bacterial artificial chromosomes containing the full length genomes of several GHV-2 strains with different oncogenic potential, a 7.7-kb region within the internal long repeat has been mapped to determine – or at least modulate – GHV-2 oncogenicity [28]. Interestingly, GHV-2 vaccination of ALV-infected birds enhances ALV-mediated disease, and recombination of ALV provirus and GHV-2 has been observed [29]. MDV is widespread and vaccination with MDV-2 or HVT is routinely performed in most poultry flocks. The virus replicates systemically upon first infection and establishes latency in activated CD4-positive T cells [30]. In infected birds, cell-free MDV is only produced in feather follicles, and its transmission is through inhalation of virus shed from the follicles [31].

Papillomaviruses

Papillomaviruses are among the best-studied oncogenic viruses and are important pathogens for humans and animals [for review, see 32]. Natural papillomavirus disease of veterinary importance occurs in horses as equine sarcoid, in cattle as bovine papillomatosis, and in dogs as canine oral papillomatosis. Other animals can also harbor papillomaviruses but these are of less clinical importance. Antiviral immunity and tumor regression are common in most of the papillomavirus-infected hosts, except in equine sarcoid or intestinal papillomatosis of cattle.

Mechanisms of Papillomavirus-Induced Oncogenesis

Papillomavirus oncogenicity has been well studied in cell culture and mouse models, predominantly using human papillomaviruses and bovine papillomavirus-1 (BPV-1). The nonstructural proteins E6, E7 and E5 play a major role in the transformation of cells and the generation of tumors. In BPV, E5 appears to be most crucial for oncogenicity.

E7 binding to the retinoblastoma protein (pRb) abolishes the function of the latter, by preventing its phosphorylation by cyclin-dependent kinases (CDKs). Phosphorylated Rb protein, however, controls as a suppressor the activity of a transcriptional regulators (E2F), which in turn stimulates transcription

of various genes required for DNA synthesis, thus driving the cell into the S-phase of the cell cycle.

The papillomavirus E6 protein is also involved in cell transformation. It binds to the p53 protein via the E6-associated protein, a ubiquitin ligase tagging p53 to a proteasome-mediated degradation. p53 is a central repressor molecule for transcription of certain cellular genes. In response of cellular damage, it becomes activated and may trigger either cell cycle arrest at G1/S, or apoptosis.

The E5 protein in BPV is known to bind the Platelet-Derived Growth Factor-β Receptor (PDGF-R), which, through a signaling cascade, results in cell growth [33]. It also binds 16k ductin, a protein that downregulates the gap junction intercellular communication (GJIC) and thereby facilitates detachment from the neighbor cell. Binding to 16k ductin also results in the retention of MHC I molecules within the Golgi apparatus, reducing the number of MHC I on the cell surface [34]. Besides that, E5 activates numerous kinases such as cyclin A-CDK2, thus interfering with cell-cycle control [35].

Cattle

In cattle, 6 BPV have been described, with types 3–6 being restricted to cattle, whereas types 1 and 2 can also infect horses. Each BPV type is associated with a distinct disease. BPV-1 and -2 cause cutaneous fibropapilloma, BPV-3 cutaneous papilloma, BPV-4 intestinal tract papilloma, BPV-5 teat fibropapilloma, and BPV-6 teat papilloma. All types are widespread. Cofactors associated with malignant papillomvirus disease are reported for BPV-1, BPV-2, and BPV-4. Ingestion of bracken fern is a major contributing factor for BPV-associated invasive carcinoma of the alimentary tract (BPV-4) or the bladder (BPV-2) in Europe and South America [32]. Besides the cases of the invasive carcinomas, BPV infections are generally self-limiting and tumors regress based on an immunological antiviral response. Vaccines prepared from warts of infected animals are used for prophylaxis in some herds.

Horse

In horses, equine papillomavirus (EPV) cause benign warts that generally regress after few months. Horses are also susceptible to infection with BPV-1 and -2, however these infections can cause an invasive fibrosarcoma-like skin tumor, commonly known as equine sarcoid. These tumors do not metastasize nor regress and are invasive and often therapy-resistant. In those tumors, the BPV protein E5 is constantly expressed. There is some controversy whether sequence variation of E5 sequences amplified from equine or bovine tumors are of etiological importance [36–38].

Dog

At least two types of canine oral papillomavirus (COPV) can induce benign cutaneous tumors in dogs, preferably at the skin and oral mucosa and, although less frequently, at the genital mucosae or conjunctivae. Based on histology, cutaneous squamous papilloma, cutaneous inverted papilloma, and canine pigmented epidermal nevus can be distinguished [39]. In a kennel with susceptible dogs, more than 25% of dogs can be affected. The disease is generally self-limiting, and there is a long-lasting antiviral immunity that renders the dog immune to re-infection. Canine papillomatosis is therefore predominantly seen in young dogs. The nature of immunity in dogs is not well understood, but preparations of warts, inactivated with formalin and administered parenterally, appear to protect dogs from disease if given three weeks before experimental inoculation [40]. Similar results have been obtained with virus-like particles or virus capsomeres produced by recombinant expression of the COPV L1 major structural protein [41, 42]. Cell-mediated immunity against nonstructural proteins such as E1, E2, and E7 is also believed to confer protection [43, 44].

Tumor Viruses of Animals as Models of Human Oncogenesis

Conceptual Work and Pioneering Studies in Oncology

Many basic concepts in molecular oncology in general and, in particular, on the role of viruses in the etiology of cancer have been derived from natural and experimental animal models. This is due to intrinsic similarities and also differences in virus-induced oncogenesis in man and animals and especially due to the experimental accessibility in animals. In addition, experimentally induced or naturally occurring tumors in animals serve often as surrogate models for prevention and therapy. In this part of the review we will discuss both aspects.

Tumor Induction by Viral and Cell-Derived Oncogenes and Additional Oncogenic Strategies of Retroviruses

The first virus known to consistently induce tumors in animals is the Rous sarcoma virus (RSV), an avian retrovirus of chicken [45]. The induction of sarcoma in chicken is an acute process taking only days to weeks for full tumor development [for review, see 46]. Subsequent studies revealed that RSV did not only induce tumors in chicken but also transformed cells from other species under cell culture conditions [47]. The subsequent molecular analysis of the basic features revealed that RSV encodes, in addition to the classical retroviral genes required for replication and particle formation, also the so-called viral v-src oncogene [48]. The v-src gene encodes a tyrosine kinase that is constitutively

active thus inducing different growth-related cellular pathways [49]. Subsequently, a highly related cellular counterpart, the cellular c-src gene was identified and characterized as an important component of intracellular signaling regulating cell growth [50]. The concept emerged that RSV had, while retaining replication competence, taken up the cellular proto-oncogene c-src. Genetic alterations, relative to its cellular progenitor, of the virally transduced v-src as well as mutations or gene amplification of the cell-encoded c-src increased the oncogenic potential of this cytoplasmic tyrosine kinase by subverting it from a growth-promoting protein to a protein inducing uncontrolled cell cycle progression. The changes inducing constitutive activity are primarily related to the loss of the autoregulatory C-terminus [49]. Thus, the constitutively active v-src induces uncontrolled replication of cells leading to rapid tumor development in RSV-infected chicken.

Similar situations where different cellular proto-oncogenes had been taken up by diverse retroviruses were subsequently identified. Yet, in all these cases the proto-oncogene transducing feline, avian, and murine retroviruses have lost replication competence since, as a consequence of proto-oncogene uptake, essential coding sequences were deleted. In general, the cell-derived proto-oncogenes carry mutations or are expressed as viral-cellular fusion proteins. The cellular genes taken up by these acutely transducing retroviruses have different functions in growth-regulating pathways; for overview see [46]. The sis oncogene corresponds to the platelet-derived growth factor (PDGF), many are derived from transcription factors (jun, fos, myc, myb, ets, etc.), receptors (erbA, erbB, fms, etc.), or are involved in cellular signal transduction (src, abl, ras, raf, etc.).

The defective oncogene-transducing retroviruses depend on the presence of genetically intact parental virus in order to replicate and to be transmitted to new hosts or host cells. Identification of oncogene-transducing retroviruses led also the way to engineer recombinant retroviruses as viral vectors for gene delivery techniques. In addition, uptake of cellular genes by viruses was subsequently also identified in other viruses, especially the large DNA viruses as mentioned above.

Another form of retrovirus-induced oncogenesis is the less frequent and non-acute outcome of the integration of the retroviral genome into the host cell DNA, the insertional mutagenesis [51]. Essential cellular tumor suppressor genes are either inactivated – or their expression suppressed – or the expression of cellular proto-oncogenes is dysregulated as consequence of retroviral genome integration. Such events have been mostly studied in different avian and murine sarcoma and leukemia viruses (MLV) and in the mouse mammary tumor virus (MMTV) [46]. Retrovirus genome integration has not only contributed to the general understanding of insertional mutagenesis and maintenance of genome integrity but was also a tool to investigate the function of cellular genes [52]. Oncogenic insertional mutagenesis of human immunodeficiency virus

(HIV) and human T cell leukemia virus-I (HTLV-I) has not been described in humans so far. However, insertion of MLV-derived retroviral vectors used to combat human X-linked severe combined immunodeficiency (SCID) turned out to induce leukemia in these patients [53]. Since retroviral vectors are potent tools in ongoing and future human gene therapy trials, the risk of insertional mutagenesis and the chances of targeting retrovirus integration to sites where most likely no cancer can be induced are currently under intense scientific investigation [54, 55].

Finally, inflammation- and infection-induced immunosuppression are now considered as important cofactors during cancer development and a clear link between HIV-induced immunosuppression and cancer development is firmly established in AIDS patients [56].

Importance of Cofactors in Virus-Induced Oncogenesis

At present, there is no tumor virus known to be capable of acutely inducing tumors in humans within weeks or days [46]. Similarly, only few animal viruses, for instance the avian src-oncogene transducing RSV, FeLV, and JSRV, can – as the single causative agent – induce acute cancer development in immunocompetent animals [46]. Therefore, these acutely transforming viruses are the exception among the known tumor viruses.

The concept that viral and nonviral oncogenesis is a multi-step process was in part deduced from studies using different virus-induced malignancies, for instance the cottontail rabbit papillomavirus (CRPV). In this experimental system, CRPV-infected rabbits develop, dependent on the genetic background of the rabbit species analyzed, papilloma that may develop into carcinoma of the skin [57]. The incubation time for tumor development was significantly reduced when CRPV-infected areas of the skin with developing warts were additionally treated with chemical carcinogens, for instance with tar or methylcholanthrene [58]. Subsequent studies showed that the activated form of the cellular proto-oncogene ras cooperated enhanced CRPV-induced carcinogenesis, confirming the multi-step process of cancer development [59].

Abortive, Nonlytic Infections Can Promote Cancer Development

Considering the power of animal experiments and comparison of human disease with similar settings in animals indicate that these systems are not always comparable and intrinsic differences remain. With the background that permissiveness of a host towards replication of a given virus is restricted by a plethora of genetic factors [60], inoculation of a human virus into animals does not necessarily reflect the natural infection of human beings. An excellent example directly related to viral oncology is tumor induction by different human adenoviruses when inoculated into hamsters. In this experimental setting, a clear oncogenic potential

could be attributed for instance to human adenovirus 12 [61]. Subsequently, the early E1A and E1B genes of different human adenoviruses were shown to immortalize or fully transform cells in culture and have a clear oncogenic potential in rodents [reviewed in 62]. Although oncogenic in vitro and in a heterologous host, epidemiological studies in humans fail to show any evidence that human adenoviruses have a corresponding oncogenic potential in their natural host; discussed in [63]. These data corroborated with studies in infected cells lead to the concept that limited, nonproductive, abortive replication of different DNA viruses bears an oncogenic potential. Mechanistically, this conclusion can be explained by two complementing and not mutually exclusive explanations.

Firstly, abortive, non-productive infections are often not lytic since the proteins related to virus formation, including fusogenic surface proteins and those related to the release of the progeny virions, often associated to apoptosis or cell lysis, are not expressed [46]. Thus, the limited gene expression in these settings allow the survival of the infected cell which would have been otherwise killed during a productive infection, for instance by adenoviruses. Abortive, nonproductive infections are also characteristic for tumor induction by CRPV as mentioned (see above). In herpesviruses, latency of the virus is a prerequisite for tumor development [64].

Secondly, DNA viruses depend on cellular factors required for genome replication and these factors, for instance the essential dNTP pools, are only fully accessible when the host cell undergoes DNA replication. Therefore, different DNA viruses including adenoviruses have developed or acquired proteins expressed early during infection. These early proteins drive the newly infected cell into DNA replication and cell division. In general, these early virus-encoded proteins display the above-discussed oncogenic properties [46, 65].

In the case of oncogenic adenoviruses, the E1A-derived proteins – besides other functions – interact and functionally inactivate the retinoblastoma protein Rb, which is a negative regulator of cell cycle progression and its E1A-mediated inactivation leads to cellular immortalization [62]. The adenovirus E1B protein is required for full transformation by inhibiting p53-mediated surveillance of genome stability and induction of apoptosis of virus-infected cells. These oncogenic functions are often also encoded and utilized by other DNA tumorviruses, examples are the papillomavirus E6 and E7 proteins or the large T antigens of papovaviruses [65].

Animal Models for Human Malignancies

Animal models for human disease are an essential and crucial cornerstone of past and present biomedical research. Often, inoculation of human viruses

was used to study their biology and to establish novel therapeutic approaches. For instance, serial passages of human viruses in laboratory animals – or animal cells attenuated their infectivity – even allows the usage of the attenuated virus as a vaccine in humans [66]. Inoculation of patient-derived human viruses into animals is routinely used for pathogen identification and characterization and, in the past, this technique was even used for virus quantification.

In modern oncology, animal models of human malignancies are of primary importance for basic research. Here, especially inbred mice and transgenic mice strains are frequently used to analyze the oncogenic potential of chemicals, defined human genes and their viral counterparts. In addition, SCID mice are often used for therapeutic studies using implanted malignant human cells.

Human hepatitis B and C viruses (HBV and HCV) induce acute and chronic liver disease in man. In a substantial portion of chronically infected patients, hepatocellular carcinoma are induced after long incubation times. Intense research on both viruses revealed that persistence of the virus with ongoing replication and the resulting chronic inflammation induced by either virus is the cause for neoplastic development [67, 68]. Thus for both viruses the major – or single – oncogenic mechanism is not related to a viral oncogene or the dysregulation of cellular genes involved in cell growth and survival. In contrast and mechanistically related to *Helicobacter pylori*-induced gastric cancer [69] were chronic inflammation induced and accompanied by the release of diverse cytokines, which defines the pathway to malignancy [67, 68].

Part of this novel view on virus-induced cancer derives from pioneering studies on the replication and cancer induction by the Woodchuck hepatitis B virus (WHBV) in its natural host, the American woodchuck [for review, see 70]. Studies of WHBV in woodchucks were used to characterize viral replication since adequate cell culture systems for HBV – allowing analysis of all steps of viral replication – have not yet been available until the recent development of a primary hepatocyte-based system [71]. In addition, the WHBV-system allowed defining (WHBV) oncogenesis as a process directly related to chronic infection with liver-specific inflammation similar to that seen in HBV-infected humans [72, 73]. Subsequently, the WHBV model was used to establish novel diagnostic procedures and technologies. [for references, see 73]. Finally, nucleotide analogs and novel gene therapy strategies have been used in woodchucks in order to establish corresponding therapies for HBV infections and hepatocellular cancer in man [73].

Adult T cell leukemia/lymphoma (ATLL) induced by HTLV-I is also responsible for inapparent infections and the nonmalignant, but also fatal HTLV-I-associated myelopathy (HAM). Apparently, different replication pathways can induce significantly different disease [74]. It is worth mentioning that HTLV-I is almost undistinguishable from the Simian T cell leukemia virus-I (STLV-I) [75]. The extremely high genetic relatedness has even led to the suggestion to call

these viruses collectively 'primate T cell leukemia viruses' (PTLV). This appears even more justified by the recent observation that transmission of PTLV between simians and humans occurs frequently in areas where man and primate cohabitate [75]. The apathogenic persistence in chronic infections and the T cell leukemia induced in different simians together with markers of STLV replication in naturally infected macaques almost fully parallels the situation seen in HTLV-I-infected carriers thus making the STLV-infected simian a very valuable animal model for the human malignancy seen in distinct geographic regions [76].

Zoonosis and Host-Species Exchange-Associated Oncogenesis

As described above, the human adenovirus type 12 (Ad 12) does not cause malignancies in man, however, upon inoculation into a heterologous host species, Ad 12 induces cancer. In contrast to this scenario, BPV which is oncogenic in cattle, retains its oncogenic potential in the heterologous host, the horse. In horses, sarcoids develop which are characterized by a restricted BPV replication. Therefore, the change of the host species resulting in restricted replication in the new environment can be accompanied by the development of virus-induced cancer. However, the recent zoonotic events, for instance HIV, severe acute respiratory syndrome (SARS) coronavirus, emerging Influenza viruses, Ebola and Marburg virus, and others resulted in all cases in productive, sometimes even lytic infections that were not directly related to cancer. Presently, the only known exception is the occurrence of different malignancies associated with the HIV-mediated immunodeficiency.

Acknowledgements

We wish to thank Harald zur Hausen and Lutz Gissmann for valuable discussions and long-standing and continuous support.

References

1 Rup BJ, Hoelzer JD, Bose HR: Helper viruses associated with avian acute leukaemia viruses inhibit the cellular immune response. Virology 1982;116:61–71.
2 Fadly AM, Payne LN: Leukosis/Sarcom group; in Saif YM (ed): Diseases of Poultry. Iowa, Iowa State Press, 2003, pp 465–516.
3 Temin HM, Rubin H: Characteristics of an assay for Rous sarcoma virus and Rous sarcoma cells in tissue culture. Virology 1958;6:669–688.
4 Liu SL, Miller AD: Transformation of Madin-Darby canine kidney (MDCK) epithelial cells by sheep retrovirus envelope proteins. J Virol 2004;79:927–933.
5 Maeda N, Palmarini M, Murgia C, Fan H: Direct transformation of rodent fibroblasts by jaagsiekte sheep retrovirus DNA. Proc Natl Acad Sci USA 2001;98:4449–4454.

6 Sigurdsson B: Adenomatosis of sheep's lungs: experimental transmission. Arch Ges Virusforsch 1958;8:51–58.
7 De las Heras M, Barsky SH, Hasleton P, Wagner M, Larson E, Egan J, Ortin A, Gimenez-Mas JA, Palmarini M, Sharp JM: Evidence for a protein related immunologically to the jaagsiekte sheep retrovirus in some human lung tumours. Eur Respir J 2000;16:330–332.
8 Hiat KM, Highsmith WE: Lack of DNA evidence for jaagsiekte retrovirus in human bronchioalveolar carcinaoma. Human Pathol 2002;33:680.
9 Perk K, Hod I, Nobel TA: Pulmonary adenomatosis of sheep (jaagsiekte). I. Ultrastructure of the tumour. J Nat Cancer Inst 1971;46:525–537.
10 Allen TE, Sherrill KJ, Crispell SM, Perrott MR, Carlson JO, DeMartini JC: The jaagsiekte sheep retrovirus envelope gene induces transformation of the avian fibroblast cell line DF-1 but does not require a conserved SH2 binding domain. J Gen Virol 2002;83:2733–2742.
11 Palmarini M, Maeda N, Murgia C, De-Fraja C, Hofacre A, Fan H: A phosphatidylinositol 3-kinase docking site in the cytoplasmic tail of the Jaagsiekte sheep retrovirus transmembrane protein is essential for envelope-induced transformation of NIH 3T3 cells. J Virol 2001;75:11002–11009.
12 Maeda N, Fu W, Ortin A, de las Heras M, Fan H: Roles of the Ras-MEK-mitogen-activated protein kinase and phosphatidylinositol 3-kinase-Akt-mTOR pathways in Jaagsiekte sheep retrovirus-induced transformation of rodent fibroblast and epithelial cell lines. J Virol 2005;79:4440–4450.
13 Wootton SK, Halbert CL, Miller AD: Sheep retrovirus structural protein induces lung tumours. Nature 2005;434:904–907.
14 Cotter SM : Feline viral neoplasia; in Greene CE (ed): Infectious Diseases of the Dog and Cat. Philadelphia, Saunders, 1998, pp 71–84.
15 Poulet H, Brunet S, Boularand C, Guiot AL, Leroy V, Tartaglia J, Minke J, Audonnet JC, Desmettre P: Efficacy of a canarypox virus-vectored vaccine against feline leukaemia. Vet Rec 2003;153:141–145.
16 Baumgartener LE, Olson C, Miller JM, van der Maaten MJ: Survey for antibodies to leukemia (C-type) virus in cattle. J Am Vet Assoc 1975;166:249–258.
17 Jacobs RM: Bovine lymphoma; in Olsen RG, Krakowka S, Blakeslee JR (eds): Comparative Pathology of Viral Disease. Boca Raton, CRC Press, 1985, vol II.
18 McGirr KM, Buehring GC: Tax and rex sequences of bovine leukaemia virus from globally diverse isolates: rex amino acid sequence more variable than tax. J Vet Med 2005;B52:8–16.
19 Rice NR, Simek SL, Dubois GC, Showalter SD, Gilden RV, Stephens RM: Expression of the bovine leukemia virus X region in virus-infected cells. J Virol 1987;61:1577–1585.
20 Debacq C, Asquith B, Kerkhofs P, Portetelle D, Burny A, Kettmann R, Willems L: Increased cell proliferation, but not reduced cell death, induces lymphocytosis in bovine leukemia virus-infected sheep. Proc Natl Acad Sci USA 2002;99:10048–10053.
21 LaPierre LA, Holzschu DH, Bowser PR, Casey JW: Sequence and transcriptional analyses of the fish retroviruses walleye epidermal hyperplasia virus types 1 and 2: evidence for a gene duplication. J Virol 1999;73:9393–9403.
22 Bowser PR, Wooster GA, Earmnest-Koons K: Effects of fish age and challenge route in experimental transmission of walleye dermal sarcoma in walleyes by cell-free tumour filtrates. J Aquat Anim Health 1997;9:274–278.
23 Nudson WA, Rovnak J, Buechner M, Quackenbush SL: Walley dermal sarcoma virus is targeted to the mitochondria. J Gen Virol 2003;84:375–381.
24 LaPierre LA, Holzschu DH, Wooster GA, Bowser PR, Casey JW: Two closely related but distinct retroviruses are associated with walleye discrete epidermal hyperplasia. J Virol 1998;72:3484–3490.
25 Holzschu D, LaPierre LA, Lairmore MD: Comparative pathogenesis of epsilonretroviruses. J Virol 2003;77:12385–12391.
26 Rovnak J, Quackenbush SL: Walley dermal sarcoma virus cyclin interacts with components of the mediator complex and the RNA polymerase II holoenzyme. J Virol 2002;76:8031–8039.
27 Osterrieder N, Vautherot JF: The genome content of Marek's disease-like viruses; in Davidson F, Nair V (eds): Marek's disease – An Evolving Problem. London, Elsevier, 2004, pp 17–31.
28 Petherbridge L, Brown AC, Baigent SJ, Howes K, Sacco MA, Osterrieder N, Nair VK: Oncogenicicty of virulent Marek's disease virus cloned as bacterial artificial chromosomes. J Virol 2004;78:13376–13380.

29 Jones D, Brunovskis P, Witter RL, Kung HJ: Retroviral insertional activation in a herpesvirus: transcriptional activation of US genes by an integrated long terminal repeat in a Marek's disease virus clone. J Virol 1996;70:2460–2467.
30 Schat KA, Chen CL, Calnek BW, Char D: Transformation of T-lymphocyte subsets by Marek's disease herpesvirus. J Virol 1991;65:1408–1413.
31 Calnek BW, Alexander AM, Kahn DE: Feather follicle epithelium: a source of enveloped and infectious cell-free herpesvirus from Marek's disease. Avian Dis 1970;14:219–233.
32 Campo MS, Grindlay GJ, O'Neil BW, Chandrachud LM, McGarvie GM, Jarrett WFH: Prophylactic and therapeutic vaccination against a mucaosal papillomavirus. J Gen Virol 1993;74: 945–953.
33 DiMaio D, Mattoon D: Mechanisms of cell transformation by papillomavirus E5 proteins. Oncogene 2001;20:7866–7873.
34 Chambers G, Ellsmore VA, O'Brien PM, Reid SW, Love S, Campo MS, Nasir L: Association of bovine papillomavirus with the equine sarcoid. J Gen Virol 2003;84:1055–1062.
35 Venuti A, Campo MS: The E5 protein of Papillomaviruses; in McCance DJ (ed): Progress in Medical Virology: Papillomaviruses. Iowa, Iowa State Press, 2002, pp 141–162.
36 Carr EA, Theon AP, Madewell BR, Griffey SM, Hitchcock E: Bovine papillomavirus DNA in neoplastic and nonneoplastic tissues obtained from horses with and without sarcoids in the Western United States. Am J Vet Res 2001;62:741–744.
37 Chambers G, Ellsmore VA, O'Brien PM, Reid SW, Love S, Campo MS, Nasir L: Sequence variants of bovine papillomavirus E5 detected in equine sarcoids. Virus Res 2003;96:141–145.
38 Reid SWJ, Smith KT, Jarrett WHF: Detection, cloning, and characterisation of papillomaviral DNA present in sarcoid tumours of *Equus asinus*. Vet Rec 1994;135:430–432.
39 Nicholls PK, Stanley MA: Canine papillomavirus – a centenary review. J Comp Path 1999;120: 219–233.
40 Chambers VC, Evans CA, Weiser RS: Canine oral papillomatosis. II. Immunologic aspects of the disease. Cancer Res 1960;20:1083–1093.
41 Suzich JA, Ghim SJ, Palmer-Hill FJ, White WI, Tamura JK, Bell JA, Newsome JA, Jenson AB, Schlegel R: Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. Proc Natl Acad Sci USA 1995;92:11553–11557.
42 Yuan H, Estes PA, Chen Y, Newsome J, Olcese VA, Garcea RL, Schlegel R: Immunisation with a pentameric L1 fusion protein protects against papillomavirus infection. J Virol 2001;75:7848–7853.
43 Campo MS: Animal models of papillomavirus pathogenesis. Virus Res 2002;89:249–261.
44 Selvakumar R, Borenstein LA, Lin YL, Ahmed R, Wettstein FO: Immunisation with non-structural proteins E1 and E2 of cottontail rabbit papillomavirus stimulates regression of virus-induced papillomas. J Virol 1995;69:602–605.
45 Rous PA: A transmissible avian neoplasm: sarcoma of the common fowl. J Exp Med 1910;12: 696–705.
46 Nevins JR: Cell transformation by viruses; in Knipe DM, Howley PM (eds): Fields Virology, ed 4. Philadelphia, Lippincott Williams & Wilkins, 2001, pp 1–245.
47 Manaker RA, Groupe V: Discrete foci of altered chicken embryo cells associated with Rous sarcoma virus in tissue culture. Virology 1956;2:838–840.
48 Dhar R, Ellis RW, Shih TY, Oroszlan S, Shapiro B, Maizel J, Lowy D, Scolnick E: Nucleotide sequence of the p21 transforming protein of Harvey murine sarcoma virus. Science 1982;217: 934–936.
49 Takeya T, Hanafusa H: Nucleotide sequence of c-src. Cell 1983;32:881–890.
50 Sefton B M, Hunter T: From c-src to v-src, or the case of the missing C terminus. Cancer Surv 1986;5:159–171.
51 Brown, PO: Integration; in Coffin JM, Hughes SH, Varmus HE (eds): Retrovirology. New York, Cold Spring Harbor Laboratory Press, 1997, p 161.
52 Lai Z, Han I, Park M, Brady RO: Design of an HIV-1 lentiviral-based gene-trap vector to detect developmentally regulated genes in mammalian cells. Proc Natl Acad Sci USA 2002;99:3651–3656.
53 von Kalle C, Fehse B, Layh-Schmitt G, Schmitt M, Kelly P, Baum C: Stem cell clonality and genotoxicity in hematopoietic cells: gene activation side effects should be avoidable. Semin Hematol 2004;41:303–318.

54 Bushman D: Targeting survival: integration site selection by retroviruses and LTR retrotransposons. Cell 2003;115:135–138.
55 Sandmeyer S: Integration by design. Proc Natl Acad Sci USA 2003;100:5586–5585.
56 Feigal EG: AIDS-associated malignancies: research perspectives. Biochim Biophys Acta 1999;1423:C1–C9.
57 Kidd JG, Rous P: A transplantable rabbit carcinoma originating in a virus-induced papilloma and containing the virus in a masked or altered form. J Exp Med 1940;71:813–837.
58 Rous P, Kidd JG: The carcinogenic effect of a virus upon tarred skin. Science 1936;83:468–469.
59 Peng X, Griffith JW, Lang CM: Reinitiated expression of EJras transgene in targeted epidermal cells of transgenic rabbits by cottontail rabbit papillomavirus infection. Cancer Lett 2001;171: 193–200.
60 Bieniasz PD: Intrinsic immunity: a front-line defense against viral attack. Nat Immunol 2004;5:1109–1115.
61 Trentin, JJ, Yabe Y, Taylor G: The quest for human cancer viruses. Science 1962;137:835–849.
62 Endter C, Dobner T: Cell transformation by human adenoviruses. Curr Top Microbiol Immunol 2004;273:163–214.
63 Pagano JS, Blaser M, Buendia MA, Damania B, Khalili K, Raab-Traub N, Roizman B: Infectious agents and cancer: criteria for a causal relation. Semin Cancer Biol 2004;14:453–471.
64 Young LS, Rickinson AB: Epstein-Barr virus: 40 years on. Nat Rev Cancer 2004;4:757–768.
65 zur Hausen H: Oncogenic DNA viruses. Oncogene 2001;20:7820–7823.
66 Murphy BR, Chanock RM: Immunisation against viral diseases; in Knipe DM, Howley PM (eds): Fields Virology, ed 4. Philadelphia, Lippincott Williams & Wilkins, 2001, p 435.
67 Hollinger FB, Liang TJ: Hepatitis B virus; in Knipe DM, Howley PM (eds): Fields Virology, ed 4. Philadelphia, Lippincott Williams and Wilkins, 2001, p 2971.
68 Major ME, Rehermann B, Feinstone SM: Hepatitis C viruses; in Knipe DM, Howley PM (eds): Fields Virology, ed 4. Philadelphia, Lippincott Williams & Wilkins, 2001, p 1127.
69 Blanchard TG, Drakes ML, Czinn SJ: Helicobacter infection: pathogenesis. Curr Opin Gastroenterol 2004;20:10–15.
70 Tennant BC: Animal models of hepadnavirus-associated hepatocellular carcinoma. Clin Liv Dis 2004;5:43–68.
71 Gripon P, Rumin S, Urban S, Le Seyec J, Glaise D, Cannie I, Guyomard C, Lucas J, Trepo C, Guguen-Guillouzo C: Infection of a human hepatoma cell line by hepatitis B virus. Proc Natl Acad Sci USA 2002;99:15655–15660.
72 Popper H, Roth L, Purcell RH, Tennant BC, Gerin JL: Hepatocarcinogenicity of the woodchuck hepatitis virus. Proc Natl Acad Sci USA 1987;84:866–870.
73 Tennant BC, Toshkov IA, Peek SF, Jacob JR, Menne S, Hornbuckle WE, Schinazi RD, Kobra BE, Cote PJ, Gerin JL: Hepatocellular carcinoma in the woodchuck model of hepatitis B virus infection. Gasteroenterology 2004;127:283–293.
74 Green PL, Chen ISY: Human T-cell leukaemia virus types 1 and 2; in Knipe DM, Howley PM (eds): Fields Virology, ed 4. Philadelphia, Lippincott Williams & Wilkins, 2001, p 1941.
75 Vandamme AM, Salemi M, Desmyter J: The simian origins of the pathogenic human T-cell lymphotropic virus type I. Trends Microbiol 1998;6:477–483.
76 Gabet AS, Gessain A, Wattel E: High simian T-cell leukemia virus type 1 proviral loads combined with genetic stability as a result of cell-associated provirus replication in naturally infected, asymptomatic monkeys. Int J Cancer 2003;107:74–83.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 118–137

The Inflammatory Tumor Microenvironment and Its Impact on Cancer Development

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Abstract

The role of the immune system during cancer development is complex involving extensive reciprocal interactions between genetically altered cells, adaptive and innate immune cells, their soluble mediators and structural components present in the neoplastic microenvironment. Each stage of cancer development is regulated uniquely by the immune system; whereas full activation of adaptive immune cells at the tumor stage may result in eradication of malignant cells, chronic activation of innate immune cells at sites of premalignant growth may actually enhance tumor development. In addition, the balance between desirable anti-tumor immune responses and undesirable pro-tumor chronic inflammatory responses largely depends on the context in which a malignancy is developing. The following chapter focuses on the inflammatory components and processes engaged during cancer development and the impact of the inflammatory microenvironment.

Tumor Micro-Environment

Cancer is a progressive disease typically requiring initial mutations in proliferating cells that are necessary but not sufficient for full neoplastic progression [1]. The cellular composition of (pre-) malignant lesions represents a heterogeneous population of cells, including genetically altered cells, as well as a diverse array of stromal cells that are activated in and/or recruited to the neoplastic microenvironment, including fibroblasts, endothelial, and mural cells forming the blood vasculature and lymphatics, and innate and adaptive immune

cells, all of which co-exist in a dynamic extracellular matrix (ECM) that together modulate cancer development [2–6].

In healthy homeostatic tissue, the three-dimensional organization and function of stroma is in balanced equilibrium. Fibroblasts, the predominant cells present in stroma, are responsible for production, deposition and remodelling of most ECM components, including collagens and structural proteoglycans, as well as for secretion of various classes of proteolytic enzymes, their inhibitors and multiple growth factors that regulate cell proliferation, survival and morphology [7, 8]. Stroma forms a structural scaffold, is crucial in crosstalk between cells, regulates presence and distribution of nutrition and waste and is a scaffold for few resident inflammatory cells such as mast cells, macrophages, immature dendritic cells that monitor the surroundings for invading pathogens [4]. The blood vasculature embedded in stroma of healthy tissue consists of quiescent mature blood vessels surrounded by uninterrupted basement membranes [9].

The functional and structural characteristics of stroma undergo dramatic changes in the presence of a developing neoplasm [4]. The quantity and composition of stroma varies considerably per tumor type and from tumor to tumor. Fibroblasts typically exhibit a higher proliferative index in the neoplastic microenvironment, as compared to fibroblasts in homeostatic tissues, and often express α-smooth muscle actin and are commonly surrounded by dense accumulated fibrillar collagens [7, 8]. Initially, fibroblasts were thought to be passive participants in neoplastic progression. However, recent data indicate they exert an active role and can promote cancer development [3, 10, 11]. As will be discussed below in more detail, stroma of (pre-) malignant lesions is frequently characterized by infiltration and activation of immune cells, in particular macrophages, mast cells, granulocytes and lymphocytes [12–18]. Reactive tumor stroma is further characterized by the presence of abnormal blood vessels that are tortuous, chaotic in organization and intrinsically unstable and leaky [19–21], by increased interstitial fluid pressure [22], and by alterations in the lymphatic architecture [23]. Growth factors and proteases produced by neoplastic cells, activated fibroblasts and inflammatory cells mediate remodeling of structural proteins of ECM and basement membranes, e.g. collagen and fibrin, resulting in disruption of tissue homeostasis and allowing cell migration and invasion [24, 25]. Moreover, tissue remodeling mediated by neoplastic and stromal cell-derived proteolytic enzymes, such as matrix metalloproteinases (MMPs), results in release and/or activation of a variety of factors with distinct biological activities that are normally sequestered within the ECM [24–27]. Thus reciprocal communication between responding ‘normal’ cells, their mediators, structural components of ECM and genetically altered neoplastic cells regulate many aspects of (pre-) malignant progression [2–6].

The Inflammatory Tumor Micro-Environment

Acute versus Chronic Inflammation

The immune system is composed of many different cells and mediators that interact in a complex and dynamic manner to ensure protection against all foreign pathogens possibly encountered during a life-time, while simultaneously maintaining tolerance towards self-antigens [28, 29]. Based on the specificity of antigen recognition and on the timing of activation, the immune system can be divided into two subsets, the adaptive immune system and the innate immune system. In order to provide optimal protection against invading pathogens, both subsets of the system are intimately linked [30, 31]. The innate immune system, also referred to as the first line of immune defense against infection, is composed of macrophages, granulocytes (neutrophils, basophils, and eosinophils), dendritic cells (DCs), mast cells, natural killer cells (NK cells) and soluble complement components. It is relatively non-specific and not intrinsically affected by prior contact with infectious agents. Cells of the innate immune system express germline encoded pattern-recognition Toll-like receptors (TLRs) with which they recognize conserved molecular patterns found on microorganisms, but not in self-tissue, e.g. lipopolysaccharide (LPS), lipoteichoic acid (LTA), mannans, unmethylated CpG DNA motifs and glycan [29, 32]. Acute inflammation in response to invading pathogens or tissue injury is a multi-step process that begins with activation of resident innate immune cells and activation of the complement cascade, resulting in release of pre-formed and newly synthesized pro-inflammatory mediators including cytokines, proteases, and membrane-perforating agents, followed by recruitment and activation of other inflammatory cells from the periphery and nonspecific lysis and phagocytosis of foreign cells and bacteria [29, 33–35]. Acute activation of the innate immune system not only forms the first line of immune defense against invading pathogens, but is also necessary for efficient activation of the more specific adaptive immune system [29, 36, 37].

The adaptive immune system – also called the acquired immune system – is comprised of B lymphocytes, CD4+ (helper) and CD8+ (cytotoxic) T lymphocytes and distinguishes itself from the innate immune system by its antigen-specificity and memory formation. B and T lymphocytes express unique, highly diverse, somatically generated antigen-specific receptors, B cell receptors (BCRs) and T cell receptors (TCRs), that are formed during their development by random rearrangement of the Immunoglobulin (Ig) and TCR gene segments, respectively [38, 39]. Thus, tremendously diverse B and T lymphocyte repertoires are generated that provide a flexible and broader range of responses to pathogens as compared to innate immune cells [38, 39]. B lymphocytes exert their effector function by secreting antibodies with the same antigen specificity

as the BCR [40, 41]. Fully activated T lymphocytes contribute to acute immune responses by cytokine production, B cell help (CD4+ T cells) and cytotoxic killing of cells expressing the antigen of specificity (CD8+ T cells). The kinetics of primary adaptive immune responses are slower than innate immune responses, because clonal expansion of antigen-specific lymphocytes is required to obtain a sufficient number of antigen-specific T and/or B lymphocytes [40, 42]. However, upon initial activation, a subset of lymphocytes differentiates into long-lived memory cells, thus forming heightened states of immune reactivity to later contact with the same antigen [42]. Acute inflammation therefore triggers a cascade of immunological events, starting with activation of innate immune responses followed by activation of antigen-specific adaptive immune responses. Such acute inflammatory responses result in removal of invading organisms and aberrant cells, resolution of inflammation and subsequent re-establishment of tissue integrity and homeostasis.

Under certain circumstances however, tissue-damaging chronic inflammatory responses develop, the underlying mechanisms of which are still poorly understood. Many chronic inflammatory states are associated with pathogens that are able to evade clearance, resulting in persistent activation of the immune system. For instance, *Helicobacter pylori* persists in the gastric epithelium and causes chronic gastritis in essentially all infected hosts, and infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is linked with chronic hepatitis [43–46]. In addition, unresolved inflammation can be a consequence of autoimmunity, exposure to toxins, e.g. asbestos and smoking, ongoing chemical or physical irritation, e.g. acid reflux disease or UV exposure, or exposure to certain dietary factors or hormones. Several studies have reported a link between increased susceptibility to chronic inflammation and specific subsets of genes and genetic polymorphisms in cytokine and signal transduction networks [47–50]. For instance, in human chronic inflammatory disorders such as asthma and diabetes, a cytokine imbalance favoring 'pro-humoral immunity' Th2 immune responses instead of 'pro-cellular immunity' Th1 immune responses may play a key role in increased susceptibility to disease [47, 48]. In addition, a dysregulated balance of regulatory T cells – a subset of T cells that suppresses specific T cell responses – might lead to chronic inflammatory conditions [51–54]. Continuous exposure of tissue to activated immune cells and their soluble mediators results in excessive tissue remodeling, loss of tissue architecture as a consequence of tissue destruction, and under certain circumstances, enhances risk for development neoplastic tissue states.

Clinical Association between Chronic Inflammation and Cancer

Infiltration of leukocytes into human and experimental (pre-) malignant lesions is a common and well-described phenomenon, although its significance

has long been a matter of debate. Over the last decade, it has become clear that increased presence of immune cells in neoplastic tissue is not merely a secondary consequence of tissue changes occurring during cancer development, but frequently a regulatory mechanism playing a central role in disease pathogenesis. Clinical observations indicate that the immune system plays a dual role in tumor development and progression; an immune balance favoring anti-tumor T lymphocyte responses has been correlated with improved disease outcome [55–61], whereas a balance favoring pro-tumor chronic innate immune cell activation in the tumor microenvironment often correlates with poor clinical outcome [12–18, 62–64]. As an example, infiltration of T lymphocytes into multiple types of human malignancies has been reported to be associated with improved clinical outcome [55–61]. Based on the idea that genetically altered cells can be recognizable targets for the adaptive immune system, substantial effort is being put into development of immunotherapeutic approaches that elicit anti-tumor adaptive immune responses [65–71]. Although multiple groups have been successful in developing vaccines that elicit tumor-specific CD8+ T cell responses in cancer patients, in only few patients such anti-tumor adaptive immune responses have led to actual stabilization or regression of the tumor [72]. One of the explanations for poor clinical therapeutic benefit of anti-cancer vaccination strategies is that the tumor microenvironment often does not favor efficient anti-tumor adaptive immune responses, but rather subverts the immune system to facilitate neoplastic progression [73]. The unique characteristics of neoplastic tissues, e.g. dysregulated cytokine production and altered oxygen levels, favor chronic inflammation. The presence of chronically activated innate immune cells, such as myeloid suppressor cells, and presence of regulatory T cells may subsequently further inhibit desirable anti-tumor adaptive immune responses both systemically as well as locally [74–78]. Consistent with this concept, it has been demonstrated that many human malignancies contain innate immune cells, and importantly, the abundance of innate immune cells, in particular macrophages and mast cells, correlates with angiogenesis and poor clinical outcome [12–18, 62–64, 79]. For example, clinical prognosis of patients with renal cell carcinoma containing high numbers of tumor-associated macrophages (TAMs) is poor [79]. Likewise, the number of mast cells and macrophages infiltrating pancreatic ductal adenocarcinomas directly correlates with intratumoral microvessel counts and lymph node metastases [18]. Gene-expression profiling of biopsies from human follicular lymphomas revealed that molecular features of nonmalignant, tumor infiltrating immune cells could be used to predict survival, again pointing out an important interplay between the host immune response and cancer development [80].

A clinical link between inflammation and cancer provided by epidemiological studies indicates that a broad spectrum of chronic inflammatory disorders,

chronic infections and chronic mechanical and chemical irritations predispose to cancer development [46, 81–84]. The best established of malignancies associated with chronic inflammation is colon carcinogenesis arising in patients with inflammatory bowel disease [84, 85]. In addition, hereditary and sporadic forms of pancreatitis predispose patients to development of pancreatic cancer and prostatitis has been associated with prostate cancer [86–89]. Moreover, 15% of all human cancers have been associated with chronic inflammation caused by infectious agents [46, 90, 91]. Many of these pathogens are known to genetically alter infected cells via activation of proto-oncogenes or integrating and inducing viral oncogene expression [46]. However, some infectious agents, e.g., *Helicobacter pylori*, HBV and HCV, indirectly promote carcinogenesis via induction of chronic inflammatory states that by continuous production of reactive oxygen species, growth factors, pro-inflammatory cytokines and extracellular proteases regulate tissue remodelling, proliferation and angiogenesis [46, 92]. Experimental and clinical studies have established that chronic gastritis induced by the gram-negative bacterium *Helicobacter pylori* predisposes to gastric cancer, the second most common cause of cancer-related mortality word-wide [93–97] and HBV and HCV are known to cause hepatocellular carcinomas (HCC) by inducing chronic inflammation in the liver [43, 92, 98, 99]. Examples of mechanical and chemical irritation-induced (pre-) malignant lesions are mesothelioma (asbestosis) [100], Barrett's esophagus and esophageal adenocarcinoma (chronic reflux of gastric acid) [101, 102], and gallbladder cancer (gallstones) [103].

Additional compelling clinical evidence for the importance of inflammation during neoplastic progression comes from studies showing a reduced risk of cancer among long-term users of nonsteroidal anti-inflammatory drugs (NSAID), e.g. aspirin and selective cyclooxygenase-2 (COX-2) inhibitors [104–110]. These studies have revealed that long-term usage of these compounds reduces colon cancer risk by about 50%, gastric and esophageal cancer risk by approximately 40% and breast cancer by approximately 20% [104–106, 108, 109, 111–113]. Thus, clinical data indicate a clear relationship between inflammation and development of cancer, and suggest that elucidation of the mechanisms by which the immune system participates in neoplasia formation may contribute to development of novel therapeutic approaches against human cancer.

Experimental Studies Linking Inflammation and Cancer

The last decade, much effort has been extended towards understanding the complex mechanisms underlying causal links between chronic inflammation and

pre-malignant progression, tumor growth and metastasis formation [6, 73, 108, 114–122]. The availability of a growing number of de novo carcinogenesis mouse models has allowed us to take the first steps towards understanding recruitment pathways of inflammatory cells into (pre-) malignant microenvironments, their modulation of such micro-environments, and their contribution to cancer progression. Using a transgenic mouse model of multistage epithelial carcinogenesis where the early region genes of the human papillomavirus type 16 (HPV16) are expressed as transgenes under control of the human keratin 14 (K14) promotor [123, 124], e.g. K14-HPV16 mice, we have reported that transgenic oncogene expression alone is not sufficient for complete cancer development. Instead, additional signals provided by immune cells are required for elaboration of the malignant state [114, 115, 122]. Genetic elimination of mast cells is sufficient to attenuate neoplastic progression in K14-HPV16 mice [115]. Recently, we found that genetic deletion of the complete adaptive immune system in K14-HPV16 mice resulted in failure to initiate chronic inflammation during pre-malignancy, resulting in attenuated pre-malignant progression and reduced carcinoma incidence [122]. Transfer of B lymphocytes or serum isolated from K14-HPV16 mice into adaptive immune-deficient/K14-HPV16 mice restored chronic inflammation in neoplastic skin as well as hallmarks of pre-malignant progression [122], indicating that B lymphocytes play a crucial role in the onset of chronic inflammation associated with pre-malignant progression, thus potentiating neoplastic cascades downstream of oncogene expression.

Tumor-promoting roles for innate immune cells downstream of oncogene expression have also been described in other experimental tumor models [125, 126]. Lin and colleagues studied the role of colony stimulating factor-1 (CSF-1) during mammary carcinoma development by comparing transgenic mice susceptible to *de novo* development of mammary carcinomas (PyMT mice) with CSF-1 deficient PyMT mice (PyMT/CSF-1$^{op/op}$) [125]. Whereas absence of CSF-1 during early neoplastic development was without apparent consequence, development of late-stage invasive carcinomas and pulmonary metastases was significantly attenuated in PyMT/CSF-1$^{op/op}$ mice, and correlated with a failure to recruit mature macrophages into neoplastic tissue in the absence of CSF-1 [125]. Macrophage recruitment was restored by transgenic CSF-1 expression in mammary epithelium in PyMT/CSF-1$^{op/op}$ mice, as was characteristic for primary and metastatic tumor development [125]. Likewise, secretion of the chemokine CXCL-8 by xenografted tumor cells is required for RasV12-dependent tumor-associated inflammation, angiogenesis and tumor growth [126]. Depletion of granulocytes attenuated angiogenesis of RasV12-expressing tumors, suggesting that ability of neoplastic cells to recruit inflammatory cells facilitates tumor outgrowth [126]. The biological effect of tumor infiltrating innate immune cells, however, depends on the local levels of pro-inflammatory cytokines, and

numbers of innate immune cells in the neoplastic microenvironment [127]. Experimental studies using tumor cells expressing the chemokine MCP-1 have revealed that low concentrations of MCP-1 elicited modest macrophage recruitment and enhanced angiogenesis and tumor growth in melanoma xenograft models [127]. In contrast, high levels of MCP-1 expression resulted in more extensive macrophage infiltration and more robust angiogenic responses and enhanced tumor growth, but eventually also in tumor regression [127]. Thus, tumor infiltrating innate immune cells can play dual roles during neoplasia development. Consistent with this, some studies have described a beneficial effect of macrophage infiltration in human cancer [61, 128]. For instance, survival of patients with colorectal cancer containing high numbers of tumor-infiltrating macrophages and CD8+ T cells was reported to be better than those with low numbers of macrophages and CD8+ T cells [61]. The exact cellular and/or molecular mechanisms underlying these contradictory correlations between macrophage infiltration and tumor progression remain to be elucidated. However, it is conceivable that the dual role of macrophages owes to their activation and differentiation status and cytokine milieu present in tumor microenvironments [129, 130].

It has recently been reported that immature myeloid suppressor GR1+CD11b+ cells accumulate in peripheral blood of cancer patients [75, 131] as well as in tumors and lymphoid organs of tumor-bearing animals [76, 132]. Myeloid suppressor cells were initially identified as cells that indirectly enhance tumorigenesis by suppressing tumor-specific adaptive immune responses [75, 76, 133]; however, it recently became clear that myeloid suppressor cells can also directly promote growth of experimental tumors by contributing to angiogenesis at the tumor site [132]. In conclusion, these studies support the concept that inflammation is not just a bystander effect of the tissue changes that occur during neoplasia formation, but rather promotes neoplastic events downstream of oncogene expression.

Inflammatory Cell-Mediated Modulation of Neoplastic Progression

As chronic inflammation is a complex and dynamic process with many different cells and soluble mediators involved, it is no surprise that multiple mechanisms have been identified via which inflammatory states can promote cancer development. The modulatory effects of immune cells on cancer development can be divided into direct effects on neoplastic cells, e.g. induction of DNA damage or paracrine regulation of signal transduction pathways inside neoplastic cells, and indirect effects on neoplastic growth, e.g. activation of angiogenesis, tissue remodeling, and suppression of anti-tumor adaptive immune responses (fig. 1).

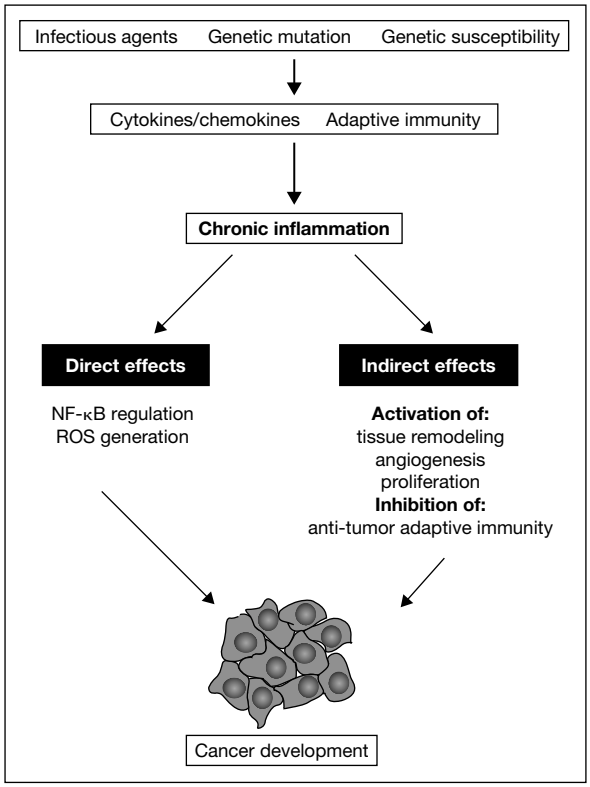

Fig. 1. Chronic inflammation and cancer development. Initiation of cancer development, e.g. by infectious agents and/or genetic mutations, often results in chronic inflammatory states that are regulated by the presence of cytokines, chemokines and components of the adaptive immune system, e.g. antibodies. Chronically activated immune cells promote cancer development via direct and indirect mechanisms. Inflammatory cells are capable of modulating expression of genes within neoplastic cells, such as NF-κB, that favor proliferation and survival in a paracrine fashion. In addition, chronic inflammation results in production of free radicals that can cause DNA damage. Immune cells indirectly modulate cancer development by production of proteolytic enzymes, cytokines, chemokines and pro-angiogenic mediator, and upregulation of COX-2. These inflammatory cell-derived mediators induce tissue remodeling, proliferation and activation of angiogenesis, thus creating a microenvironment that is permissive for primary tumor development and secondary metastasis formation. Myeloid suppressor cells and regulatory T cells present in tumor-bearing hosts suppress anti-tumor adaptive immune responses, and thus contribute to immune evasion.

Direct Effect on Neoplastic Cells

Nuclear factor-κB (NF-κB), a pro-inflammatory transcription factor that regulates cell proliferation, survival, and growth arrest, has been implicated as

a link between inflammation and cancer in two independent mouse models of inflammation-associated cancer [116, 119, 134]. Using a mouse model of inflammation-associated hepatocellular carcinogenesis, Pikarsky et al. [119] reported that inflammatory cells present in the neoplastic microenvironment control hepatocyte NF-κB activation via production of tumor necrosis factor-α (TNF-α). Greten et al. [116] reported that specific deletion of IKKβ – a key intermediary of NF-κB – in myeloid cells decreased tumor growth in a mouse model of colitis-associated cancer through reduced production of tumor-promoting paracrine factors [116]. These elegant studies indicate that inflammatory cells are capable of modulating expression of genes within neoplastic cells that favor proliferation and survival in a paracrine fashion.

Another mechanism by which chronic inflammation directly influences cancer development is by generating reactive oxygen and nitrogen species that can cause DNA damage in proliferating cells [135, 136]. Repeated tissue damage and regeneration of tissue in the presence of highly reactive nitrogen and oxygen species released from inflammatory cells can result in permanent genomic alterations, e.g. point mutations, deletions or rearrangements that further neoplastic programs of growth [136].

Indirect Effect on Neoplastic Cells

Besides directly influencing proliferation and survival of neoplastic cells, infiltrating inflammatory cells also indirectly regulate tumorigenesis. The two most prominent changes that occur in the neoplastic microenvironment besides recruitment and activation of inflammatory cells are tissue remodeling and activation of angiogenesis. These processes are crucial for cancer development. Breakdown of ECM molecules allows expansion of neoplastic tissues and increases bioavailability of growth factors and cytokines, and formation of new blood vessels is critical as expansion of tissue requires supply of oxygen and nutrition. Both activation of angiogenesis and tissue remodeling are modulated by inflammatory cells present in the neoplastic microenvironment [6, 73].

Innate immune cells produce numerous soluble growth factors, cytokines and chemokines as well as various types of extracellular or cell-associated proteinases, such as MMPs, that have pro-inflammatory, pro-angiogenic and pro-tissue remodeling capacities. As an example, several experimental cancer models have revealed that inflammatory cells functionally contribute to tumorigenesis via secretion of MMPs [25]. To date, about 26 human secreted or transmembrane MMPs have been identified [137–139]. MMPs collectively possess enzymatic activity against virtually all ECM components, and each MMP family member has distinct, but often overlapping, substrate specificities [24, 25, 139, 140]. MMPs regulate tumor development by remodeling ECM components as well as non-ECM substrates such as cytokines, growth factors, cell-cell

and cell-matrix adhesion molecules, and thus contribute to angiogenesis, inflammation and proliferation [24, 25]. MMP-9 deficiency in K14-HPV16 mice resulted in reduced skin carcinogenesis [114], and characteristics of neoplastic development were restored in MMP-9-deficient K14-HPV16 mice by reconstitution with wild type bone marrow-derived cells [114]. Likewise, in a transgenic mouse model of pancreatic islet cell cancer where MMP-9 could only be detected in infiltrating inflammatory cells and not in neoplastic cells, MMP-9 was reported to contribute to the angiogenic switch [141]. Growth, vascularization and macrophage infiltration of xenografted MMP-9 expressing human ovarian cancer cells were clearly reduced in MMP-9-deficient nude mice [142]. Reconstitution with MMP-9 expressing spleen cells resulted in increased angiogenesis and tumorigenicity [142]. A study by Hiratsuka et al. [143] revealed that a primary tumor can specifically direct MMP-9 expression in macrophages and endothelial cells in distant pre-metastatic lung. In line with these findings, patients with distant tumors displayed significantly elevated levels of MMP-9 in lung tissue as compared to those from tumor-free patients [143]. Thus, these studies indicate that inflammatory cells contribute to carcinogenesis by creating an environment that is permissive for primary tumor development and secondary metastasis formation.

Another mechanism by which inflammatory cells regulate angiogenesis and consequently enhance tumorigenesis is production of pro-angiogenic mediators such as vascular endothelial growth factor-A (VEGF-A) [18, 144]. In addition, Gr+CD11b+ myeloid immune suppressor cells can differentiate into endothelial cells and directly incorporate into tumor endothelium in tumor-bearing animals [132]. Other inflammatory cell-derived soluble mediators that are known to modulate cancer development are serine- and cysteine-proteases, membrane-perforating agents, TNF-α, interleukins and interferons [114–116, 119, 132, 143, 145, 146]. Together, host-derived soluble mediators are known to evoke innate immune cell recruitment and/or activation, tissue remodeling and angiogenesis, and together, create a microenvironment favoring cell proliferation, genomic instability and expansion of cell populations into ectopic tissue microenvironments, culminating in malignant conversion and cancer development.

Inflammation is also known to up-regulate COX-2, a key enzyme in the synthesis of prostaglandins from arachidonic acid, and there is accumulating evidence that COX-2 up-regulation plays an important role in neoplasia formation [109, 147, 148]. In general, COX-2 is not expressed in quiescent tissues. However, its expression is induced in many human cancers [149–151]. Chronic presence of COX-2 in (pre-) malignant microenvironments results in production of prostaglandins that are known to mediate many effects, including but not limited to promotion of proliferation while reducing apoptosis, activation of

angiogenesis, induction of pro-inflammatory chemokines, and suppression of immune surveillance mechanisms [148]. This is underscored by the observation that transgenic overexpression of COX-2 is sufficient to drive hyperplasia and carcinomas in several tissues, e.g. mammary glands and the urinary bladder [152–154] and enhances chemical-induced skin carcinogenesis [155]. Genetic deletion or selective inhibition of COX-2 has been reported to decrease development of several human and experimental cancers [113, 156, 157]. Great efforts have been employed to develop selective COX-2 inhibitors, and the therapeutic safety and efficacy of such selective COX-2 inhibitors in cancer prevention have been and are being tested in clinical trials [113, 158, 159].

In addition, chronic inflammation promotes cancer development via suppression of anti-tumor adaptive immune responses, allowing tumor escape from host immune surveillance. A subset of innate immune cells, e.g. myeloid suppressor GR+CD11b+ cells of myeloid macrophage/dendritic cell lineage, accumulates in tumors and lymphoid organs [75, 76, 133]. These myeloid suppressor cells actively inhibit anti-tumor adaptive immunity via induction of T lymphocyte dysfunction by direct cell-cell contact and by production of immunosuppressive factors [75, 133, 160, 161]. In addition, malignant lesions attract regulatory T cells that are known to suppress effector functions of cytotoxic T cells [78, 162]. In an elegant study by Curiel and colleagues, it was revealed that tumor micro-environmental macrophages derived from patients with ovarian cancer produce CCL22, a chemokine that was shown to mediate trafficking of regulatory T cells to the tumor [78]. These regulatory T cells in ovarian cancer patients suppressed tumor-specific T cell immunity, and their presence correlated with reduced survival [78]. Thus in the presence of a growing neoplasm, the balance between innate and adaptive immunity is often disrupted in favor of tumor outgrowth.

In conclusion, there are many means by which inflammation contributes to carcinogenesis. Which mechanism is involved in a particular situation will likely depend on the stage of neoplastic progression, the tumor type, the genetic make-up and immune status of the patient and previous exposure to therapies.

Concluding Remarks and Perspectives

Compelling clinical and experimental studies implicate adaptive and/or innate immune cells as critical regulators of cancer development. In this chapter, we have provided an overview of the many facets of inflammation associated with neoplastic programming of tissues. Each stage of cancer development is regulated uniquely by the immune system, whereas full activation of adaptive immune cells at the tumor stage may result in eradication of malignant cells,

chronic activation of innate immune cells at the site of a pre-malignant lesion during earlier stages may actually facilitate tumor progression. Likewise, different types of malignancies are differentially effected by the presence or activation state of immune cells. Progress in understanding the dual roles of the adaptive and innate immune system during neoplastic progression will set the stage for development of therapeutic approaches that prevent inflammation-induced cancer development and/or activate effective anti-tumor immune responses. As stated above, the efficacy of NSAIDs and selective COX-2 inhibitors [108, 109] in cancer prevention argues for anti-inflammatory therapy at the earliest stages of neoplastic progression. A deeper understanding of the underlying mechanisms regulating inflammation at the neoplastic site, and a more thorough translation of the data obtained so far using experimental mouse models into the human situation will help in designing therapeutics that can change the balance from a chronic inflammatory state into an acute inflammatory response.

Acknowledgements

We thank members of the Coussens laboratory for insightful discussions. Karin E. de Visser is supported by a fellowship from the Dutch Cancer Society. Lisa M. Coussens is supported by grants from the National Institutes of Health, Center for Proteolytic Pathways, the National Cancer Institute and the Department of Army, BCCOE.

References

1 Hanahan D, Weinberg RA: The hallmarks of cancer. Cell 2000;100:57–70.
2 Bissell MJ, Radisky D: Putting tumors in context. Nat Rev Cancer 2001;1:46–54.
3 Tlsty TD: Stromal cells can contribute oncogenic signals. Semin Cancer Biol 2001;11:97–104.
4 Mueller MM, Fusenig NE: Friends or foes – bipolar effects of the tumor stroma in cancer. Nat Rev Cancer 2004;4:839–849.
5 Kiaris H, Chatzistamou I, Kalofoutis C, Koutselini H, Piperi C, Kalofoutis A: Tumor-stroma interactions in carcinogenesis: basic aspects and perspectives. Mol Cell Biochem 2004;261:117–122.
6 Coussens LM, Werb Z: Inflammation and cancer. Nature 2002;420:860–867.
7 Sappino AP, Schurch W, Gabbiani G: Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulations. Lab Invest 1990;63:144–161.
8 Tlsty TD, Hein PW: Know thy neighbour: stromal cells can contribute oncogenic signals. Curr Opin Genet Dev 2001;11:54–59.
9 Kalluri R: Basement membranes: structure, assembly and role in tumor angiogenesis. Nat Rev Cancer 2003;3:422–433.
10 Allinen M, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J, Huang H, Porter D, Hu M, Chin L, Richardson A, Schnitt S, Sellers WR, Polyak K: Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell 2004;6:17–32.
11 De Wever O, Mareel M: Role of tissue stroma in cancer cell invasion. J Pathol 2003;200:429–447.
12 Duncan LM, Richards LA, Mihm MC: Increased mast cell density in invasive melanoma. J Cutan Pathol 1998;25:11–15.

13 Takanami I, Takeuchi K, Naruke M: Mast cell density is associated with angiogenesis and poor prognosis in pulmonary adenocarcinoma. Cancer 2000;88:2686–2692.
14 Tomita M, Matsuzaki Y, Onitsuka T: Effect of mast cells on tumor angiogenesis in lung cancer. Ann Thorac Surg 2000;69:1686–1690.
15 Toth-Jakatics R, Jimi S, Takebayashi S, Kawamoto N: Cutaneous malignant melanoma: correlation between neovascularisation and peritumor accumulation of mast cells overexpressing vascular endothelial growth factor. Hum Pathol 2000;31:955–960.
16 Imada A, Shijubo N, Kojima H, Abe S: Mast cells correlate with angiogenesis and poor outcome in stage I lung adenocarcinoma. Eur Respir J 2000;15:1087–1093.
17 Benitez-Bribiesca L, Wong A, Utrera D, Castellanos E: The role of mast cell tryptase in neoangiogenesis of premalignant and malignant lesions of the uterine cervix. J Histochem Cytochem 2001;49:1061–1062.
18 Esposito I, Menicagli M, Funel N, Bergmann F, Boggi U, Mosca F, Bevilacqua G, Campani D: Inflammatory cells contribute to the generation of an angiogenic phenotype in pancreatic ductal adenocarcinoma. J Clin Pathol 2004;57:630–636.
19 McDonald DM, Baluk P: Significance of blood vessel leakiness in cancer. Cancer Res 2002;62: 5381–5385.
20 Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, Jain RK, McDonald DM: Openings between defective endothelial cells explain tumor vessel leakiness. Am J Pathol 2000;156:1363–1380.
21 Bergers G, Benjamin LE: Angiogenesis: tumorigenesis and the angiogenic switch. Nat Rev Cancer 2003;3:401–410.
22 Heldin CH, Rubin K, Pietras K, Ostman A: High interstitial fluid pressure – an obstacle in cancer therapy. Nat Rev Cancer 2004;4:806–813.
23 Achen MG, McColl BK, Stacker SA: Focus on lymphangiogenesis in tumor metastasis. Cancer Cell 2005;7:121–127.
24 Egeblad M, Werb Z: New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002;2:161–174.
25 de Visser KE, Coussens LM: Inflammation and matrix metalloproteinases: implications for cancer development; in Morgan DW, Forssmann UJ, Nakada MT (eds): Cancer and Inflammation. Basel, Birkhauser Verlag, 2004, pp 71–97.
26 Bergers G, Coussens LM: Extrinsic regulators of epithelial tumor progression: metalloproteinases. Curr Opin Genet Dev 2000;10:120–127.
27 McCawley LJ, Matrisian LM: Matrix metalloproteinases: they're not just for matrix anymore! Curr Opin Cell Biol 2001;13:534–540.
28 Janeway CA Jr: How the immune system works to protect the host from infection: a personal view. Proc Natl Acad Sci USA 2001;98:7461–7468.
29 Janeway CA Jr, Medzhitov R: Innate immune recognition. Annu Rev Immunol 2002;20:197–216.
30 Murphy JE, Robert C, Kupper TS: Interleukin-1 and cutaneous inflammation: a crucial link between innate and acquired immunity. J Invest Dermatol 2000;114:602–608.
31 Luster AD: The role of chemokines in linking innate and adaptive immunity. Curr Opin Immunol 2002;14:129–135.
32 Akira S, Takeda K: Toll-like receptor signalling. Nat Rev Immunol 2004;4:499–511.
33 Zlotnik A, Yoshie O: Chemokines: a new classification system and their role in immunity. Immunity 2000;12:121–127.
34 Walport MJ: Complement. First of two parts. N Engl J Med 2001;344:1058–1066.
35 Walport MJ: Complement. Second of two parts. N Engl J Med 2001;344:1140–1144.
36 Carroll MC: The complement system in regulation of adaptive immunity. Nat Immunol 2004;5: 981–986.
37 Belardelli F, Ferrantini M: Cytokines as a link between innate and adaptive antitumor immunity. Trends Immunol 2002;23:201–208.
38 Goldrath AW, Bevan MJ: Selecting and maintaining a diverse T-cell repertoire. Nature 1999;402: 255–262.
39 Tonegawa S: Somatic generation of antibody diversity. Nature 1983;302:575–581.
40 McHeyzer-Williams MG: B cells as effectors. Curr Opin Immunol 2003;15:354–361.

41 Janeway CA, Travers P, Walport M, Shlomchik M: Immunobiology, ed 5. New York and London, Garland Publishing, 2001.
42 Sprent J, Surh CD: T cell memory. Annu Rev Immunol 2002;20:551–579.
43 Brechot C: Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: Old and new paradigms. Gastroenterology 2004;127:S56–S61.
44 Ernst P: Review article: the role of inflammation in the pathogenesis of gastric cancer. Aliment Pharmacol Ther 1999;13(suppl 1):13–18.
45 De Luca A, Iaquinto G: *Helicobacter pylori* and gastric diseases: a dangerous association. Cancer Lett 2004;213:1–10.
46 Pagano JS, Blaser M, Buendia MA, Damania B, Khalili K, Raab-Traub N, Roizman B: Infectious agents and cancer: criteria for a causal relation. Semin Cancer Biol 2004;14:453–471.
47 Urcelay E, Santiago JL, de la Calle H, Martinez A, Figueredo A, Fernandez-Arquero M, de la Concha EG: Interleukin-10 polymorphisms in Spanish type 1 diabetes patients. Genes Immun 2004;5:306–309.
48 Renauld JC: New insights into the role of cytokines in asthma. J Clin Pathol 2001;54:577–589.
49 Podolsky DK: Inflammatory bowel disease. N Engl J Med 2002;347:417–429.
50 Ohyauchi M, Imatani A, Yonechi M, Asano N, Miura A, Iijima K, Koike T, Sekine H, Ohara S, Shimosegawa T: The polymorphism interleukin 8 -251 A/T influences the susceptibility of *Helicobacter pylori* related gastric diseases in the Japanese population. Gut 2005;54:330–335.
51 Belkaid Y, Rouse BT: Natural regulatory T cells in infectious disease. Nat Immunol 2005;6: 353–360.
52 Hori S, Carvalho TL, Demengeot J: CD25+CD4+ regulatory T cells suppress CD4+ T cell-mediated pulmonary hyperinflammation driven by Pneumocystis carinii in immunodeficient mice. Eur J Immunol 2002;32:1282–1291.
53 Aseffa A, Gumy A, Launois P, MacDonald HR, Louis JA, Tacchini-Cottier F: The early IL-4 response to *Leishmania* major and the resulting Th2 cell maturation steering progressive disease in BALB/c mice are subject to the control of regulatory CD4+CD25+ T cells. J Immunol 2002;169:3232–3241.
54 Maloy KJ, Salaun L, Cahill R, Dougan G, Saunders NJ, Powrie F: CD4+CD25+ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. J Exp Med 2003; 197:111–119.
55 Chiba T, Ohtani H, Mizoi T, Naito Y, Sato E, Nagura H, Ohuchi A, Ohuchi K, Shiiba K, Kurokawa Y, Satomi S: Intraepithelial CD8+ T-cell-count becomes a prognostic factor after a longer follow-up period in human colorectal carcinoma: possible association with suppression of micrometastasis. Br J Cancer 2004;91:1711–1717.
56 Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC, Coukos G: Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003;348:203–213.
57 Oshikiri T, Miyamoto M, Shichinohe T, Suzuoki M, Hiraoka K, Nakakubo Y, Shinohara T, Itoh T, Kondo S, Katoh H: Prognostic value of intratumoral CD8+ T lymphocyte in extrahepatic bile duct carcinoma as essential immune response. J Surg Oncol 2003;84:224–228.
58 Abe M, Kondo S, Hirano S, Ambo Y, Tanaka E, Morikawa T, Okushiba S, Katoh H: Long-term survival after radical resection of advanced pancreatic cancer: a case report with special reference to CD8+ T-cell infiltration. Int J Gastrointest Cancer 2003;33:107–110.
59 Wakabayashi O, Yamazaki K, Oizumi S, Hommura F, Kinoshita I, Ogura S, Dosaka-Akita H, Nishimura M: CD4(+) T cells in cancer stroma, not CD8(+) T cells in cancer cell nests, are associated with favorable prognosis in human non-small cell lung cancers. Cancer Sci 2003;94:1003–1009.
60 Nakakubo Y, Miyamoto M, Cho Y, Hida Y, Oshikiri T, Suzuoki M, Hiraoka K, Itoh T, Kondo S, Katoh H: Clinical significance of immune cell infiltration within gallbladder cancer. Br J Cancer 2003;89:1736–1742.
61 Funada Y, Noguchi T, Kikuchi R, Takeno S, Uchida Y, Gabbert HE: Prognostic significance of CD8+ T cell and macrophage peritumoral infiltration in colorectal cancer. Oncol Rep 2003;10: 309–313.

62 Lin EY, Pollard JW: Macrophages: modulators of breast cancer progression. Novartis Found Symp 2004;256:158–168.
63 Bingle L, Brown NJ, Lewis CE: The role of tumor-associated macrophages in tumor progression: implications for new anticancer therapies. J Pathol 2002;196:254–265.
64 Yano H, Kinuta M, Tateishi H, Nakano Y, Matsui S, Monden T, Okamura J, Sakai M, Okamoto S: Mast cell infiltration around gastric cancer cells correlates with tumor angiogenesis and metastasis. Gastric Cancer 1999;2:26–32.
65 de Visser KE, Schumacher TN, Kruisbeek AM: CD8+ T cell tolerance and cancer immunotherapy. J Immunother 2003;26:1–11.
66 Overwijk WW, Restifo NP: Creating therapeutic cancer vaccines: notes from the battlefield. Trends Immunol 2001;22:5–7.
67 Dudley ME, Rosenberg SA: Adoptive-cell-transfer therapy for the treatment of patients with cancer. Nat Rev Cancer 2003;3:666–675.
68 Spadaro M, Lanzardo S, Curcio C, Forni G, Cavallo F: Immunological inhibition of carcinogenesis. Cancer Immunol Immunother 2004;53:204–216.
69 Dranoff G: Coordinated tumor immunity. J Clin Invest 2003;111:1116–1118.
70 Pardoll DM: Does the immune system see tumors as foreign or self? Annu Rev Immunol 2003;21:807–839.
71 Finn OJ: Cancer vaccines: between the idea and the reality. Nat Rev Immunol 2003;3:630–641.
72 Rosenberg SA, Yang JC, Restifo NP: Cancer immunotherapy: moving beyond current vaccines. Nat Med 2004;10:909–915.
73 Balkwill F, Charles KA, Mantovani A: Smoldering and polarised inflammation in the initiation and promotion of malignant disease. Cancer Cell 2005;7:211–217.
74 Bronte V, Apolloni E, Cabrelle A, Ronca R, Serafini P, Zamboni P, Restifo NP, Zanovello P: Identification of a CD11b(+)/Gr-1(+)/CD31(+) myeloid progenitor capable of activating or suppressing CD8(+) T cells. Blood 2000;96:3838–3846.
75 Serafini P, De Santo C, Marigo I, Cingarlini S, Dolcetti L, Gallina G, Zanovello P, Bronte V: Derangement of immune responses by myeloid suppressor cells. Cancer Immunol Immunother 2004;53:64–72.
76 Kusmartsev S, Gabrilovich DI: Immature myeloid cells and cancer-associated immune suppression. Cancer Immunol Immunother 2002;51:293–298.
77 Sakaguchi S: Naturally arising Foxp3-expressing CD25(+)CD4(+) regulatory T cells in immunological tolerance to self and non-self. Nat Immunol 2005;6:345–352.
78 Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W: Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 2004;10:942–949.
79 Hamada I, Kato M, Yamasaki T, Iwabuchi K, Watanabe T, Yamada T, Itoyama S, Ito H, Okada K: Clinical effects of tumor-associated macrophages and dendritic cells on renal cell carcinoma. Anticancer Res 2002;22:4281–4284.
80 Dave SS, Wright G, Tan B, et al: Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med 2004;351:2159–2169.
81 Weitzman SA, Gordon LI: Inflammation and cancer: Role of phagocyte-generated oxidants in carcinogenesis. Blood 1990;76:655–663.
82 Ness RB, Cottreau C: Possible role of ovarian epithelial inflammation in ovarian cancer. J Natl Cancer Inst 1999;91:1459–1467.
83 Thun MJ, Henley SJ, Gansler T: Inflammation and cancer: an epidemiological perspective. Novartis Found Symp 2004;256:6–21.
84 Shacter E, Weitzman SA: Chronic inflammation and cancer. Oncology 2002;16:217–226.
85 Eaden JA, Abrams KR, Mayberry JF: The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut 2001;48:526–535.
86 Nelson WG, De Marzo AM, Isaacs WB: Prostate cancer. N Engl J Med 2003;349:366–381.
87 Maisonneuve P, Lowenfels AB: Chronic pancreatitis and pancreatic cancer. Dig Dis 2002;20: 32–37.

88 Whitcomb DC: Inflammation and cancer. V. Chronic pancreatitis and pancreatic cancer. Am J Physiol 2004;287:G315–G319.
89 Farrow B, Evers BM: Inflammation and the development of pancreatic cancer. Surg Oncol 2002;10:153–169.
90 Kuper H, Adami HO, Trichopoulos D: Infections as a major preventable cause of human cancer. J Intern Med 2000;248:171–183.
91 Pisani P, Parkin DM, Munoz N, Ferlay J: Cancer and infection: estimates of the attributable fraction in 1990. Cancer Epidemiol Biomarkers Prev 1997;6:387–400.
92 Nakamoto Y, Guidotti LG, Kuhlen CV, Fowler P, Chisari FV: Immune pathogenesis of hepatocellular carcinoma. J Exp Med 1998;188:341–350.
93 Ernst PB, Gold BD: The disease spectrum of *Helicobacter pylori*: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. Annu Rev Microbiol 2000;54:615–640.
94 Eurogast Study Group: An international association between *Helicobacter pylori* infection and gastric cancer. Lancet 1993;341:1359–1362.
95 Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK: *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 1991;325: 1127–1131.
96 Honda S, Fujioka T, Tokieda M, Satoh R, Nishizono A, Nasu M: Development of *Helicobacter pylori*-induced gastric carcinoma in Mongolian gerbils. Cancer Res 1998;58:4255–4259.
97 Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M: *Helicobacter pylori* infection induces gastric cancer in Mongolian gerbils. Gastroenterology 1998;115:642–648.
98 Heathcote EJ: Prevention of hepatitis C virus-related hepatocellular carcinoma. Gastroenterology 2004;127:S294–S302.
99 Donato F, Boffetta P, Puoti M: A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. Int J Cancer 1998;75:347–354.
100 Manning CB, Vallyathan V, Mossman BT: Diseases caused by asbestos: mechanisms of injury and disease development. Int Immunopharmacol 2002;2:191–200.
101 Falk GW: Barrett's esophagus. Gastroenterology 2002;122:1569–1591.
102 Lagergren J, Bergstrom R, Lindgren A, Nyren O: Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. N Engl J Med 1999;340:825–831.
103 Wistuba, II, Gazdar AF: Gallbladder cancer: lessons from a rare tumor. Nat Rev Cancer 2004;4: 695–706.
104 Garcia-Rodriguez LA, Huerta-Alvarez C: Reduced risk of colorectal cancer among long-term users of aspirin and nonaspirin nonsteroidal anti-inflammatory drugs. Epidemiology 2001;12: 88–93.
105 Sharpe CR, Collet JP, McNutt M, Belzile E, Boivin JF, Hanley JA: Nested case-control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage. Br J Cancer 2000;83:112–120.
106 Cotterchio M, Kreiger N, Sloan M, Steingart A: Nonsteroidal anti-inflammatory drug use and breast cancer risk. Cancer Epidemiol.Biomarkers Prev 2001;10:1213–1217.
107 Mann JR, DuBois RN: Cyclooxygenase-2 and gastrointestinal cancer. Cancer J 2004;10:145–152.
108 Clevers H: At the crossroads of inflammation and cancer. Cell 2004;118:671–674.
109 Turini ME, DuBois RN: Cyclooxygenase-2: a therapeutic target. Annu Rev Med 2002;53:35–37.
110 Koki AT, Masferrer JL: Celecoxib: a specific COX-2 inhibitor with anticancer properties. Cancer Control 2002;9:28–35.
111 Meier CR, Schmitz S, Jick H: Association between acetaminophen or nonsteroidal anti-inflammatory drugs and risk of developing ovarian, breast, or colon cancer. Pharmacotherapy 2002;22:303–309.
112 Akre K, Ekstrom AM, Signorello LB, Hansson LE, Nyren O: Aspirin and risk for gastric cancer: a population-based case-control study in Sweden. Br J Cancer 2001;84:965–968.
113 Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su LK, Levin B: The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 2000;342:1946–1952.
114 Coussens LM, Tinkle CL, Hanahan D, Werb Z: MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell 2000;103:481–490.

115 Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, Caughey GH, Hanahan D: Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. Genes Dev 1999;13:1382–1397.
116 Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M: IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 2004;118:285–296.
117 Balkwill F, Mantovani A: Inflammation and cancer: Back to Virchow? Lancet 2001;357:539–545.
118 Giraudo E, Inoue M, Hanahan D: An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. J Clin Invest 2004;114:623–633.
119 Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y: NF-kappaB functions as a tumor promoter in inflammation-associated cancer. Nature 2004;431:461–466.
120 Daniel D, Meyer-Morse N, Bergsland EK, Dehne K, Coussens LM, Hanahan D: Immune enhancement of skin carcinogenesis by CD4+ T cells. J Exp Med 2003;197:1017–1028.
121 de Visser KE, Korets LV, Coussens LM: Early neoplastic progression is complement independent. Neoplasia 2004;6:768–776.
122 de Visser KE, Korets LV, Coussens LM: De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. Cancer Cell 2005;7:411–423.
123 Arbeit JM, Munger K, Howley PM, Hanahan D: Progressive squamous epithelial neoplasia in K14-human papillomavirus type 16 transgenic mice. J Virol 1994;68:4358–4368.
124 Coussens LM, Hanahan D, Arbeit JM: Genetic predisposition and parameters of malignant progression in K14- HPV16 transgenic mice. Am J Path 1996;149:1899–1917.
125 Lin EY, Nguyen AV, Russell RG, Pollard JW: Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med 2001;193:727–740.
126 Sparmann A, Bar-Sagi D: Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. Cancer Cell 2004;6:447–458.
127 Nesbit M, Schaider H, Miller TH, Herlyn M: Low-level monocyte chemoattractant protein-1 stimulation of monocytes leads to tumor formation in nontumorigenic melanoma cells. J Immunol 2001;166:6483–6490.
128 Noguchi T, Wada S, Takeno S, Moriyama H, Kimura Y, Uchida Y: Lymph node metastasis could be predicted by evaluation of macrophage infiltration and hsp70 expression in superficial carcinoma of the esophagus. Oncol Rep 2003;10:1161–1164.
129 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M: The chemokine system in diverse forms of macrophage activation and polarisation. Trends Immunol 2004;25:677–686.
130 Mantovani A, Allavena P, Sica A: Tumor-associated macrophages as a prototypic type II polarised phagocyte population: role in tumor progression. Eur J Cancer 2004;40:1660–1667.
131 Almand B, Clark JI, Nikitina E, van Beynen J, English NR, Knight SC, Carbone DP, Gabrilovich DI: Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. J Immunol 2001;166:678–689.
132 Yang L, Debusk LM, Fukuda K, Fingleton B, Green-Jarvis B, Shyr Y, Matrisian LM, Carbone DP, Lin PC: Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. Cancer Cell 2004;6:409–421.
133 Gabrilovich DI, Velders MP, Sotomayor EM, Kast WM: Mechanism of immune dysfunction in cancer mediated by immature Gr-1+ myeloid cells. J Immunol 2001;166:5398–5406.
134 Balkwill F, Coussens LM: Cancer: an inflammatory link. Nature 2004;431:405–406.
135 Maeda H, Akaike T: Nitric oxide and oxygen radicals in infection, inflammation, and cancer. Biochemistry 1998;63:854–865.
136 Ohshima H: Genetic and epigenetic damage induced by reactive nitrogen species: implications in carcinogenesis. Toxicol Lett 2003;140–141:99–104.
137 Sternlicht MD, Werb Z: ECM Proteinases; in Kries T, Vale R (eds): Guidebook to the Extracellular Matrix. Oxford, Oxford University Press, 1999, pp 503–562.
138 Overall CM: Molecular determinants of metalloproteinase substrate specificity: matrix metalloproteinase substrate binding domains, modules, and exosites. Mol Biotechnol 2002;22:51–86.
139 Puente XS, Sanchez LM, Overall CM, Lopez-Otin C: Human and mouse proteases: a comparative genomic approach. Nat Rev Genet 2003;4:544–558.

140 Lopez-Otin C, Overall CM: Protease degradomics: a new challenge for proteomics. Nat Rev Mol Cell Biol 2002;3:509–519.
141 Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, Hanahan D: Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2000;2:737–744.
142 Huang S, Van Arsdall M, Tedjarati S, McCarty M, Wu W, Langley R, Fidler IJ: Contributions of stromal metalloproteinase-9 to angiogenesis and growth of human ovarian carcinoma in mice. J Natl Cancer Inst 2002;94:1134–1142.
143 Hiratsuka S, Nakamura K, Iwai S, Murakami M, Itoh T, Kijima H, Shipley JM, Senior RM, Shibuya M: MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. Cancer Cell 2002;2:289–300.
144 Barbera-Guillem E, Nyhus JK, Wolford CC, Friece CR, Sampsel JW: Vascular endothelial growth factor secretion by tumor-infiltrating macrophages essentially supports tumor angiogenesis, and IgG immune complexes potentiate the process. Cancer Res 2002;62:7042–7049.
145 Balkwill F: Cancer and the chemokine network. Nat Rev Cancer 2004;4:540–550.
146 Balkwill F: Tumor necrosis factor or tumor promoting factor? Cytokine Growth Factor Rev 2002;13:135–141.
147 Smith WL, DeWitt DL, Garavito RM: Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000;69:145–182.
148 Cao Y, Prescott SM: Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. J Cell Physiol 2002;190:279–286.
149 Ristimaki A, Sivula A, Lundin J, Lundin M, Salminen T, Haglund C, Joensuu H, Isola J: Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. Cancer Res 2002;62:632–635.
150 Subbaramaiah K, Norton L, Gerald W, Dannenberg AJ: Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer: evidence for involvement of AP-1 and PEA3. J Biol Chem 2002;277:18649–18657.
151 Choy H, Milas L: Enhancing radiotherapy with cyclooxygenase-2 enzyme inhibitors: a rational advance? J Natl Cancer Inst 2003;95:1440–1452.
152 Klein RD, Van Pelt CS, Sabichi AL, Dela Cerda J, Fischer SM, Furstenberger G, Muller-Decker K: Transitional cell hyperplasia and carcinomas in urinary bladders of transgenic mice with keratin 5 promoter-driven cyclooxygenase-2 overexpression. Cancer Res 2005;65:1808–1813.
153 Liu CH, Chang SH, Narko K, Trifan OC, Wu MT, Smith E, Haudenschild C, Lane TF, Hla T: Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. J Biol Chem 2001;276:18563–18569.
154 Muller-Decker K, Berger I, Ackermann K, Ehemann V, Zoubova S, Aulmann S, Pyerin W, Furstenberger G: Cystic duct dilatations and proliferative epithelial lesions in mouse mammary glands upon keratin 5 promoter-driven overexpression of cyclooxygenase-2. Am J Pathol 2005;166:575–584.
155 Muller-Decker K, Neufang G, Berger I, Neumann M, Marks F, Furstenberger G: Transgenic cyclooxygenase-2 overexpression sensitizes mouse skin for carcinogenesis. Proc Natl Acad Sci USA 2002;99:12483–12488.
156 Koehne CH, Dubois RN: COX-2 inhibition and colorectal cancer. Semin Oncol 2004;31:12–21.
157 Jacoby RF, Seibert K, Cole CE, Kelloff G, Lubet RA: The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. Cancer Res 2000;60:5040–5044.
158 Gasparini G, Longo R, Sarmiento R, Morabito A: Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents? Lancet Oncol 2003;4:605–615.
159 Giardiello FM, Yang VW, Hylind LM, Krush AJ, Petersen GM, Trimbath JD, Piantadosi S, Garrett E, Geiman DE, Hubbard W, Offerhaus GJ, Hamilton SR: Primary chemoprevention of familial adenomatous polyposis with sulindac. N Engl J Med 2002;346:1054–1059.
160 Kusmartsev SA, Li Y, Chen SH: Gr-1+ myeloid cells derived from tumor-bearing mice inhibit primary T cell activation induced through CD3/CD28 costimulation. J Immunol 2000;165:779–785.

161 Bronte V, Serafini P, Mazzoni A, Segal DM, Zanovello P: *L*-Arginine metabolism in myeloid cells controls T-lymphocyte functions. Trends Immunol 2003;24:302–306.
162 Woo EY, Yeh H, Chu CS, Schlienger K, Carroll RG, Riley JL, Kaiser LR, June CH: Cutting edge: regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. J Immunol 2002;168:4272–4276.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 138–155

Co-Opting Macrophage Traits in Cancer Progression: A Consequence of Tumor Cell Fusion?

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Abstract

Tumor-associated macrophages (TAMs) play multiple roles in tumor initiation and progression. Tumors frequently appear in areas of chronic inflammation. This is likely aided by the mutagenic actions of macrophages. Tumor growth and progression is supported by macrophage-induced neoangiogenesis and stroma production, and macrophages produce tumor-stimulating growth factors. In most cancers a high density of TAMs predicts poor outcome. But not only do cancer cells depend upon macrophages for growth and invasion, they also co-opt macrophage traits. These include a wide diversity of molecules and pathways regulating adhesion, matrix alterations, neoangiogenesis, motility, chemotaxis, immune signaling pathways and even multidrug resistance proteins. Evidence is presented that these traits could be generated through macrophage-tumor cell fusion. Fusion has been reported in numerous animal tumor models and was recently documented in 2 human cases. Fusion could also account for the high degree of aneuploidy and plasticity in cancer, and for immune evasion. One common trait of myeloid-tumor fusion is the high expression of β1,6-branched N-glycans, used by macrophages in systemic migration. β1,6-branched oligosaccharides have long been associated with metastasis in animal models and were recently found to be common in a wide diversity of human cancers. We suggest that β1,6-branched oligosaccharides in human cancer may reflect widespread tumor cell fusion. Viewing the cancer cell as a myeloid hybrid provides new approaches towards understanding and treating this complex disease.

Introduction

Tumor-associated macrophages (TAMs) facilitate both cancer initiation and progression [1–4]. Macrophages are attracted through chemotactic signals to tumors where they exert their abilities for matrix degradation, tissue remodeling, stroma deposition, tropism and neoangiogenesis. These are normally employed in functions such as wound healing, osteogenesis, and embryogenesis [3]. Since similar microenvironments exist within tumors, it is thought that macrophages become recruited to these 'wounds that never heal' [2]; or 'tissues that never cease to develop' [3]. Indeed, macrophages are recruited to existing tumors by inflammatory cytokines and growth factors normally produced following wounding or infection (e.g. chemotactic chemokine CCL2; colony-stimulating factor, CSF-1; vascular endothelial growth factor, VEGF-A) [3, 5]. Macrophages initiate neoplasia through release of reactive oxygen and nitrogen species that are mutagenic and carcinogenic [4]. Tumor microenvironment cytokines – transforming growth factor-β1 (TGF-β1), interleukin-10 (IL-10), and macrophage-colony stimulating factor (M-CSF) – induce macrophages to differentiate from M1 to M2-type cells that produce tumor growth-promoting factors and stimulate angiogenesis [4]. Macrophages accumulate in hypoxic regions of tumors through HIF-1-mediated upregulation of the chemokine receptor CXCR4 [6].

The density of TAMs correlates with poor outcome in more than 80% of human cancers, most notably in carcinomas of the breast, prostate, ovary and cervix [3, 7]. In these cancers, potential benefits from TAM anticancer immune functions were apparently dominated by the TAM tumor-promoting abilities. This was demonstrated in a mouse mammary tumor model where mice carrying a null mutation for CSF-1 showed a marked reduction in TAM density. Mammary tumors developed normally in the null mutants but unlike those in wild type mice they tended not to metastasize [8]. Thus, the presence of TAMs was a key requirement for metastasis in this model.

However, tumor progression is not completely explained by the presence of TAMs. During transition to a metastatic phenotype, tumor cells notoriously co-opt leukocytic traits [3, 9–11]. Malignant cells are chemotactic, responding to chemokines and exhibiting their own matrix-degrading and angiogenic capabilities. Like migratory leukocytes, metastatic cells exhibit loss of homotypic adhesion, and the ability to transverse a basement membrane, migrate through the mesodermal matrix, intravasate into lymphatics or the blood circulatory system, extravasate from these vessels, and colonize lymph nodes and distant organs [12–13]. But unlike normal leukocytes, cancer cells have deregulated mitotic cycles and their numbers continually increase, killing the host if left unchecked.

During this process, invasive carcinomas and melanomas often lose differentiated traits such as E-cadherin expression, homotypic cell-cell adhesion, and

cytokeratin or melanin production, while gaining mesodermal traits normally attributed to fibroblasts such as production of fibronectin and vimentin, loose adherence, mesenchymal motility mechanisms, and mesoderm-associated pathways such as the uPA/uPAR and HGF/cmet pathways [14–19]. This is known as the epithelial-mesenchymal transition (EMT), and thought to be a process where cancer cells mimic the pathways through which the mesoderm is formed from the epithelium in early development [14–17]. A developmental connection to EMT in cancer was shown through analyses of transcription factors such as the *Snail*/*Slug* superfamily and *Twist* that control EMT in embryogenesis. These factors regulate mesoderm formation during gastrulation, and were also associated with cancer progression [16, 20–22]. It has thus been proposed that the complex processes in metastasis may be explained by the action of master regulatory genes normally associated with development [14–16, 20–21, 23].

However, a uniform phenotype for EMT in cancer has not yet been described. Carcinomas and melanomas are notoriously heterogeneous, particularly as primary tumors [13, 24–27]. Many invasive and metastatic carcinomas and melanomas continue to produce cytokeratins or melanin, and not all invasive and/or metastatic carcinomas lose E-cadherin [14, 15, 22]. *Twist* expression is not universal. In human breast carcinoma, *Twist* upregulation is associated with invasive lobular carcinomas, but not with invasive ductal carcinomas, which make up 80% of breast cancers and which metastasize at a similar rate as the lobular [16, 22, 28]. If EMT defines tumor progression, why is it not expressed more uniformly [29]? One explanation could be that EMT is transient: For example, metastases may regain differentiated traits in the process of colonizing lymph nodes or distant organs in a reversal process known as MET (mesenchymal-epithelial transition) [14–15, 17, 21].

Another explanation could be that EMT is a consequence of tumor cell-myeloid cell fusion. Monocytes/macrophages and other myeloid cells are of mesenchymal origin, as shown in *Drosophila*, where double mutants in the mesoderm regulators *Twist* and *Snail* lack macrophages [30]. There is a growing list of myeloid-type traits that are shared by malignant cells. These include loss of homotypic adhesion, chemotactic motility, matrix degradation, immune signaling pathways, systemic migration, neoangiogenesis, and even multidrug resistance [19, 31–107] (table 1). A key example is amoeboid motility, a characteristic of bone marrow-derived leukocytes, stem cells, and metastatic cancer cells alike. Amoeboid motility is required for migration through the stroma and dissemination via the circulatory system [33–35]. In amoeboid motility, cells are highly deformable and because of their lack of stable focal adhesions can move at high velocities. The ability to undergo rapid shape change allows for migration through tissue without the need to degrade matrix [31, 33]. Moreover, monocytes/ macrophages and malignant tumor cells both show high plasticity, such as the ability to differentiate

Table 1. Examples of myeloid traits expressed by cancer cells

Trait	Reference
Amoeboid, single cell motilities	19, 31–33
Chemotaxis, chemokines, chemokine receptors	34–37
Endothelial differention	38–41
FGF/FGFR	42–45
Fibronectin	46
Focal adhesion kinase	47–49
GnT-V, β1,6-branched oligosaccharides	50–53
HGF/cMet pathways	54–56
Hypoxia inducible factors	57–59
β-Integrins	60–63
MAP kinases	64–68
Mesenchymal differentiation	40
MMPs	60, 69–72
Multidrug resistance, *p*-glycoprotein, ABC transporters	73–75
NF-kappaB1	76–82
Neurotrophin and neurotrophin receptors	83–85
Osteopontin	86–87
Phagocytic, vesicular phenotype	24, 51, 53, 88–92
RAGE/HMGB1	93–96
STATs	97–99
Toll-like receptors	100–101
uPA/uPAR pathways	102–104
VEGFs, angiogenic factors	72, 105–106
Vimentin	107

into fibroblastic or endothelial-like cells and to exhibit vascular mimicry [38–41]. This is mediated in part through focal adhesion kinase (FAK), a monocyte/macrophage-associated enzyme whose expression is associated with both vascular mimicry and metastasis [47–49]. Similarly, neurotrophins and neurotrophin receptors are expressed by macrophages and are also associated with cancer anoikis resistance and metastasis [83–85]. Melanoma and colon carcinoma cell lines express the macrophage-associated Toll receptor-4 (TR-4) and are responsive to LPS [100]. The expression of Toll-like receptors could facilitate evasion of immune surveillance of metastatic cells [100–101]. Cancer cells and macrophages both express multidrug-resistance proteins (ABC transporters) such as p-glycoprotein and other MDR proteins that confer chemotherapeutic resistance [73–75].

In other examples, freshly excised human lung carcinoma cells expressing macrophage-specific antigens were so common that the authors proposed they might have been of hematopoietic rather than of lung cell origin [108]. Human

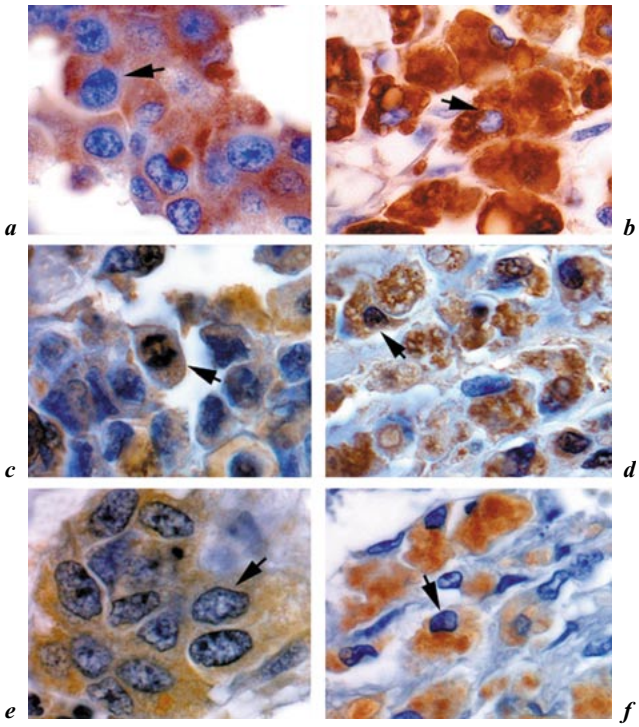

Fig. 1. Malignant melanoma cells and TAMs stained for three metastasis-associated markers: β1,6-branched oligosaccharides, GnT-V, and matriptase. Slides were bleached to decolorize melanin and stained by the immunoperoxidase reaction with the lectin LPHA for β1,6-branched oligosaccharides (***a***, tumor cells; ***b***, TAMs), with anti-GnT-V (***c***, tumor cells; ***d***, TAMs), or anti-matriptase (***e***, tumor cells; ***f***, TAMs). All fields were from the same tumor. TAMs were further verified by S100/azure blue staining [51; Handerson and Pawelek, unpubl.]. Arrows denote nuclear size differences between cancer cells and macrophages. See online version for color.

ovarian carcinoma cells co-expressing CD68 (KP-1) and cytokeratin were cited as evidence of macrophage-tumor cell fusion [109]. Immunomarkers in the identification of macrophages such as CD68, α_1-antitrypsin, MAC387, and Ham56 are often expressed in melanomas and other cancers [reviewed in 9, 110]. Phagocytic activity equal to that in macrophages was observed in a variety of human cancers, and was associated with an aggressive phenotype in breast carcinoma and melanoma [88–92].

To illustrate marker co-expression, melanoma cells and TAMs from the same histological section of a metastatic melanoma are each shown expressing GnT-V (EC 2.4.1.155; N-acetylglucoseaminyltransferase V), β1,6-branched oligosaccharides, and matriptase, a GnT-V substrate (fig. 1). These markers

play key roles in both macrophage and cancer cell migration, and all three are prognostic indicators for metastasis and poor outcome in human cancers [51, 92, 111–113]. Their high expression must have been acquired at some point during or following neoplastic transformation, since normal cutaneous melanocytes were negative (not shown).

From these and additional examples, it is here proposed that EMT in cancer may better be described as the acquisition of a myeloid-type phenotype rather than a fibroblastic one [15, 22]. As shown below, this phenomenon could be explained by myeloid cell-tumor cell fusion and genomic hybridization. Rather than reversion of cancer cells to earlier developmental pathways, the phenotype of myeloid-tumor fusion hybrids would be defined by the sum of gene expression in hybrid genomes from cells of different developmental lineages, each fusion partner being imprinted for gene expression from its tissue of origin (e.g. myeloid-epithelial, myeloid-melanocytic). Metastatic cells would arise when the migratory abilities of myeloid cells, and the uncontrolled proliferation of tumor cells were co-expressed in hybrids. Aneuploidy and heterogeneity would occur through variations in the hybrid genome, which would likely differ between individual hybrids [9, 11, 26].

Cell Fusion in Normal and Cancerous Tissues

In normal tissues, it is now known that bone marrow-derived cells contribute to a wide variety of normal tissues such as liver, brain, and heart. This is due at least in part to cell fusion, shown in some cases to be with monocyte/macrophage-lineage cells [114–124]. In cancer, Aichel proposed nearly a century ago that fusion with macrophages might cause tumor spread [9, 11, 125]. The first experimental support was with human astrocytic glioma cells implanted in a hamster cheek pouch [126]. These formed aggressive metastases that through karyotype analyses contained individual cells with both human and hamster chromosomes. In later studies, Kerbel et al. [127] demonstrated sarcoma-bone marrow hybrids in mice receiving allogenic bone marrow transplants. Larizza et al. [128] described a highly metastatic varient generated in vivo from a mouse T cell lymphoma that was likely to have been derived via fusion with a host macrophage. Similar results were reported in mice for spontaneous macrophge-sarcoma hybrids [129], bone marrow-insulinoma hybrids [130], and host-melanoma hybrids [131]. Hybrid melanoma cells expressed upregulated GnT-V and β1,6-branched oligosaccharides [132] along with a coarse vesicular phenotype, marked pigmentation, and high chemotactic motility (see below). These traits were identical to those of experimental macrophage-melanoma hybrids fused in vitro, suggesting that the host fusion partner in vivo had been a macrophage [131].

Putative BMT Tumor Cell Hybrids in Humans

Genetic evidence for bone marrow tumor cell hybrids in human cancer was recently obtained in 2 cases where individuals developed renal cell carcinoma following allogeneic bone marrow transplants. In each case, DNA from bone marrow transplant donors was detected in recipient tumor cells [133, 134]. In the first, tumor DNA was analyzed from a child who, after a BMT from his 6-year-old brother, developed renal cell carcinoma and then metastases [133]. The BMT donor ABO blood group genotype was A/O, while that of the recipient was O/O. Tumor cells from a nodal metastasis were microdissected free of blood cells and the DNA was PCR amplified using specific A and O allele primers. All 16 of 16 samples, taken from throughout the tumor, were shown through PCR to contain the donor A allele. Since the donor was a child and had remained cancer-free for more than 10 years, it was unlikely that carcinoma cells were transferred via the BMT. The BMT recipient was also a child but his prior history of radiation put him at elevated risk of developing solid tumors.

In the second case, a primary renal cell carcinoma was obtained from a female patient who two years prior to detection of the tumor had received a BMT from her cancer-free 15-year-old son [134]. As in the first case, the patient's prior treatment history placed her at elevated risk for malignancies. Karyotyping revealed that some of her tumor cells contained a trisomic chromosome 17 (trisomy 17). Through FISH analyses, three or more 17s and the donor Y chromosome were visualized together in individual nuclei of primary carcinoma cells, providing direct genetic evidence for BMT tumor hybrids.

Thus, in both of the human cases, the BMT donor DNA had in some manner become engrafted in the recipient tumor cells. It was concluded that the data best fit the clinical and pathological diagnoses that the tumor arose de novo in the patient, and the carcinoma cells containing BMT donor markers were donor-recipient fusion hybrids. However, there were other possible explanations, including transfer of cryptic carcinoma cells via the BMT or differentiation of BMT cells into renal carcinoma cells following the transplant. Transfer of cryptic carcinoma cells was not supported by the case histories, since the BMT donors were cancer-free children and have remained so post-transplant. Likewise, although differentiation of BMT cells into renal carcinoma cells could not be ruled out, it seemed more likely that the tumors had arisen de novo since each of the BMT recipients had received prior treatments placing them at elevated risk for de novo malignancies.

The mechanisms for cell fusion in vivo are as yet unknown. It was proposed that this might occur through aberrant phagocytosis of cancer cells by macrophages [9, 11, 135]. In vitro transfer of oncogenes during phagocytosis of apoptotic cells and oncogenic transformation was demonstrated [136]. Another consideration is that fusion is a natural function of macrophages, e.g. in the

production of osteoclasts and multinucleated giant cells [137], suggesting that under appropriate environmental conditions macrophages may have a natural propensity to fuse with tumor cells, even those in a non-apoptotic state. There is also evidence for virus-induced fusion in cancer [138]. Other potential fusion partners of monocytic origin to consider are dendritic cells, Langerhans cells and granulocytes. Fibroblasts, epithelial or endothelial cells might become induced into a phagocytic state when a neighboring cancer cell becomes apoptotic or in some other manner fuse with cancer cells [136]. It is also possible that cancer stem cells are involved in fusion events [139]. Different phenotypes would be expected depending on the fusion partners.

Gene Expression in Artificial Fusion Hybrids

Regarding gene expression, tumor hybrids generated in vitro between cancer cells and normal epithelial cells or fibroblasts were suppressed in tumorigenicity and the expression of differentiated functions, leading to the identification of tumor suppressor genes [9, 11]. But when myeloid cells were the fusion partners with cancer cells 'transactivation' of genes and differentiated traits between parental genomes was seen. Experimental macrophage-melanoma hybrids showed multiple phenotypic changes characteristic of each parental fusion partner, including accentuated pigmentation (melanocytic) and markedly enhanced chemotactic motility (myeloid) [140–141]. This was accompanied by increased metastatic potential in mice [131, 135]. One underlying cause for this involved expression of myeloid-type N-glycosylation [132, 140]. In particular, the myeloid-associated GnT-V and its enzymatic product, β1,6-branched oligosaccharides, along with a coarse vesicular cytoplasm were all prominent in macrophage-melanoma hybrids with high metastatic potential [9, 11, 53, 131, 140].

β1,6-Branched Oligosaccharides and Coarse Vesicles in Human BMT Tumor Hybrids

Prompted by these observations, β1,6-branched oligosaccharides (stained with the lectin leukocyte phytohemagglutinin (LPHA) and coarse vesicles were investigated in the 2 human cases of putative BMT tumor hybrids in renal cell carcinomas described above [133, 134]. In both cases, the tumors were LPHA-positive with a coarse vesicular cytoplasm. In the case of lymph node metastasis in a child receiving a BMT from his brother [133], tumor cells were nearly homogeneous for this phenotype (fig. 2a, left), while adjacent nodal lymphocytes were negative (fig. 2a, right). The homogeneity of staining with LPHA correlated with the donor A allele being distributed throughout this metastasis

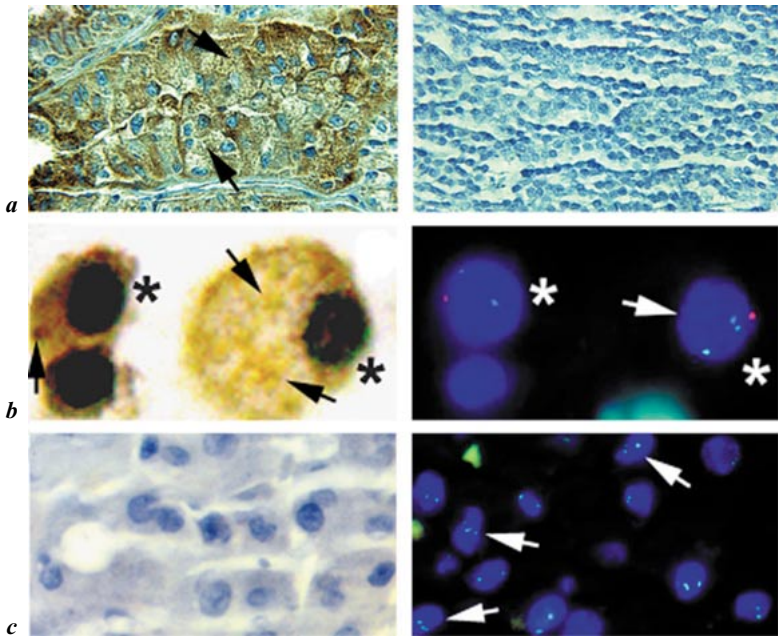

Fig. 2. Putative human BMT-tumor hybrids exhibit a phenotype of β1,6-branched oligosaccharides and coarse vesicles. ***a*** A section of a renal cell carcinoma lymph node metastasis from a child who had received a prior BMT from his 6-year-old brother [133]. The section was stained with the lectin LPHA (brown) and counterstained with hematoxylin (blue). Left, renal carcinoma cells staining with LPHA. Arrows denote coarse vesicles. Right, adjacent normal lymphoid cells in the same section were negative for LPHA. ***b*** A section of a primary renal cell carcinoma from a female who had received a prior BMT from her 15-year-old son [134]. The section was first stained with LPHA, LPHA positive cells were photographed, and the section was processed by FISH for the Y (red) and 17 (green) chromosomes. Left: three LPHA-positive carcinoma cells with coarse vesicles denoted by black arrows. Right: the corresponding FISH-stained nuclei. One nucleus showed no FISH signal, while two showed the Y (red, asterix) and 17 (green), with one of the Y-containing cells also containing a trisomy 17 (white arrow). ***c*** A region of the same tumor in ***b*** [134] that was devoid of LPHA-positive cells. Left: LPHA-negative carcinoma cells. Right: a FISH-labelled sequential section of the same region with the LPHA-negative cells displaying only chromosome 17 and not the Y [Yilmaz and Pawelek, unpubl.]. See online version for color.

[133]. In the case of the primary tumor from a female patient with a male BMT [134], Y-containing carcinoma cells were in the minority, yet it was chiefly these cells that expressed an LPHA-positive, coarse vesicular phenotype. For example, a field with three LPHA-positive carcinoma cells with coarse vesicles is shown (fig. 2b, left). The corresponding FISH-stained nuclei revealed that one showed no FISH signal, while the other two showed the Y and 17, with one of

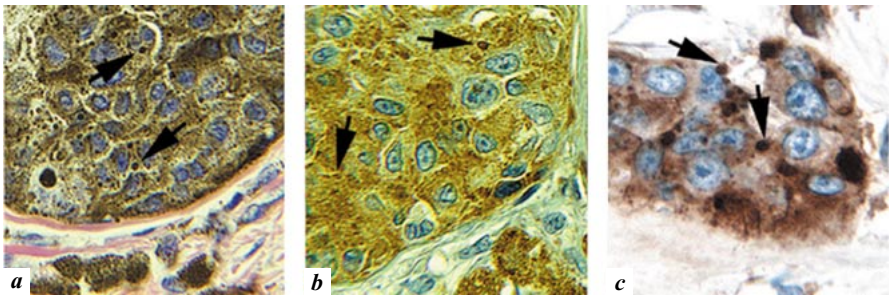

Fig. 3. Archival pathology specimens of primary melanoma and breast carcinoma displaying a phenotype of LPHA-positive coarse vesicles. ***a*** A histological section of a human malignant melanoma stained with HE. Arrows denote coarse melanin-containing vesicles. Melanophages are labeled 'mac'. ***b*** A sequential section from the same melanoma that was first bleached to decolorize melanin and then stained for β1,6-branched oligosaccharides with LPHA. ***c*** A primary breast carcinoma stained with LPHA. Arrows denote coarse vesicles. See online version for color.

the Y-containing cells also containing a trisomy 17 (fig. 2b, right). Of the 70 LPHA-positive cells studied in this manner, 46 nuclei gave positive FISH signals, and of these 37 (80%) contained a Y chromosome. The majority of tumor cells were LPHA-negative and displayed 17 but not the Y (fig. 2c). Therefore, in each of the two human cases above, putative hybrid cells were the main source of tumor cell-associated β1,6-branched oligosaccharides and these structures were associated in part with coarse cytoplasmic vesicles.

β1,6-Branched Oligosaccharides and Coarse Vesicles Are Common in Human Cancers

From these results, we initiated a survey for β1,6-branched oligosaccharides and coarse vesicles in human cancers [51]. In melanoma, LPHA-positive, melanin-containing coarse vesicles were common. Amelanotic melanomas contained similar structures but lacking melanin. Further, LPHA-positive coarse vesicles were readily found in all 22 different cancers surveyed, including carcinomas of the lung, colon, breast, ovary, prostate, kidney, liver, and a variety of lymphomas [51]. To illustrate, an in situ cutaneous melanoma filled with coarse melanin is shown (fig. 3a). When a sequential section was bleached to decolorize melanin and stained with LPHA, the coarse vesicles were highlighted (fig. 3b). The tumor was bordered by dermal melanophages that also displayed melanized vesicles, thought to be phagolysosomes containing partially digested melanoma cells. Melanophages, like tumor cells, were LPHA-positive (fig. 3b); however,

nearby lymphocytes and fibroblasts were negative (not shown). A phenotype remarkably similar to that of the melanoma is seen in a primary breast carcinoma stained with LPHA (fig. 3c). In this and other non-melanized tumors the vesicles were far less apparent by standard HE staining. In patient-matched specimens, LPHA-positive, coarse vesicle-containing cells could be found in primary tumors that were morphologically identical to those in metastases, suggesting that these cells were the source of metastases. In breast cancer microarrays the phenotype was most pronounced in nodal metastases where it was found at near homogeneity in more than 95% of cases [92]. In primary breast carcinomas, the phenotype was a prognostic indicator of poor outcome [92, 142].

The biological implications of this phenotype in human cancer are as yet unknown. It is possible that it is related to the high phagocytic activity associated with human cancers [88–92]. However, the relationship between high phagocytic activity in vitro and coarse vesicles in fixed pathology specimens will require further study. From the results herein it is tempting to speculate that the widespread appearance of coarse vesicles and β1,6-branched oligosaccharides in human cancers is a reflection of macrophage-tumor hybridization. But the extent of tumor hybridization in humans and its potential as an initiator of metastasis remain to be determined.

Conclusions

Tumor progression as a process of cellular evolution is fundamental to our current view of metastasis [143–147]. Studies in evolutionary biology revealed that the evolution of both prokaryotic and eukaryotic cells proceeded not only stepwise, through single mutations, but also through mechanisms involving horizontal gene transfer and endosymbiosis, where large clusters of genes were pooled from parents of disparate genetic backgrounds. It is proposed here that cell fusion provides analogous genetic mechanisms in the progression of human cancer. Fusion with myeloid cells could explain the high prevalence of myeloid-lineage traits in cancer. Spontaneous myeloid cell-tumor cell hybrids have been reported in animals and humans. One phenotype of myeloid-tumor cell hybrids, the expression of β1,6-branched oligosaccharides and coarse vesicles, is widespread in human cancer, raising the possibility that this phenotype represents a histological correlate of myeloid-tumor hybrids. However, the extent of tumor hybridization in humans, and its potential as an initiator of metastasis are as yet unknown. It is hoped that the evidence and concepts summarized here may foster more research in this important area. Further studies of cases where patients who develop cancer following allogeneic BMT would appear to be one fruitful direction to follow.

Acknowledgements

We are grateful to Stefano Sodi and James Platt for their excellent technical contributions and to Drs David Bermudes, Jean Bolognia, and Lynn Margulis for their many stimulating discussions on macrophage-tumor hybridization. Portions of this article are reprinted from [11]. Funded in part through an unrestricted gift from Vion Pharmaceuticals, New Haven, Conn., USA.

References

1 Condeelis J, Pollard JW: Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 2006;124:263–266.
2 Balkwill F, Mantovani A: Inflammation and cancer: back to Virchow? Lancet 2001;357:539–545.
3 Pollard JW: Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 2004;4:71–78.
4 Balkwill F, Charles KA, Mantovani A: Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 2005;7:211–217.
5 Goswami S, Sahai E, Wyckoff JB, Cammer M, Cox D, Pixley FJ, Stanley ER, Segall JE, Condeelis JS: Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop. Cancer Res 2005;65:5278–5283. Erratum in: Cancer Res 2005;65:7031.
6 Schioppa T, Uranchimeg B, Saccani A, Biswas SK, Doni A, Rapisarda A, Bernasconi S, Saccani S, Nebuloni M, Vago L, Mantovani A, Melillo G, Sica A: Regulation of the chemokine receptor CXCR4 by hypoxia. J Exp Med 2003;198:1391–1402.
7 Bingle L, Brown NJ, Lewis CE: The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol 2002;196:254–265.
8 Lin EY, Nguyen AV, Russell RG, Pollard JW: Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med 2001;193:727–740.
9 Pawelek JM: Tumor cell hybridization and metastasis revisited. Melanoma Res 2000;10: 507–514.
10 Chakraborty AK, Pawelek JM: GnT-V, macrophage and cancer metastasis: a common link. Clin Exp Metastasis 2003;20:365–373.
11 Pawelek JM: Tumor cell fusion as a source of myeloid traits in cancer. Lancet Oncol 2005;6: 988–993.
12 Chambers AF, Groom AC, MacDonald IC: Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2002;2:563–572.
13 Fidler IJ: The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. Nat Rev Cancer 2003;3:453–458.
14 Thiery JP: Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002;2: 442–454.
15 Thiery JP: Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol 2003;15:740–746.
16 Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA: Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 2004;117:927–939.
17 Kang Y, Massague J: Epithelial-mesenchymal transitions: twist in development and metastasis. Cell 2004;118:277–279.
18 Wang D, Shen Q, Chen YQ, Wang MH: Collaborative activities of macrophage-stimulating protein and transforming growth factor-beta1 in induction of epithelial to mesenchymal transition: roles of the RON receptor tyrosine kinase. Oncogene 2004;23:1668–1680.
19 Friedl P: Prespecification and plasticity: shifting mechanisms of cell migration. Curr Opin Cell Biol 2004;16:14–23.

20 Nieto MA: The snail superfamily of zinc-finger transcription factors. Nat Rev Mol Cell Bio 2002;3:1551–1566.
21 Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M, Hung MC: Dual regulation of Snail by GSK-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat Cell Biol 2004;6:931–940.
22 Thiery JP, Morgan M: Breast cancer progression with a Twist. Nat Med 2004;10:777–778.
23 Kerr CA: A twist to metastasis. Lancet Oncol 2004;5:465.
24 Banerjee SS, Harris M: Morphological and immunophenotypic variations in malignant melanoma. Histopathol 2000;36:387–402.
25 Fidler IJ, Hart IR: Biological diversity in metastatic neoplasms: origins and implications. Science 1982;217:998–1003.
26 Warner TFCS: Cell hybridization: an explanation for the phenotypic diversity of certain tumours. Med Hypothesis 1975;1:51–57.
27 Clark WH, Mastangelo MJ, Ainsworth AM, Berd D, Bellet RE, Bernardino EA: Current concepts of the biology of human cutaneous malignant melanoma. Adv Cancer Res 1977;24: 267–338.
28 Ferlicot S, Vincent-Salomon A, Medioni J, Genin P, Rosty C, Sigal-Zafrani B, Freneaux P, Jouve M, Thiery JP, Sastre-Garau X: Wide metastatic spreading in infiltrating lobular carcinoma of the breast. Eur J Cancer 2004;40:336–341.
29 Thompson EW, Newgreen DF, Tarin D: Carcinoma invasion and metastasis: a role for epithelial-mesenchymal transition? Cancer Res 2005;65:5991–5995 (discussion 5995).
30 Tepass U, Fessler LI, Aziz A, Hartenstein V: Embryonic origin of hemocytes and their relationship to cell death in *Drosophila*. Development 1994;120:1829–1837.
31 Friedl P, Wolf K: Proteolytic and non-proteolytic migration of tumour cells and leucocytes. Biochem Soc Symp 2003;70:277–285.
32 Condeelis J, Segall JE: Intravital imaging of cell movement in tumours. Nat Rev Cancer 2003;3: 921–930.
33 Wolf K, Mazo I, Leung H, Engelke K, von Andrian UH, Deryugina EI, Strongin AY, Brocker EB, Friedl P: Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. J Cell Biol 2003;160:267–277.
34 Panzer U, Uguccioni M: Prostaglandin E_2 modulates the functional responsiveness of human monocytes to chemokines. Eur J Immunol 2004;34:3682–3689.
35 Kulbe H, Levinson NR, Balkwill F, Wilson JL: The chemokine network in cancer – much more than directing cell movement. Int J Dev Biol 2004;48:489–496.
36 Coates PT, Colvin BL, Ranganathan A, Duncan FJ, Lan YY, Shufesky WJ, Zahorchak AF, Morelli AE, Thomson AW: CCR and CC chemokine expression in relation to Flt3 ligand-induced renal dendritic cell mobilization. Kidney Int 2004;66:1907–1917.
37 Bursill CA, Choudhury RP, Ali Z, Greaves DR, Channon KM: Broad-spectrum CC-chemokine blockade by gene transfer inhibits macrophage recruitment and atherosclerotic plaque formation in apolipoprotein E-knockout mice. Circulation 2004;110:2460–2466.
38 Schmeisser A, Garlichs CD, Zhang H, Eskafi S, Graffy C, Ludwig J, Strasser RH, Daniel WG: Monocytes coexpress endothelial and macrophagocytic lineage markers and form cord-like structures in Matrigel under angiogenic conditions. Cardiovasc Res 2001;49:671–680.
39 Hendrix MJ, Seftor EA, Hess AR, Seftor RE: Vasculogenic mimicry and tumour-cell plasticity: lessons from melanoma. Nat Rev Cancer 2003;3:4114–4121.
40 Kuwana M, Okazaki Y, Kodama H, Izumi K, Yasuoka H, Ogawa Y, Kawakami Y, Ikeda Y: Human circulating CD14+ monocytes as a source of progenitors that exhibit mesenchymal cell differentiation. J Leukoc Biol 2003;74:833–845.
41 Streubel B, Chott A, Huber D, Exner M, Jager U, Wagner O, Schwarzinger I: Lymphoma-specific genetic aberrations in microvascular endothelial cells in B-cell lymphomas. N Engl J Med 2004; 351:250–259.
42 Berardi AC, Wang A, Abraham J, Scadden DT: Basic fibroblast growth factor mediates its effects on committed myeloid progenitors by direct action and has no effect on hematopoietic stem cells. Blood 1995;86:2123–2129.

43 Jones M, Tussey L, Athanasou N, Jackson DG: Heparan sulfate proteoglycan isoforms of the CD44 hyaluronan receptor induced in human inflammatory macrophages can function as paracrine regulators of fibroblast growth factor action. J Biol Chem 2000;275:7964–7974.
44 Zuo J, Jiang J, Dolce C, Holliday LS: Effects of basic fibroblast growth factor on osteoclasts and osteoclast-like cells. Biochem Biophys Res Commun 2004;318:162–167.
45 Chikazu D, Katagiri M, Ogasawara T, Ogata N, Shimoaka T, Takato T, Nakamura K, Kawaguchi H: Regulation of osteoclast differentiation by fibroblast growth factor 2: stimulation of receptor activator of nuclear factor kappaB ligand/osteoclast differentiation factor expression in osteoblasts and inhibition of macrophage colony-stimulating factor function in osteoclast precursors. J Bone Miner Res 2001;16:2074–2081.
46 Armbrust T, Kreissig M, Tron K, Ramadori G: Modulation of fibronectin gene expression in inflammatory mononuclear phagocytes of rat liver after acute liver injury. J Hepatol 2004;40:638–645.
47 Hess AR, Postovit LM, Margaryan NV, Seftor EA, Schneider GB, Seftor RE, Nickoloff BJ, Hendrix MJ: Focal adhesion kinase promotes the aggressive melanoma phenotype. Cancer Res 2005;65:9851–9860.
48 Rovida E, Lugli B, Barbetti V, Giuntoli S, Olivotto M, Dello Sbarba P: Focal adhesion kinase is redistributed to focal complexes and mediates cell spreading in macrophages in response to M-CSF. Biol Chem 2005;386:919–929.
49 McLean GW, Carragher NO, Avizienyte E, Evans J, Brunton VG, Frame MC: The role of focal-adhesion kinase in cancer – a new therapeutic opportunity. Nat Rev Cancer 2005;5:505–515.
50 Fukuda M, Spooncer E, Oates JE, Dell A, Klock JC: Structure of sialylated fucosyl lactosaminoglycan isolated from human granulocytes. J Biol Chem 1984;25:10925–10935.
51 Handerson T, Pawelek JM: β1,6-branched oligosaccharides and coarse vesicles: a common and pervasive phenotype in melanoma and other human cancers. Cancer Res 2003;63:5363–5369.
52 Miyake Y, Matsumoto H, Yokoo M, Miyazawa K, Kimura N, Tunjung WA, Shimizu T, Sasada H, Aso H, Yamaguchi T, Sato E: Expression and glycosylation with polylactosamine of CD44 antigen on macrophages during follicular atresia in pig ovaries. Biol Reprod 2005;Nov 23 (epub ahead of print).
53 Rupani R, Handerson T, Pawelek JM: Co-localization of β1,6-branched oligosaccharides and coarse melanin in macrophage-melanoma fusion hybrids and human melanoma cells in vitro. Pigment Cell Res 2004;17:281–288.
54 Beilmann M, Odenthal M, Jung W, Vande Woude GF, Dienes HP, Schirmacher P: Neoexpression of the c-met/hepatocyte growth factor-scatter factor receptor gene in activated monocytes. Blood 1997;90:4450–4458.
55 Ren Y, Cao B, Law S, Xie Y, Lee PY, Cheung L, Chen Y, Huang X, Chan HM, Zhao P, Luk J, Vande Woude G, Wong J: Hepatocyte growth factor promotes cancer cell migration and angiogenic factors expression: a prognostic marker of human esophageal squamous cell carcinomas. Clin Cancer Res 2005;11:6190–6197.
56 Chakraborty AK, Kolesnikova N, Sousa Jde F, Espreafico EM, Peronni KC, Pawelek J: Expression of c-Met proto-oncogene in metastatic macrophage x melanoma fusion hybrids: implication of its possible role in MSH-induced motility. Oncol Res 2003;14:163–174.
57 Acker T, Plate KH: Hypoxia and hypoxia inducible factors (HIF) as important regulators of tumor physiology. Cancer Treat Res 2004;117:219–248.
58 Knowles H, Leek R, Harris AL: Macrophage infiltration and angiogenesis in human malignancy. Novartis Found Symp 2004;256:189–200; discussion 200–204, 259–269.
59 Cramer T, Yamanishi Y, Clausen BE, Forster I, Pawlinski R, Mackman N, Haase VH, Jaenisch R, Corr M, Nizet V, Firestein GS, Gerber HP, Ferrara N, Johnson RS: HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell 2003;112:645–657. Erratum in: Cell 2003;113:419.
60 Jacob SS, Sudhakaran PR: Molecular mechanism involved in matrix dependent upregulation of matrix metalloproteinases in monocyte/macrophage. J Biochem Mol Biol Biophys 2002;6:335–340.
61 Barringhaus KG, Phillips JW, Thatte JS, Sanders JM, Czarnik AC, Bennett DK, Ley KF, Sarembock IJ: Alpha4beta1 integrin (VLA-4) blockade attenuates both early and late leukocyte recruitment and neointimal growth following carotid injury in apolipoprotein E (−/−) mice. J Vasc Res 2004;41:252–260.
62 McDowall A, Inwald D, Leitinger B, Jones A, Liesner R, Klein N, Hogg N: A novel form of integrin dysfunction involving beta1, beta2, and beta3 integrins. J Clin Invest 2003;111:51–60.

63 Semel AC, Seales EC, Singhal A, Eklund EA, Colley KJ, Bellis SL: Hyposialylation of integrins stimulates the activity of myeloid fibronectin receptors. J Biol Chem 2002;277:32830–32836.
64 Zhang L, Fang B: Mechanisms of resistance to TRAIL-induced apoptosis in cancer. Cancer Gene Ther 2005;12:228–237.
65 Otsuka M, Tsuchiya S, Aramaki Y: Involvement of ERK, a MAP kinase, in the production of TGF-beta by macrophages treated with liposomes composed of phosphatidylserine. Biochem Biophys Res Commun 2004;324:1400–1405.
66 Beinke S, Robinson MJ, Hugunin M, Ley SC: Lipopolysaccharide activation of the TPL-2/MEK/extracellular signal-regulated kinase mitogen-activated protein kinase cascade is regulated by IkappaB kinase-induced proteolysis of NF-kappaB1 p105. Mol Cell Biol 2004;24: 9658–9667.
67 Shepherd EG, Zhao Q, Welty SE, Hansen TN, Smith CV, Liu Y: The function of mitogen-activated protein kinase phosphatase-1 in peptidoglycan-stimulated macrophages. J Biol Chem 2004;279: 54023–54031.
68 Jiang W, Reich III CF, Pisetsky DS: Mechanisms of activation of the RAW264.7 macrophage cell line by transfected mammalian DNA. Cell Immunol 2004;229:31–40.
69 Bicknell CD, Peck D, Alkhamesi NA, Cowling MG, Clark MW, Goldin R, Foale R, Jenkins MP, Wolfe JH, Darzi AW, Cheshire NJ: Relationship of matrix metalloproteinases and macrophages to embolization during endoluminal carotid interventions. J Endovasc Ther 2004;11: 483–493.
70 Lessner SM, Martinson DE, Galis ZS: Compensatory vascular remodeling during atherosclerotic lesion growth depends on matrix metalloproteinase-9 activity. Arterioscler Thromb Vasc Biol 2004;24:123–129.
71 Dreier R, Grassel S, Fuchs S, Schaumburger J, Bruckner P: Pro-MMP-9 is a specific macrophage product and is activated by osteoarthritic chondrocytes via MMP-3 or a MT1-MMP/MMP-13 cascade. Exp Cell Res 2004;297:303–312.
72 Giraudo E, Inoue M, Hanahan D: An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. J Clin Invest 2004;114:623–633.
73 Breier A, Barancik M, Sulova Z, Uhrik B: P-glycoprotein – implications of metabolism of neoplastic cells and cancer therapy. Curr Cancer Drug Targets 2005;5:457–468.
74 Jorajuria S, Dereuddre-Bosquet N, Becher F, Martin S, Porcheray F, Garrigues A, Mabondzo A, Benech H, Grassi J, Orlowski S, Dormont D, Clayette P: ATP binding cassette multidrug transporters limit the anti-HIV activity of zidovudine and indinavir in infected human macrophages. Antivir Ther 2004;9:519–528.
75 Michot JM, Van Bambeke F, Mingeot-Leclercq MP, Tulkens PM: Active efflux of ciprofloxacin from J774 macrophages through an MRP-like transporter. Antimicrob Agents Chemother 2004; 48:2673–2682.
76 Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y: NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 2004;431:461–466.
77 Liu CA, Wang MJ, Chi CW, Wu CW, Chen JY: Rho/Rhotekin-mediated NF-kappaB activation confers resistance to apoptosis. Oncogene 2004;23:8731–8742.
78 Aggarwal BB: Nuclear factor-kappaB: the enemy within. Cancer Cell 2004;6:203–208.
79 Ito T, Morimatsu M, Oonuma T, Shiina T, Kitamura H, Syuto B: Transcriptional regulation of the MAIL gene in LPS-stimulated RAW264 mouse macrophages. Gene 2004;342:137–143.
80 Han Y, Englert JA, Yang R, Delude RL, Fink MP: Ethyl pyruvate inhibits NF-{kappa}B-dependent signaling by directly targeting p65. J Pharmacol Exp Ther 2005;312:1097–1101
81 Jang BC, Paik JH, Kim SP, Bae JH, Mun KC, Song DK, Cho CH, Shin DH, Kwon TK, Park JW, Park JG, Baek WK, Suh MH, Lee SH, Baek SH, Lee IS, Suh SI: Catalase induces the expression of inducible nitric oxide synthase through activation of NF-kappaB and PI3K signaling pathway in Raw 264.7 cells. Biochem Pharmacol 2004;68:2167–2176.
82 Maddali S, Stapleton PP, Freeman TA, Smyth GP, Duff M, Yan Z, Daly JM: Neuroendocrine responses mediate macrophage function after trauma. Surgery 2004;136:1038–1046.
83 Geiger TR, Peeper DS: The neurotrophic receptor TrkB in anoikis resistance and metastasis: a perspective. Cancer Res 2005;65:7033–7036.

84 Ricci A, Greco S, Mariotta S, Felici L, Amenta F, Bronzetti E: Neurotrophin and neurotrophin receptor expression in alveolar macrophages: an immunocytochemical study. Growth Factors 2000;18:193–202.
85 Barouch R, Appel E, Kazimirsky G, Brodie C: Macrophages express neurotrophins and neurotrophin receptors. Regulation of nitric oxide production by NT-3. J Neuroimmunol 2001;112:72–77.
86 Rittling SR, Chambers AF: Role of osteopontin in tumour progression. Br J Cancer 2004;90: 1877–1881.
87 Wai PY, Kuo PC: The role of osteopontin in tumor metastasis. J Surg Res 2004;121:1228–1241.
88 Damiani MT, Colombo MI: Microfilaments and microtubules regulate recycling from phagosomes. Exp Cell Res 2003;289:152–161.
89 Coopman PJ, Do MT, Thompson EW, Mueller SC: Phagocytosis of cross-linked gelatin matrix by human breast carcinoma cells correlates with their invasive capacity. Clin Cancer Res 1998;4: 507–515.
90 Montcourrier P, Mangeat PH, Valembois C, Salazar G, Sahuquet A, Duperray C, Rochefort H: Characterization of very acidic phagosomes in breast cancer cells and their association with invasion. J Cell Sci 1994;107:2381–2391.
91 Lugini L, Lozupone F, Matarrese P, Funaro C, Luciani F, Malorni W, Rivoltini L, Castelli C, Tinari A, Piris A, Parmiani G, Fais S: Potent phagocytic activity discriminates metastatic and primary human malignant melanomas: a key role of ezrin. Lab Invest 2003;83:1555–1567.
92 Handerson T, Camp R, Harigopal M, Rimm D, Pawelek J: β1,6-Branched oligosaccharides are associated with metastasis and predict poor outcome in breast carcinoma. Clin Can Res 2005;11:2969–2973.
93 Kokkola R, Andersson A, Mullins G, Ostberg T, Treutiger CJ, Arnold B, Nawroth P, Andersson U, Harris RA, Harris HE: RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages. Scand J Immunol 2005;61:1–9.
94 DeMarco RA, Fink MP, Lotze MT: Monocytes promote natural killer cell interferon gamma production in response to the endogenous danger signal HMGB1. Mol Immunol 2005;42:433–444.
95 Ishiguro H, Nakaigawa N, Miyoshi Y, Fujinami K, Kubota Y, Uemura H: Receptor for advanced glycation end products (RAGE) and its ligand, amphoterin are overexpressed and associated with prostate cancer development. Prostate 2005;64:92–100.
96 Takada M, Hirata K, Ajiki T, Suzuki Y, Kuroda Y: Expression of receptor for advanced glycation end products (RAGE) and MMP-9 in human pancreatic cancer cells. Hepatogastroenterology 2004;51:928–930.
97 Ghilardi N, Li J, Hongo JA, Yi S, Gurney A, de Sauvage FJ: A novel type I cytokine receptor is expressed on monocytes, signals proliferation, and activates STAT-3 and STAT-5. J Biol Chem 2002;277:16831–16836.
98 Chappell VL, Le LX, LaGrone L, Mileski WJ: Stat proteins play a role in tumor necrosis factor alpha gene expression. Shock 2000;14:400–402; discussion 402–403.
99 Makuta Y, Sonoda Y, Yamamoto D, Funakoshi-Tago M, Aizu-Yokota E, Takebe Y, Kasahara T: Interleukin-10-induced CCR5 expression in macrophage like HL-60 cells: involvement of Erk1/2 and STAT-3. Biol Pharm Bull 2003;26:1076–1081.
100 Molteni M, Marabella D, Orlandi C, Rossetti C: Melanoma cell lines are responsive in vitro to lipopolysaccharide and express TLR-4. Cancer Lett. E-pub, 2005.
101 Huang B, Zhao J, Li H, He KL, Chen Y, Mayer L, Unkeless JC, Xiong H: Toll-like receptors on tumor cells facilitate evasion of immune surveillance. Cancer Res 2005;65:5009–5014.
102 Pluskota E, Soloviev DA, Bdeir K, Cines DB, Plow EF: Integrin alphaMbeta2 orchestrates and accelerates plasminogen activation and fibrinolysis by neutrophils. J Biol Chem 2004;279: 18063–18072.
103 Siren V, Immonen I: uPA, tPA and PAI-1 mRNA expression in periretinal membranes. Curr Eye Res 2003;27:261–267.
104 Pejler G, Winberg JO, Vuong TT, Henningsson F, Uhlin-Hansen L, Kimata K, Kolset SO: Secretion of macrophage urokinase plasminogen activator is dependent on proteoglycans. Eur J Biochem 2003;270:3971–3980.
105 Aharinejad S, Paulus P, Sioud M, Hofmann M, Zins K, Schafer R, Stanley ER, Abraham D: Colony-stimulating factor-1 blockade by antisense oligonucleotides and small interfering RNAs

suppresses growth of human mammary tumor xenografts in mice. Cancer Res 2004;64: 5378–5384.
106 Ren Y, Tsui HT, Poon RT, Ng IO, Li Z, Chen Y, Jiang G, Lau C, Yu WC, Bacher M, Fan ST: Macrophage migration inhibitory factor: roles in regulating tumor cell migration and expression of angiogenic factors in hepatocellular carcinoma. Int J Cancer 2003;107:22–29.
107 Mor-Vaknin N, Punturieri A, Sitwala K, Markovitz DM: Vimentin is secreted by activated macrophages. Nat Cell Biol 2003;5:59–63.
108 Ruff MR, Pert DB: Small cell carcinoma of the lung: macrophage-specific antigens suggest hemopoietic stem cell origin. Science 1984;225:1034–1036.
109 Kerschmann RL, Woda BA, Majno G: The fusion of tumor cells with host cells: reflections on an ovarian tumor. Persp Biol Med 1995;38:467–472.
110 Kluk MJ, Grant-Kels JM, Kerr P, Hoss D, Berke A, Claffey KP, Murphy M: Melanoma on the move: the progression of melanoma: novel concepts with histologic correlates. Am J Dermatopathol 2004;26:504–510.
111 Fernandes B, Sagman U, Auger M, Demetrio M, Dennis JW: β1,6-branched oligosaccharides as a marker of tumor progression in human breast and colon neoplasia. Cancer Res 1991;51:718–723.
112 Kang JY, Dolled-Filhart M, Ocal IT, Singh B, Lin CY, Dickson RB, Rimm DL, Camp RL: Tissue microarray analysis of hepatocyte growth factor/Met pathway components reveals a role for Met, matriptase, and hepatocyte growth factor activator inhibitor 1 in the progression of node-negative breast cancer. Cancer Res 2003;63:1101–1105.
113 Ihara S, Miyoshi E, Nakahara S, Sakiyama H, Ihara H, Akinaga A, Honke K, Dickson RB, Lin CY, Taniguchi N: Addition of beta1–6 GlcNAc branching to the oligosaccharide attached to Asn 772 in the serine protease domain of matriptase plays a pivotal role in its stability and resistance against trypsin. Glycobiology 2004;14:139–146.
114 Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Olson S, Grompe M: Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 2003;422:897–901.
115 Vassilopoulos G, Wang PR, Russell DW: Transplanted bone marrow regenerates liver by cell fusion. Nature 2003;422:901–904.
116 Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, Alvarez-Buylla A: Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 2003;425:968–973.
117 Weimann JM, Johansson CB, Trejo A, Blau HM: Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. Nat Cell Biol 2003;5:959–566.
118 Kozorovitsky Y, Gould E: Stem cell fusion in the brain. Nat Cell Biol 2003;5:952–954.
119 Camargo FD, Chambers SM, Goodell MA: Stem cell plasticity: from transdifferentiation to macrophage fusion. Cell Prolif 2004;37:55–65.
120 Camargo FD, Finegold M, Goodell MA: Hematopoietic myelomonocytic cells are the major source of hepatocyte fusion partners. J Clin Invest 2004;113:1266–1270.
121 Corti S, Locatelli F, Donadoni C, Guglieri M, Papadimitriou D, Strazzer S, Del Bo R, Comi GP: Wild-type bone marrow cells ameliorate the phenotype of SOD1-G93A ALS mice and contribute to CNS, heart and skeletal muscle tissues. Brain 2004;127:2518–2532.
122 Willenbring H, Bailey AS, Foster M, Akkari Y, Dorrell C, Olson S, Finegold M, Fleming WH, Grompe M: Myelomonocytic cells are sufficient for therapeutic cell fusion in liver. Nat Med 2004;10:744–748.
123 Daley GQ: Alchemy in the liver: fact or fusion? Nat Med 2004;10:671–672.
124 Rodic N, Rutenberg MS, Terada N: Cell fusion and reprogramming: resolving our transdifferences. Trends Mol Med 2004;10:93–96.
125 Aichel O: In Roux W (ed): Vorträge und Aufsätze über Entvickelungsmechanik Der Organismen. Leipzig, Wilhelm Engelmann, 1911, pp 1–115.
126 Goldenberg DM, Pavia RA, Tsao MC: In vivo hybridization of human tumour and normal hamster cells. Nature 1974;250:649–651.
127 Kerbel RS, Lagarde AE, Dennis J, Donaghue TP: Spontaneous fusion in vivo between normal host and tumor cells: possible contribution to tumor progression metastasis studied with a lectin-resistant mutant tumor. Mol Cell Biol 1983;3:523–538.

128 Larizza L, Schirrmacher V, Graf L, Pfluger E, Peres-Martinez M, Stohr M: Suggestive evidence that the highly metastatic variant ESb of the T-cell lymphoma Eb is derived from spontaneous fusion with a host macrophage. Int J Cancer 1984;34:699–707.
129 Busund LT, Killie MK, Bartnes K, Seljelid R: Spontaneously formed tumorigenic hybrids of Meth A sarcoma cells and macrophages in vivo. Int J Cancer 2003;106:153–159.
130 Direkze NC, Hodivala-Dilke K, Jeffery R, Hunt T, Poulsom R, Oukrif D, Alison MR, Wright NA: Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. Cancer Res 2004;64:8492–8495.
131 Chakraborty A, Sodi S, Rachkovsky M, Kolesnikova N, Platt JT, Bolognia JL, Pawelek JM: A spontaneous murine melanoma lung metastasis comprised of host x tumor hybrids. Cancer Res 2000;60:2512–2519.
132 Chakraborty AK, Pawelek J, Ikeda Y, Miyoshi E, Kolesnikova N, Funasaka Y, Ichihashi M, Taniguchi N: Fusion hybrids with macrophage and melanoma cells up-regulate N-acetylglucosaminyltransferase V, beta1–6 branching, and metastasis. Cell Growth Differ 2001;12:623–630.
133 Chakraborty A, Lazova R, Davies S, Bäckvall H, Ponten F, Brash D, Pawelek J: Donor DNA in a renal cell carcinoma metastasis from a bone marrow transplant recipient. Bone Marrow Transplant 2004;34:183–186.
134 Yilmaz Y, Lazova R, Qumsiyeh M, Cooper D, Pawelek J: Donor Y chromosome in renal carcinoma cells of a female BMT recipient. Bone Marrow Transplant 2005;35:1021–1024.
135 Rachkovsky MS, Sodi S, Chakraborty A, Avissar Y, Bolognia J, McNiff JM, Platt J, Bermudes D, Pawelek J: Enhanced metastatic potential of melanoma x macrophage fusion hybrids. Clin Exp Metastasis 1998;16:299–312.
136 Holmgren L, Bergsmedh A, Spetz AL: Horizontal transfer of DNA by the uptake of apoptotic bodies. Vox Sang 2002;83(suppl 1):305–306.
137 Vignery A: Macrophage fusion: are somatic and cancer cells possible partners? Trends Cell Biol 2005;4:188–193.
138 Duelli DM, Hearn S, Myers MP, Lazebnik Y: A primate virus generates transformed human cells by fusion. J Cell Biol 2005;171:493–503.
139 Bjerkvig R, Tysnes BB, Aboody KS, Najbauer J, Terzis AJ: Opinion: the origin of the cancer stem cell: current controversies and new insights. Nat Rev Cancer 2005;5:899–904.
140 Pawelek JM, Chakraborty AK, Rachkovsky M, Orlow SJ, Bolognia JL, Sodi SA: Altered N-glycosylation in macrophage x melanoma fusion hybrids. Cell Mol Biol 2000;45:1011–1027.
141 Rachkovsky M, Pawelek J: Acquired melanocyte stimulating hormone-inducible chemotaxis following macrophage fusion with Cloudman S91 melanoma cells. Cell Growth Differ 1999;10:515–524.
142 Siddiqui SF, Pawelek J, Handerson T, Lin CY, Dickson RB, Rimm DL, Camp RL: Coexpression of beta1,6-N-acetylglucosaminyltransferase V glycoprotein substrates defines aggressive breast cancers with poor outcome. Cancer Epidemiol Biomarkers Prev 2005;14:2517–2523.
143 Fidler IJ: Selection of successive tumour lines for metastasis. Nat New Biol 1973;24:148–149.
144 Nowell, PC: The clonal evolution of tumor cell populations. Science 1976;194:23–28.
145 Fidler IJ, Kripke ML: Metastasis results from preexisting variant cells with a malignant tumor. Science 1977;197:893–895.
146 Van Noorden CJF, Meade-Tollin LC, Bosman FT: Metastasis. Amer Sci 1989;86:130–141.
147 Marte B: Cell division and cancer. Nature 2004;432:293.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 156–169

Carcinogenesis Driven by Bone Marrow-Derived Stem Cells

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Abstract

The overall mechanism of bone marrow-derived stem cell (BMDC) trans-differentiation seems to be simple: BMDCs trans-differentiate as referred to the blueprint, which is given by the tissue itself. Thereby, the blueprint can be the local tissue micro-environment (defined by the tissue-specific cytokine, chemokine, adhesion molecule pattern, etc.), it can be a single cell (cell fusion), or it can be a combination of both. In fact stem cell trans-differentiation is a complex not yet fully understood process. In between the start- and stop-points of trans-differentiation several gene reprogramming steps have to occur in a sequential step-by-step manner, for which a defined set of instructions is a prerequisite to ensure an accurate trans-differentiation. However, a recent study indicated that the ability of BMDCs – to adopt tissue function by reading its blueprint – seems to be a double-edged sword since BMDCs that have received a faulty blueprint, provided by chronically inflamed tissue, trans-differentiated into a neoplastic phenoytpe. Here, we review the importance of an accurate blueprint for BMDC trans-differentiation and discuss a model showing that BMDCs might contribute to overall tumor development due to recruitment to tumor tissue.

The plasticity of bone marrow-derived stem cells (BMDCs) such as hematopoietic stem cells and mesenchymal stem cells has opened new perspectives for novel clinical therapeutic strategies to restore the function of damaged tissues by using these particular cell types. Within one decade, lots of studies have broadened our horizon on the differentiation capacities of BMDCs, how they are recruited to damaged tissue and how trans-differentiation of BMDCs into tissue cells is instructed. Although the overall mechanism of trans-differentiation sounds simple – the blueprint for trans-differentiation is given the local tissue micro-environment (defined by a tissue-specific cytokine, chemokine, adhesion molecule, etc., pattern), a single cell (cell fusion), or a combination of both – the process in detail is complex and poorly understood.

Trans-differentiation can be subdivided into an initiation, maintenance and finalization phase, whereby the complete process is accompanied by several gene reprogramming steps in which stem cell related genes are successively switched off and tissue specific genes are sequentially turned on. Thus, with ongoing trans-differentiation BMDCs lose more and more stem cell qualities, but gain more and more tissue specific features. All these steps must be directed and controlled in a proper manner to ensure an accurate stem cell trans-differentiation for which a correct blueprint is fundamental.

The fact that a correct blueprint is prerequisite for an accurate trans-differentiation tends to the question what could be the phenotype of a BMDC that has trans-differentiated as referred to a faulty blueprint as it is given by chronically inflamed tissue and/or tumor tissue? Answers to this question will be discussed here.

Instruction of Stem Cell Trans-Differentiation

The first hints that BMDCs or extrahepatic stem cells can trans-differentiate into hepatocytes were derived from cross-gender and whole transplantation studies in rodents [1, 2]. In these studies the authors were able to identify male-derived cells, as indicated by the presence of the Y chromosome, in female liver tissue displaying typical liver characteristics such as albumin expression [1]. The presence of the Y chromosome indicated that BMDCs of male origin must have migrated into liver tissue and subsequently differentiated into hepatocytes. Based on these studies, Alison et al. [3] were able to show that such a mechanism also exists in humans. By analyzing liver biopsies of females, who once received a bone marrow transplantation from a male donor, the authors detected Y chromosome-positive cytokeratin 8-expressing cells in the female liver tissue. Similar results were reported by Korbling et al. [4] showing that circulating stem cells can differentiate into mature hepatocytes and epithelial cells of the skin and the gastrointestinal tract.

However, in the past years it has been shown that BMDCs can trans-differentiate into a variety of tissues including skeletal muscle [5–7], hepatocytes [8–11], epithelial cells [12], neurons [13–15], endothelial cells [16, 17] and cardiomyocytes [16, 17]. The capacity to trans-differentiate has also been shown for mesenchymal stem cells from bone marrow [18, 19], processed lipoaspirate (PLA) cells from adipose tissue [20], neural stem cells [21], and even for cells of the myelomonocytic lineage [22–25]. The process of trans-differentiation presupposes the presence of a defined set of instructions that directs, for example, the differentiation of a hematopoietic stem cell into a liver cell. To date, there are two known mechanisms that direct stem cell trans-differentiation

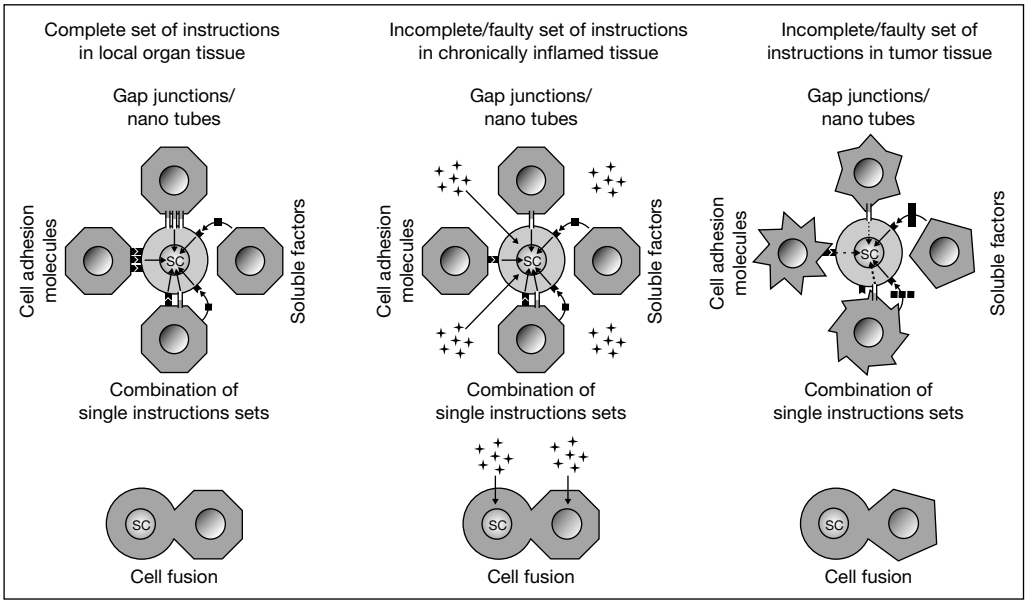

Fig. 1. Instructions of stem cell trans-differentiation are given by the local tissue milieu. BMDCs can differentiate to new local cells. Thereby, the complete blueprint, which directs BMDC trans-differentiation to the organ cell is given by the organ specific milieu itself, by an organ cell, or by a combination of both. The question is what might happen to a BMDC within chronically inflamed or tumor tissue. Due to the hypothesized incomplete or faulty blueprint (indicated by different arrow styles) given by chronically inflamed tissue or tumor tissue BMDC, trans-differentiation might deviate from normal organ tissue.

(fig. 1). Both mechanisms have been validated by several in vitro and in vivo experiments. In the first mechanism, trans-differentiation is instructed by the local tissue milieu [26–28]; in the second mechanism, trans-differentiation is directed by fusion of a BMDC with a tissue cell [8, 10, 18, 22, 24, 29–33].

The instructions of the local tissue milieu for stem cell trans-differentiation can be delivered by several mechanisms such as (1) soluble factors secreted by tissue cells [28]; (2) intercellular communication via gap junctions [34] and/or nanotubular highways [35–37], and (3) adhesion molecules [38–43]. It can be assumed that all of these mechanisms participate in trans-differentiation, but it still remains unclear to which extent they are involved in this process. Co-culture experiments with injured murine liver tissue and murine hematopoietic stem cells, both separated by a trans-well membrane (pore size 0.4 μm), revealed that proteins are detectable in hematopoietic stem cells within 48 h that are characteristically expressed during the differentiation to liver (e.g. GATA4, NHF4)

and are found in mature liver (e.g. cytokeratin 18, albumin) [26]. These data indicate that soluble factors can at least induce and maintain stem cell trans-differentiation. It is well-recognized that intercellular gap junction communication (GJIC) plays a crucial role in mediating several cellular functions including control of cell growth and differentiation [44], adaptive functions of differentiated cells [45] and apoptosis [46]. The diameter of one gap junction is around 2 nm and the cut-off level of molecules is around 1–2 kDa, which is sufficient for the intercellular exchange of ions, nucleotides [47, 48] and even small proteins [49]. An impaired or lack of GJIC is associated with severe diseases including visceroatrial heterotaxia [50], hereditary non-syndromic sensorineural deafness [51] and even cancer [52–54]. Studies of Trosko et al. [34] indicated that stem cells do not appear to have gap junctions, but GJIC of tissue stem cells increases with successive differentiation steps. Thus, it might be speculated that GJIC occurs at a later stage of stem cell trans-differentiation and that it might be involved in finalizing the process. In addition to the exchange of information between two cells via gap junctions, a recent study indicated that two cells can also exchange information via so-called tunneling nanotubes (TNTs) [37]. Those TNTs form a cytoplasma bridge between two cells large enough for the transport of even whole cell organelles.

The question whether cell fusion is the principle source of bone marrow-derived hepatocytes, as stated by Wang et al. [11] in their work, is still a subject of controversial discussions. On the one hand, two independent studies have shown that mouse progenitor cells of the central nervous system [33] as well as mouse bone marrow cells [8] can spontaneously fuse with mouse embryonic stem cells and subsequently adopt the phenotype of the recipient cell. Additionally, the tetraploid hybrids exhibit full pluripotent character, including multilineage contribution to chimaeras [33]. Further studies by Alvarez-Dolado et al. [55] demonstrated that BMDCs fuse in vivo with hepatocytes in liver, Purkinje neurons in the brain and cardiac muscle in the heart, resulting in the formation of multinucleated cells. Studies by Wang et al. [11] and Vassilopoulos et al. [10] revealed that liver function is completely restored after fusion of hepatocytes with BMDCs. Similar to these studies, two independent groups have recently shown that hematopoietic myelomonocytic cells are the major source of hepatocyte fusion partners, thereby restoring liver function [22, 24]. However, although cell fusion is an appealing descriptive model of how a stem cell adopts the phenotype of a tissue cell, several questions remain unanswered. First, multinucleated cells are predominantly found in those tissues known to be harboring polyploid cells such as brain, liver and heart. Other tissues were negative for multinucleated cells. Thus, it remains unclear whether cell fusion might be a general mechanism for stem cell trans-differentiation or if cell fusion is restricted to certain cell types and tissues. However, Ying et al. [33] reported that

fusion of mouse progenitor cells of the central nervous system with mouse embryonic stem cells can give rise to chimaeras and that these chimaeras harboring tetraploid cells. But, this cell fusion occurred under in vitro conditions and severe selection conditions were employed to obtain fused cells. Thus it is difficult to transfer these findings to the in vivo situation. Second, those studies demonstrating liver restoration by cell fusion used the mouse model of tyrosinemia type I for their studies [10, 11, 22, 24]. These mice are dominant negative for the fumarylacetoacetate hydrolase (FaH), which is a key enzyme in the tyrosine degradation process. Mutant mice have progressive liver failure and renal tubular damage unless treated with 2-(2-nitro-4-trifluoro-methyl-benzoyl)-1,3-cyclohexadione (NTBC) [56]. In all studies, NTBC application was withdrawn immediately after introduction of bone marrow cells of FaH-positive mice. Again, severe selection conditions were employed and thus it may be speculated that only under these unusual conditions cell fusion might play a role in tissue restoration. As mentioned above, several studies have indicated that BMDCs can trans-differentiate without fusion [26–28].

In the context of cell fusion as a potential trans-differentiation mechanism, the question about the genetic stability of hybrid cells is of pivotal interest. Both, Wang et al. [11] and Vassilopoulos et al. [10] showed that both FaH−/− wild-type and hybrid cells carried an aneuploid karyotype. For instance, Wang et al. [11] identified a single hybrid cell containing 167,5X3Y chromosomes and Vassilopoulos et al. [10] detected hybrid cells showing a karyotype of up to 18N. It is not yet clear whether this aneuploid karyotype is attributed to an unequal chromosome segregation in hybrid cells or if it is caused due to genomic instability because of FaH deficiency itself [57]. Nonetheless, the finding of an aneuploid karyotype might bear a risk in view of the hypothesis that aneuploidy might play a key role in carcinogenesis [58, 59]. Willenbring et al. [24] observed no malignant transformation in their study, but suggested that the potential hazard posed by aneuploidy demands further investigations.

What Happens if BMDC Trans-Differentiation Is Directed by a Faulty Blueprint

Trans-differentiation of stem cells is a complex mechanism and presupposes the presence of a defined set of instructions regulating the initiation, the maintenance, and the finalization of this process. Although the exact mechanism in detail of stem cell differentiation remains unclear, it is generally accepted that a stem cell is a fragile cell type susceptible to various factors, agents, or compounds with differentiating capacities. For instance, Trosko [see Trosko and Tai, this vol, pp 45–65] postulated in his stem cell theory of

carcinogenesis that a neoplastic phenotype could emerge from a tissue stem cell due to carcinogen exposition [53, 54]. Here, the carcinogen acts as an additional differentiating agent on the maturing tissue stem cell and interferes with a correct tissue stem cell differentiation process, which ultimately can give rise to the evolution of a malignant phenotype [60]. In other words, stem cells receiving a faulty set of instructions can (trans-)differentiate into a neoplastic phenotype.

Several recent studies now have proven this hypothesis and the results indicate that cancer might be a disease that originated from stem cells that received a faulty set of instructions. A recent study has shown that bronchioalveolar stem cells were identified as the putative cells of origin for lung adenocarcinoma [61]. Additionally, Theise et al. [62] demonstrated that hepatocellular carcinoma and cholangiocarcinoma are derived from hepatic progenitor cells. These tumors were identified in livers with features of chronic hepatitis caused by chronic hepatitis B virus, or hepatitis C virus infection, or chronic alcohol liver injury, which let assume that the chronic inflamed micro-environment and/or the chronic virus infection might be responsible for the delivery of the faulty set of instructions. In this connection, a recent study by Houghton et al. [63] is of interest by showing that gastric cancer is of BMDC origin. Here the authors showed that a chronic infection of C57BL/6 mice with *Helicobacter felix*, a known carcinogen, induced the repopulation of the stomach with BMDCs. Subsequently, these cells progress through metaplasia and dysplasia to intraepithelial cancer [63].

The finding that tissue progenitor cells or recruited BMDCs can give rise to a malignant phenotype within a chronically inflamed micro-environment indicate that the faulty set of instructions must be delivered by the chronically inflamed tissue itself. Thereby, chronic inflammation is characterized by the presence of various cell types including monocytes/macrophages, neutrophil granulocytes, lymphocytes, mast cells, fibroblasts, epithelial cells and endothelial cells, and the complex interplay between them mediated by chemokine, cytokines, reactive oxygen and nitrogen species, and proteases [64, 65]. Reactive oxygen and nitrogen species can cause DNA damage, can prevent DNA repair, and can lead to the inactivation of tumor suppressor genes. The growth and survival of malignant cells is facilitated by cytokines and chemokines, whereas the tissue is remodelled by proteases. Moreover, the interplay of chemokines, cytokines, reactive oxygen and nitrogen species, and proteases does also have an impact on the modulation of cell-cell communication via gap junctions and adhesion molecules. Thus, a chronically inflamed microenvironment possibly results in an alteration of the instructions, which are required for a correct (trans-)differentiation of tissue stem cells or recruited BMDCs.

What Happens if Tumor Tissue Is the Blueprint for BMDC Trans-Differentiation?

The scenario 'what happens if a BMDC has moved into tumor tissue' might be speculative, but the rationale of this hypothesis is given on the one hand due to the heterogeneity of tumor tissue [58, 66–71]. As a consequence, each single tumor cell expresses a defined set of proteins including cytokines, chemokines, proteases, and adhesion molecules. Thus, like in chronically inflamed tissue the instructions for stem cell trans-differentiation are likely different in tumor tissue as compared to normal tissue. On the other hand, tumor tissue consists not only of tumor cells but rather is a mixture of various cell types including tumor cells, fibroblasts, lymphocytes and macrophages. Thereby, a pivotal role in tumor growth has been attributed to the so-called tumor-associated macrophages (TAMs), which are a major component of most, if not all, tumors [72]. Evidence has emerged for a symbiotic relationship between tumor cells and TAMs, in which tumor cells attract TAMs and sustain their survival, with TAMs then responding to micro-environmental factors in tumors such as hypoxia by producing important mitogens as well as various growth factors and enzymes that stimulate tumor angiogenesis [73]. However, in addition to those factors mediating the growth of tumor cells and the neovascularization of tumor tissue, TAMs also secrete proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6 as well as various chemokines including monocyte chemoattractant protein-1, -2, and -3 (MCP-1, MCP-2, MCP-3) [65, 74]. Thus, tumor tissue resembles in a way to chronically inflamed tissue and therefore tumors were often described as wounds that do not heal [75]. However, the finding that tumor growth is putatively associated with a latent chronically inflamed micro-environment might have the consequence that stem cells recruited by tumor tissue befall the same fate as stem cells that have been recruited to chronically inflamed tissue. Young et al. [76, 77] have shown that CD34+ hematopoietic progenitor cells are recruited to tumor tissue by tumor cell-derived vascular endothelial growth factor (VEGF). Recent studies clearly indicate that stromal cell-derived factor-1α (SDF-1α), which to date is the most prominent chemoattractant for hematopoietic stem cells [78], is as well expressed during inflammatory conditions [63, 79, 80] and is suggested to be a key mediator in BMDC recruitment to damaged tissue. Thereby, SDF-1α is expressed by various cell types including dendritic cells [81] and endothelial cells [80, 81]. Additionally, SDF-1α itself can also be expressed directly by tumor tissue [82, 83].

If we consider the local tissue milieu as the instructor for stem cell trans-differentiation [26–28] then the trans-differentiation of tumor-recruited BMDCs would take place in a substantially altered setting. This assumption is

in view with a recent study of Mengel et al. [84] showing that metanephric adenomas demonstrated microchimerism consisting of both donor- and recipient-derived tumor cells. The authors conclude from their results that except for metanephric adenomas, tumors arising in renal transplants originate completely from graft cells. Thus, the mixed derivation of metanephric adenomas indicates an incorporation of recipient-derived progenitor cells suggesting that adult stem cells can assume neoplastic phenotypes [84]. Similar results were reported from the Pawelek group studying solid tumors in patients who have received a bone marrow transplantation. Two cases were reported and in both cases the authors were able to identify donor-specific DNA in host-derived renal cell carcinomas [85, 86]. In accordance with that, Bhatia et al. [87] have recently shown that normal human prostate (NHP) epithelial cells, that also express several prostate progenitor/stem cell markers, can spontaneously fuse with 293T and other tumor cells, and thereby become immortalized and transformed.

Conclusion

The data presented in this chapter indicate that the ability of BMDCs to adopt tissue function – simply by following its instructions written down in the blueprint – is a double-edged sword: On the one hand, BMDCs can give rise to healthy tissue and on the other, a neoplastic phenotype can evolve. It only depends on the blueprint.

The process of BMDC recruitment into tumor tissue is not a static but rather a dynamically permanent process. Due to latent chronically inflammation conditions inside the tumor tissue and/or generation of BMDC recruiting cytokines/chemokines by the tumor tissue itself, a continuous recruitment of BMDCs into the tumor tissue should be the consequence. Due to the increasing number of mis-trans-differentiated BMDC within the tumor tissue newly recruited naive BMDCs should not only receive their blueprint from tumor cells, but additionally from mis-trans-differentiated BMDCs. The result of this process would be a heterogeneous tissue comprising of tumor cells and a pool of mis-trans-differentiated BMDCs (fig. 2a). However, due to the limited data available, it is difficult to elicit how and even if such BMDC-derived neoplastic cells will contribute to overall cancer development and even metastasis. It might be speculated that those cells, although neoplastic, will remain inside the tumor tissue without any action. However, it is also conceivable that those cells would boost tumor development and metastasis formation since mis-trans-differentiated BMDCs could still exhibit stem cell properties including telomerase activity and/or the ability to migrate in response to cytokines and chemokines. Moreover, BMDC trans-differentiation should be accompanied with an upregulation of cell-to-cell

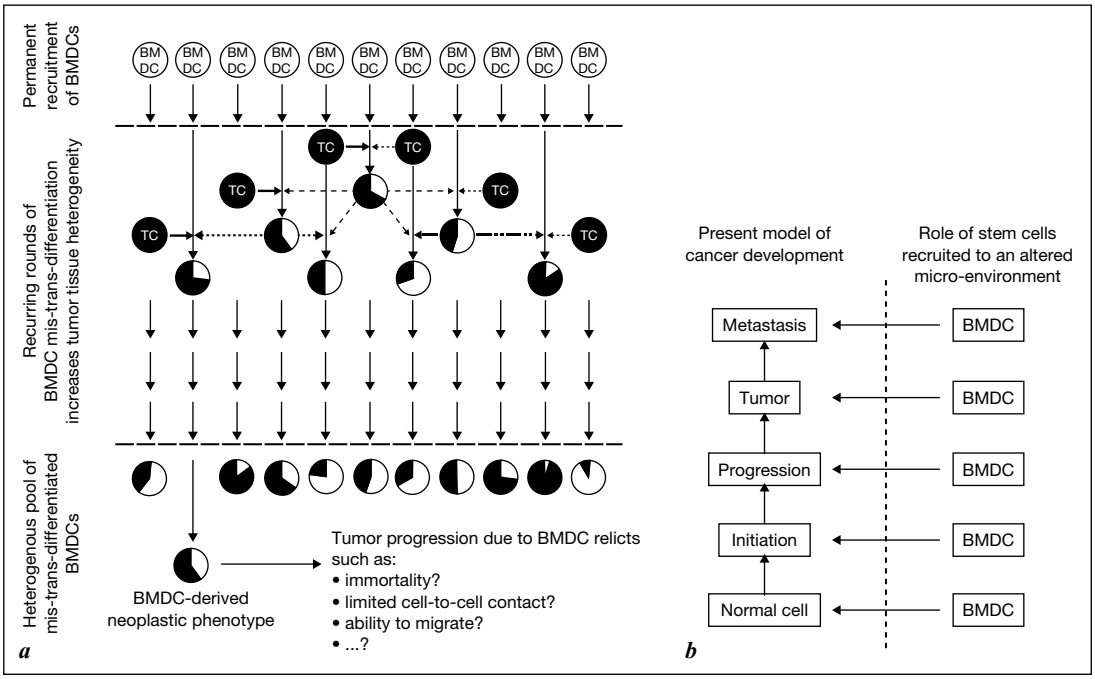

Fig. 2. Hypothesized role of stem cells in cancer development. ***a*** Due to heterogeneity of tumor tissue (indicated by different arrow styles) BMDC trans-differentiation should take place in a substantially altered setting. The resulting cell has adopted parts of surrounding tumor tissue (black), but still harbors stem cell features (white). Due to latent chronic inflammation and/or secretion of BMDC recruiting factor by the tumor tissue BMDCs are permanently recruited. Because of the increasing number of mis-trans-differentiated BMDCs, trans-differentiation of newly recruited naïve BMDCs should also be directed by mis-trans-differentiated BMDCs. The result of this process should be an increased tissue heterogeneity due to pools of mis-trans-differentiated BMDCs (indicated by varying amounts of black and white). Some of them ultimately can give rise to a neoplastic phenotype. It is conceivable that these cells contribute to overall tumor development due to possible persistent stem cell relicts such as immortality, the ability to migrate, and/or a reduced expression of cell adhesion molecules. ***b*** The mechanism shown in (***a***) can occur at every stage of tumor development.

contacts via cell adhesion molecules. Thus a limited number of cell adhesion molecules, due to an incorrect trans-differentiation, could result in a limited number of cell-to-cell contacts, which could promote the detachment of single cells from a cell cluster or tissue and facilitating metastasis (fig. 2a). Furthermore, due to the universal mechanism of BMDC recruitment and trans-differentiation the above-mentioned scenario is not restricted to the primary tumor alone, but rather can take place at any stage of tumor development (fig. 2b).

In summary, the universal mechanism of BMDC trans-differentiation – namely that the blueprint for this process is given by the tissue itself – might at the same time be the Achilles heel. BMDCs are 'blind', they do not differ between degenerated healthy tissue and degenerated malignant tissue. Once they have been recruited and receive the appropriate signals, BMDCs trans-differentiate in accordance to the instructions which are given by the tissue itself. In case of chronically inflamed tissue it was shown that BMDCs could be a source of neoplastic cells, which indicates a possible involvement of BMDCs in overall tumor development and even in metastasis formation.

Acknowledgments

The authors are grateful for the technical assistance of Silvia Keil. This work was supported by the Fritz-Bender-Foundation, Munich, and the Westermann-Westorp-Foundation, Essen, Germany.

References

1 Theise ND, Badve S, Saxena R, Henegariu O, Sell S, Crawford JM, Krause DS: Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. Hepatology 2000;31:235–240.
2 Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP: Bone marrow as a potential source of hepatic oval cells. Science 1999;284:1168–1170.
3 Alison MR, Poulsom R, Jeffery R, Dhillon AP, Quaglia A, Jacob J, Novelli M, Prentice G, Williamson J, Wright NA: Hepatocytes from non-hepatic adult stem cells. Nature 2000; 406:257.
4 Korbling M, Katz RL, Khanna A, Ruifrok AC, Rondon G, Albitar M, Champlin RE, Estrov Z: Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. N Engl J Med 2002;346:738–746.
5 Camargo FD, Green R, Capetenaki Y, Jackson KA, Goodell MA: Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. Nat Med 2003;9:1520–1527.
6 Fukada S, Miyagoe-Suzuki Y, Tsukihara H, Yuasa K, Higuchi S, Ono S, Tsujikawa K, Takeda S, Yamamoto H: Muscle regeneration by reconstitution with bone marrow or fetal liver cells from green fluorescent protein-gene transgenic mice. J Cell Sci 2002;115:1285–1293.
7 Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F: Muscle regeneration by bone marrow-derived myogenic progenitors. Science 1998;279: 1528–1530.
8 Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW: Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 2002;416:542–545.
9 Grompe M: The role of bone marrow stem cells in liver regeneration. Semin Liver Dis 2003;23:363–372.
10 Vassilopoulos G, Wang PR, Russell DW: Transplanted bone marrow regenerates liver by cell fusion. Nature 2003;422:901–904.
11 Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Olson S, Grompe M: Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 2003;422:897–901.

12 Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ: Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 2001;105:369–377.
13 Locatelli F, Corti S, Donadoni C, Guglieri M, Capra F, Strazzer S, Salani S, Del Bo R, Fortunato F, Bordoni A, Comi GP: Neuronal differentiation of murine bone marrow Thy-1- and Sca-1-positive cells. J Hematother Stem Cell Res 2003;12:727–734.
14 Mezey E, Chandross KJ: Bone marrow: a possible alternative source of cells in the adult nervous system. Eur J Pharmacol 2000;405:297–302.
15 Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR: Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science 2000;290:1779–1782.
16 Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, Entman ML, Michael LH, Hirschi KK, Goodell MA: Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest 2001;107:1395–1402.
17 Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P: Bone marrow cells regenerate infarcted myocardium. Nature 2001; 410:701–705.
18 Spees JL, Olson SD, Ylostalo J, Lynch PJ, Smith J, Perry A, Peister A, Wang MY, Prockop DJ: Differentiation, cell fusion, and nuclear fusion during ex vivo repair of epithelium by human adult stem cells from bone marrow stroma. Proc Natl Acad Sci USA 2003;100:2397–2402.
19 Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM: Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41–49.
20 Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH: Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002;13: 4279–4295.
21 Bjornson CR, Rietze RL, Reynolds BA, Magli MC, Vescovi AL: Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo. Science 1999;283:534–537.
22 Camargo FD, Finegold M, Goodell MA: Hematopoietic myelomonocytic cells are the major source of hepatocyte fusion partners. J Clin Invest 2004;113:1266–1270.
23 Kuwana M, Okazaki Y, Kodama H, Izumi K, Yasuoka H, Ogawa Y, Kawakami Y, Ikeda Y: Human circulating CD14+ monocytes as a source of progenitors that exhibit mesenchymal cell differentiation. J Leukoc Biol 2003;74:833–845.
24 Willenbring H, Bailey AS, Foster M, Akkari Y, Dorrell C, Olson S, Finegold M, Fleming WH, Grompe M: Myelomonocytic cells are sufficient for therapeutic cell fusion in liver. Nat Med 2004;10:744–748.
25 Zhao Y, Glesne D, Huberman E: A human peripheral blood monocyte-derived subset acts as pluripotent stem cells. Proc Natl Acad Sci USA 2003;100:2426–2431.
26 Jang YY, Collector MI, Baylin SB, Diehl AM, Sharkis SJ: Hematopoietic stem cells convert into liver cells within days without fusion. Nat Cell Biol 2004;6:532–539.
27 Ianus A, Holz GG, Theise ND, Hussain MA: In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest 2003;111:843–850.
28 Newsome PN, Johannessen I, Boyle S, Dalakas E, McAulay KA, Samuel K, Rae F, Forrester L, Turner ML, Hayes PC, Harrison DJ, Bickmore WA, Plevris JN: Human cord blood-derived cells can differentiate into hepatocytes in the mouse liver with no evidence of cellular fusion. Gastroenterology 2003;124:1891–1900.
29 Kozorovitskiy Y, Gould E: Stem cell fusion in the brain. Nat Cell Biol 2003;5:952–954.
30 Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD: Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc Natl Acad Sci USA 2003;100:12313–12318.
31 Reinecke H, Minami E, Poppa V, Murry CE: Evidence for fusion between cardiac and skeletal muscle cells. Circ Res 2004;94:e56–e60.
32 Shi D, Reinecke H, Murry CE, Torok-Storb B: Myogenic fusion of human bone marrow stromal cells, but not hematopoietic cells. Blood 2004;104:290–294.

33 Ying QL, Nichols J, Evans EP, Smith AG: Changing potency by spontaneous fusion. Nature 2002;416:545–548.
34 Trosko JE, Chang CC, Wilson MR, Upham B, Hayashi T, Wade M: Gap junctions and the regulation of cellular functions of stem cells during development and differentiation. Methods 2000;20: 245–264.
35 Francis K, Ramakrishna R, Holloway W, Palsson BO: Two new pseudopod morphologies displayed by the human hematopoietic KG1a progenitor cell line and by primary human CD34(+) cells. Blood 1998;92:3616–3623.
36 Fruehauf S, Srbic K, Seggewiss R, Topaly J, Ho AD: Functional characterization of podia formation in normal and malignant hematopoietic cells. J Leukoc Biol 2002;71:425–432.
37 Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH: Nanotubular highways for intercellular organelle transport. Science 2004;303:1007–1010.
38 Aplin AE, Howe A, Alahari SK, Juliano RL: Signal transduction and signal modulation by cell adhesion receptors: the role of integrins, cadherins, immunoglobulin-cell adhesion molecules, and selectins. Pharmacol Rev 1998;50:197–263.
39 Aplin AE, Juliano RL: Integrin and cytoskeletal regulation of growth factor signaling to the MAP kinase pathway. J Cell Sci 1999;112:695–706.
40 Ben-Ze'ev A, Shtutman M, Zhurinsky J: The integration of cell adhesion with gene expression: the role of beta-catenin. Exp Cell Res 2000;261:75–82.
41 Reyes-Reyes M, Mora N, Gonzalez G, Rosales C: Beta1 and beta2 integrins activate different signalling pathways in monocytes. Biochem J 2002;363:273–280.
42 Rosales C, O'Brien V, Kornberg L, Juliano R: Signal transduction by cell adhesion receptors. Biochim Biophys Acta 1995;1242:77–98.
43 Yarwood SJ, Woodgett JR: Extracellular matrix composition determines the transcriptional response to epidermal growth factor receptor activation. Proc Natl Acad Sci USA 2001;98: 4472–4477.
44 Loewenstein WR: Junctional intercellular communication: the cell-to-cell membrane channel. Physiol Rev 1981;61:829–913.
45 Meda P, Bruzzone R, Chanson M, Bosco D, Orci L: Gap junctional coupling modulates secretion of exocrine pancreas. Proc Natl Acad Sci USA 1987;84:4901–4904.
46 Trosko JE, Goodman JI: Intercellular communication may facilitate apoptosis: implications for tumor promotion. Mol Carcinog 1994;11:8–12.
47 Sirac Dilber M, Edvard Smith CI: Suicide genes and bystander killing: local and distant effects. Gene Ther 1997;4:273–274.
48 Freeman SM, Abboud CN, Whartenby KA, Packman CH, Koeplin DS, Moolten FL, Abraham GN: The 'bystander effect': tumor regression when a fraction of the tumor mass is genetically modified. Cancer Res 1993;53:5274–5283.
49 Neijssen J, Herberts C, Drijfhout JW, Reits E, Janssen L, Neefjes J: Cross-presentation by intercellular peptide transfer through gap junctions. Nature 2005;434:83–88.
50 Dasgupta C, Escobar-Poni B, Shah M, Duncan J, Fletcher WH: Misregulation of connexin43 gap junction channels and congenital heart defects. Novartis Found Symp 1999;219:212–221; discussion 221–225.
51 Kitamura K, Takahashi K, Tamagawa Y, Noguchi Y, Kuroishikawa Y, Ishikawa K, Hagiwara H: Deafness genes. J Med Dent Sci 2000;47:1–11.
52 Trosko JE, Ruch RJ: Gap junctions as targets for cancer chemoprevention and chemotherapy. Curr Drug Targets 2002;3:465–482.
53 Trosko JE: The role of stem cells and gap junctional intercellular communication in carcinogenesis. J Biochem Mol Biol 2003;36:43–48.
54 Trosko JE, Chang CC: Stem cell theory of carcinogenesis. Toxicol Lett 1989;49:283–295.
55 Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, Alvarez-Buylla A: Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 2003;425:968–973.
56 Grompe M, Lindstedt S, al-Dhalimy M, Kennaway NG, Papaconstantinou J, Torres-Ramos CA, Ou CN, Finegold M: Pharmacological correction of neonatal lethal hepatic dysfunction in a murine model of hereditary tyrosinaemia type I. Nat Genet 1995;10:453–460.

57 Jorquera R, Tanguay RM: Fumarylacetoacetate, the metabolite accumulating in hereditary tyrosinemia, activates the ERK pathway and induces mitotic abnormalities and genomic instability. Hum Mol Genet 2001;10:1741–1752.
58 Duesberg P, Rasnick D, Li R, Winters L, Rausch C, Hehlmann R: How aneuploidy may cause cancer and genetic instability. Anticancer Res 1999;19:4887–4906.
59 Li R, Sonik A, Stindl R, Rasnick D, Duesberg P: Aneuploidy vs. gene mutation hypothesis of cancer: recent study claims mutation but is found to support aneuploidy. Proc Natl Acad Sci USA 2000;97:3236–3241.
60 Trosko JE: Human stem cells as targets for the aging and diseases of aging processes. Med Hypotheses 2003;60:439–447.
61 Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T: Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell 2005;121:823–835.
62 Theise ND, Yao JL, Harada K, Hytiroglou P, Portmann B, Thung SN, Tsui W, Ohta H, Nakanuma Y: Hepatic 'stem cell' malignancies in adults: four cases. Histopathology 2003;43:263–271.
63 Houghton J, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR, Wang TC: Gastric cancer originating from bone marrow-derived cells. Science 2004;306:1568–1571.
64 Balkwill F, Mantovani A: Inflammation and cancer: back to Virchow? Lancet 2001;357:539–545.
65 Coussens LM, Werb Z: Inflammation and cancer. Nature 2002;420:860–867.
66 Cahill DP, Kinzler KW, Vogelstein B, Lengauer C: Genetic instability and Darwinian selection in tumours. Trends Cell Biol 1999;9:M57–M60.
67 Lengauer C, Kinzler KW, Vogelstein B: Genetic instability in colorectal cancers. Nature 1997;386:623–627.
68 Lengauer C, Kinzler KW, Vogelstein B: DNA methylation and genetic instability in colorectal cancer cells. Proc Natl Acad Sci USA 1997;94:2545–2550.
69 Lengauer C, Kinzler KW, Vogelstein B: Genetic instabilities in human cancers. Nature 1998;396:643–649.
70 Shih IM, Zhou W, Goodman SN, Lengauer C, Kinzler KW, Vogelstein B: Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. Cancer Res 2001;61:818–822.
71 Duesberg P, Li R, Rasnick D, Rausch C, Willer A, Kraemer A, Yerganian G, Hehlmann R: Aneuploidy precedes and segregates with chemical carcinogenesis. Cancer Genet Cytogenet 2000;119:83–93.
72 Mantovani A, Bottazzi B, Colotta F, Sozzani S, Ruco L: The origin and function of tumor-associated macrophages. Immunol Today 1992;13:265–270.
73 Bingle L, Brown NJ, Lewis CE: The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol 2002;196:254–265.
74 Balkwill F, Foxwell B, Brennan F: TNF is here to stay! Immunol Today 2000;21:470–471.
75 Dvorak HF: Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986;315:1650–1659.
76 Young MR, Kolesiak K, Wright MA, Gabrilovich DI: Chemoattraction of femoral CD34+ progenitor cells by tumor-derived vascular endothelial cell growth factor. Clin Exp Metastasis 1999;17:881–888.
77 Young MR, Petruzzelli GJ, Kolesiak K, Achille N, Lathers DM, Gabrilovich DI: Human squamous cell carcinomas of the head and neck chemoattract immune suppressive CD34(+) progenitor cells. Hum Immunol 2001;62:332–341.
78 Aiuti A, Webb IJ, Bleul C, Springer T, Gutierrez-Ramos JC: The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. J Exp Med 1997;185:111–120.
79 Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, Penn MS: Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003;362:697–703.
80 Kollet O, Shivtiel S, Chen YQ, Suriawinata J, Thung SN, Dabeva MD, Kahn J, Spiegel A, Dar A, Samira S, Goichberg P, Kalinkovich A, Arenzana-Seisdedos F, Nagler A, Hardan I, Revel M,

Shafritz DA, Lapidot T: HGF, SDF-1, and MMP-9 are involved in stress-induced human CD34+ stem cell recruitment to the liver. J Clin Invest 2003;112:160–169.
81 Pablos JL, Amara A, Bouloc A, Santiago B, Caruz A, Galindo M, Delaunay T, Virelizier JL, Arenzana-Seisdedos F: Stromal-cell derived factor is expressed by dendritic cells and endothelium in human skin. Am J Pathol 1999;155:1577–1586.
82 Sun YX, Wang J, Shelburne CE, Lopatin DE, Chinnaiyan AM, Rubin MA, Pienta KJ, Taichman RS: Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) in vivo. J Cell Biochem 2003;89:462–473.
83 Zou W, Machelon V, Coulomb-L'Hermin A, Borvak J, Nome F, Isaeva T, Wei S, Krzysiek R, Durand-Gasselin I, Gordon A, Pustilnik T, Curiel DT, Galanaud P, Capron F, Emilie D, Curiel TJ: Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. Nat Med 2001;7:1339–1346.
84 Mengel M, Jonigk D, Wilkens L, Radermacher J, von Wasielewski R, Lehmann U, Haller H, Mihatsch M, Kreipe H: Chimerism of metanephric adenoma but not of carcinoma in kidney transplants. Am J Pathol 2004;165:2079–2085.
85 Yilmaz Y, Lazova R, Qumsiyeh M, Cooper D, Pawelek J: Donor Y chromosome in renal carcinoma cells of a female BMT recipient: visualization of putative BMT-tumor hybrids by FISH. Bone Marrow Transplant 2005;35:1021–1024.
86 Chakraborty A, Lazova R, Davies S, Backvall H, Ponten F, Brash D, Pawelek J: Donor DNA in a renal cell carcinoma metastasis from a bone marrow transplant recipient. Bone Marrow Transplant 2004;34:183–186.
87 Bhatia B, Schneider-Broussard R, Calhoun T, Tang S, Zhou J, Schroeder L, Shen J, Multani AS, Pathak S, Chang S, Tang DT: Replicative senescence and cell fusion-mediated immortalization and tumorigenic transformation of normal human prostate (NHP) epithelial cells: implications in prostate tumorigenesis and tumor heterogeneity. Proc Am Assoc Cancer Res 2005;46:2060.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 170–190

Chemokine-Directed Metastasis

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Abstract

Over the last 20 years, the biology of chemokines has expanded beyond their initial role in mediating migration of specific subsets of leukocytes. Chemokines have been found to display pleiotropic effects for enhancing immunity to tumor-associated antigens, regulating angiogenesis, promoting proliferation/anti-apoptosis of tumor cells; and mediating tumor cell invasion and trafficking in an organ-specific manner that leads to metastases. Here, we review the importance of chemokines, especially CXC chemokines in regulating angiogenesis, tumor cell invasion and metastases; and demonstrate why they can be seen as important therapeutic targets for intervention in cancer.

Chemokines are chemotactic cytokines that are classified into four groups based on the position of the first two cysteine amino acid residues within the amino terminus: CXC, CC, C and CX_3C. CXC chemokines act largely on neutrophils, lymphocytes, and endothelial cells, whereas CC chemokines act on several cell types including monocytes, dendritic cells, basophils, eosinophils, and lymphocytes. Chemokines are important in directed cell migration, which is achieved through a seven transmembrane chemokine receptor on cells. Upon activation and signaling of the chemokine receptor, cells then traffic in response to a chemokine ligand gradient. CXC chemokines function to enhance innate and adaptive immunity, regulate angiogenesis, prevent apoptosis, promote proliferation and mediate tumor cell metastases. The functions of CXC chemokines have a direct impact on both the biology of cancer and the host's response to the tumor. Tumor growth, tumor-associated angiogenesis, invasion, and metastasis to distant organs are dependent on a highly orchestrated series of events that include: pre-neoplastic to neoplastic cellular transformation;

establishment of a pro-angiogenic environment; local tumor cell growth; loss of adherence to adjacent cells and/or extracellular matrix (ECM) followed by local invasion through ECM/vascular basement membrane and entry into the circulation. This tumor cell trafficking, extravasation, and growth as metastases in distant organs was first described by Paget [1] in 1889 and is known as Paget's theory of 'seed and soil', where tumor cells ('the seeds') metastasize and find a new niche in a specific organ ('the soil').

The above events that destine a tumor cell to invade and metastasize to distant organs in a specific manner are analogous to leukocyte maturation, subsequent entry into the circulation, and eventual homing to specific tissue sites. Over the last 20 years it has been recognized that chemokines have an increasingly important role in mediating the trafficking of populations of leukocytes under both conditions of homeostasis and inflammatory/immunological responses. In addition, numerous studies over the last decade have demonstrated that specific expression of chemokines and their receptors in the context of cancer are essential events that appear to be important in either promoting tumor growth, tumor-associated angiogenesis, and metastasis; or for inhibiting tumor growth via attenuation of tumor-associated angiogenesis. These studies highlight the expanding role that chemokines play in promoting autocrine, paracrine, and hormonal influence for successful tumor growth, tumor-associated angiogenesis, tumor invasion, and metastasis to distant organs. In this chapter, we will review the role that chemokines, especially CXC chemokines, play in regulating tumor-associated angiogenesis, tumor cell invasion, and metastasis (fig. 1).

Angiogenesis

Tumor growth, invasion and metastasis are dependent on a pro-angiogenic environment. While several factors have been found to promote angiogenesis, specific CXC chemokines have increasingly been demonstrated to significantly contribute to net angiogenesis in a variety of tumors. CXC chemokines are heparin binding proteins that contain four highly conserved cysteine amino acid residues with the first two cysteines separated by a non-conserved amino acid residue [2–4]. Several CXC chemokines also possess three amino acid residues – Glu-Leu-Arg; the so-called 'ELR' motif – at the NH2 terminus preceding the first cysteine amino acid residue. Chemokines that contain the ELR motif – ELR-positive – are pro-angiogenic, whereas members that lack the ELR motif – ELR-negative – and are in general interferon-inducible inhibit angiogenesis [2, 3, 5].

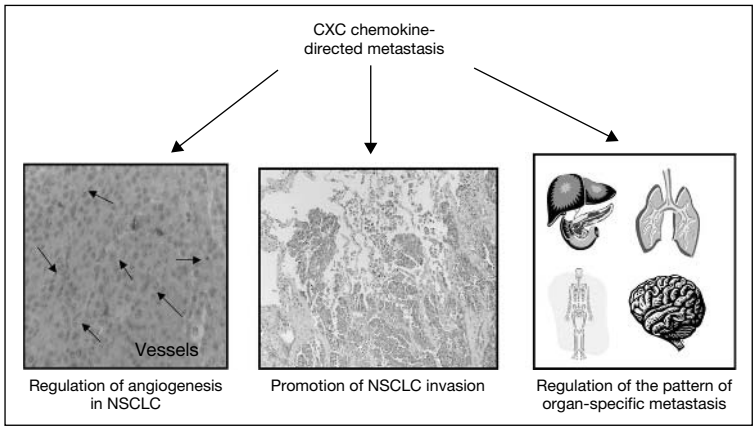

Fig. 1. CXC chemokines are pleiotropic cytokines in cancer biology. Chemokines are important in the regulation of angiogenesis, the promotion of tumor cell invasion, and the regulation of the pattern of organ-specific metastasis.

ELR Positive CXC Chemokines Promote Angiogenesis

The CXC chemokine family members that are ELR positive and promote angiogenesis are CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8 (table 1) [2–4, 6]. Angiogenic factors in a local microenvironment can function in a direct, parallel, or serial manner to promote angiogenesis. For example, a serial mechanism for the maintenance of an angiogenic micro-environment has been demonstrated by vascular endothelial cell growth factor (VEGF)-dependent activation of endothelial cells which can lead to up-regulation of the anti-apoptotic molecule Bcl-2 that in turn promotes the expression of endothelial cell-derived CXCL8 [7]. The upregulated expression of CXCL8 functions in an autocrine and paracrine manner to maintain the angiogenic phenotype of the endothelial cell [7]. Moreover, the upregulation of Bcl-2 expression in human endothelial cells that constitute tumor microvessels enhances intratumoral microvascular survival and density. Furthermore, co-implantation of human endothelial cells over-expressing Bcl-2 and tumor cells resulted in a 3-fold enhancement of tumor growth when compared with the co-implantation of control human endothelial cells and tumor cells. CXCL8-neutralizing antibodies attenuated this angiogenic activity in vitro and in vivo [7].

The autocrine and paracrine effect of VEGF in promoting the expression of CXCL8 has been further substantiated in a recent finding for neutrophils in the promotion of angiogenesis by a paracrine feed-forward mechanism involving endothelial cell-derived CXCL8. Activation of neutrophils with N-formyl-Met-Leu-Phe (fMLP) resulted in the generation of pro-angiogenic activity that was

Table 1. CXC chemokine family members that regulate angiogenesis in cancer

Angiogenic ELR-positive CXC chemokines[1]	
CXCL8 (interleukin-8; IL-8)	[20, 25, 28, 31, 33, 35]
CXCL5 (epithelial cell-derived neutrophil attractant-78; ENA-78)	[25, 28]
CXCL1 (growth-regulated oncogene-α; GRO-α)	
CXCL2 (growth-regulated oncogene-β; GRO-β)	[18, 19]
CXCL3 (growth-regulated oncogene-γ; GRO-γ)	
CXCL6 (GCP-2)	[2–4, 6]
Platelet basic protein (PBP), precursor protein of:	
Connective tissue-activating protein-III (CTAP-III)	[2–4, 6]
β-Thromboglobulin (β-TG)	[2–4, 6]
CXCL7 (neutrophil-activating protein-2; NAP-2)	[2–4, 6]
Angiostatic non-ELR positive CXC chemokines[2]	
CXCL4, CXCL4L1 (platelet factor 4; PF4)	
CXCL14 (breast and kidney cell chemokine; BRAK)	
Interferon-inducible non-ELR-positive CXC chemokines	
CXCL10 (interferon-inducible protein-10; IP-10)	[66, 70]
CXCK9 (monokine-induced by γ-interferon, MIG)	[68, 75]
CXCL11 (interferon-inducible T cell α-chemoattractant; ITAC)	

[1]CXCR2 is the putative receptor for their biological activity.
[2]CXCR3 is the putative receptor for their biological activity (except CXCL14).

related to VEGF and CXCL8 [8]. Moreover, VEGF induced the expression of CXCL8 from endothelial cells that was associated with formation of CXCL8-dependent capillary-like structures. In addition, there are other serial pathways that promote CXC chemokine-mediated angiogenesis through activation of seven transmembrane G protein coupled receptors and receptor tyrosine kinases (RTKs) that contribute to the expression of angiogenic CXC chemokines via NF-κB activation in cancer cells and enhanced tumor-associated angiogenesis [9–12]. These results demonstrate the existence of novel paracrine and autocrine signal pathways that lead to enhanced neovascularization that are related to angiogenic ELR-positive CXC chemokines.

The ability of all ELR positive CXC chemokines to bind to CXCR2 supports the notion that this receptor mediates the angiogenic activity of all the ELR-positive CXC chemokines. While CXCR1 and CXCR2 are detected in endothelial cells [13–15], the expression of CXCR2 has been found to be the primary functional chemokine receptor in endothelial cell chemotaxis. Heidemann et al. [16] found that endothelial cells respond to CXCL8 by rapid stress fiber assembly, chemotaxis, enhanced proliferation and phosphorylation of ERK1/2 through activation of CXCR2. Blocking the function of CXCR2

by using specific neutralizing antibodies or inhibiting downstream signalling with inhibitors of ERK1/2 or PI3kinase impaired CXCL8-induced stress fiber assembly, chemotaxis and endothelial tube formation in endothelial cells. In addition, lung cancer placed into CXCR2−/− mice, as compared to CXCR2+/+ mice demonstrated reduced tumor growth, increased tumor-associated necrosis, decreased tumor-associated angiogenesis, and reduced metastatic potential [17].

The Role of ELR-Positive CXC Chemokines in Tumorigenesis

The ELR positive CXC chemokines are important mediators of tumorigenesis related to their angiogenic properties. Using melanoma tumor models, studies have demonstrated that CXCL1, CXCL2, and CXCL3 play a significant role in mediating tumorigenesis related to both their mitogenic and angiogenic activities [18]. For example, ELR positive CXC chemokines have been found to be highly expressed in human melanomas [18]. To determine the biological significance of the presence of these ELR positive CXC chemokines in melanoma, human CXCL1, CXCL2, and CXCL3 genes were transfected into immortalized murine melanocytes [18, 19]. The persistent expression of CXCL1, CXCL2, or CXCL3 in these cells transformed their phenotype to one with anchorage-independent growth and the ability to form tumors in immuno-competent mice [18, 19]. The tumors were highly vascular and demonstrated similar vascularity to that of B16 melanoma controls [18, 19]. When tumors were depleted of CXCL1, CXCL2, or CXCL3 there was a marked reduction in tumor-derived angiogenesis which was directly related to the inhibition of tumor growth [18, 19]. These findings support the notion that the ELR-positive CXC chemokines have the ability to act as both autocrine growth factors for melanoma and as potent paracrine mediators in the promotion of tumor-associated angiogenesis.

The progression and growth of ovarian carcinoma is also dependent on angiogenesis, and CXCL8 has been determined to play a significant role in mediating human ovarian carcinoma-derived angiogenesis and tumorigenesis [20]. The expression of CXCL8, basic fibroblast growth factor (bFGF), and VEGF was examined in different human ovarian carcinoma cell lines [20]. All cell lines expressed similar levels of bFGF in vitro, however, these cells expressed either high or low levels of CXCL8 or VEGF. When implanted into the peritoneum of immuno-incompetent mice, the high expressing CXCL8 tumors were associated with early death in all animals (<51 days) [20]. The expression of CXCL8 was directly correlated with neovascularization and inversely correlated with survival, whereas VEGF expression was only correlated with production of ascites [20]. No correlation was found for bFGF with either tumor neovascularization or survival [20]. This study has been substantiated in patients with ovarian cancer, where ascites fluid demonstrated

angiogenic activity directly correlated to CXCL8 [21]. These findings support the notion that angiogenic ELR-positive CXC chemokines play a greater role than bFGF and VEGF in mediating angiogenesis associated with ovarian cancer.

CXCL8 is markedly elevated and contributes to the overall angiogenic activity of non-small-cell lung cancer (NSCLC) [22]. Using an in vivo model system of human tumorigenesis (i.e. human NSCLC/severe combined immunodeficiency (SCID) mouse chimera) [23], tumor-derived CXCL8 correlated directly with tumorigenesis [23]. Tumor-bearing animals depleted of CXCL8 demonstrated a >40% reduction in tumor growth and a reduction in spontaneous metastases [23]. The attenuation of tumor growth and metastases was directly correlated to reduced tumor-associated angiogenesis [23]. This study has been further corroborated using several human NSCLC cell lines grown in nude mice. NSCLC cell lines that constitutively express CXCL8 displayed greater tumorigenicity that correlated directly with angiogenesis [24].

While CXCL8 was the first angiogenic ELR positive CXC chemokine to be discovered in NSCLC, CXCL5 has a higher degree of correlation with NSCLC-derived angiogenesis [25]. Surgical specimens of NSCLC tumors demonstrate a direct correlation of CXCL5 with tumor angiogenesis. Using a SCID mouse model of human NSCLC tumorigenesis, CXCL5 expression directly correlated with tumor growth [25]. Moreover, when NSCLC tumor bearing animals were depleted of CXCL5, both tumor growth and spontaneous metastases were markedly attenuated [25]. The reduction of angiogenesis was also accompanied by an increase in tumor cell apoptosis, consistent with the previous observation that inhibition of tumor-derived angiogenesis is associated with increased tumor cell apoptosis [25]. While a significant correlation of CXCL5 exists with tumor-derived angiogenesis, tumor growth and metastases, CXCL5 depletion did not completely inhibit tumor growth [25]. This suggests that the angiogenic activity of NSCLC tumors is related to many overlapping and potentially redundant factors acting in a parallel or serial manner. However, when all ELR positive CXC chemokines are evaluated in human NSCLC, their presence directly correlates with patient mortality [26, 27].

Cyclooxygenase-2 (COX-2) has been shown to contribute to the progression of NSCLC tumorigenesis by enhancing the expression of angiogenic chemokines CXCL8 and CXCL5. COX-2 over-expressing NSCLC cell lines enhanced the in vitro expression of both CXCL8 and CXCL5. In contrast, specific COX-2 inhibition decreased the production of both chemokines as well as nuclear translocation of NF-κB. In a SCID mouse model of human NSCLC, the enhanced tumor growth of COX-2-overexpressing tumors was inhibited by neutralizing anti-CXCL5 and anti-CXCL8 antibodies, which was directly correlated with a reduction in tumor-associated angiogenesis [28].

Prostate cancer tumorigenesis and metastasis is also dependent on angiogenesis [29, 30]. Serum levels of CXCL8 have been found to be markedly elevated in patients with prostate cancer. These levels were highly correlated with the stage of the disease, independent of the ratio of free/total prostate-specific antigen (PSA) [30]. In fact, the combined use of free/total PSA and CXCL8 levels was more effective in distinguishing prostate cancer from benign prostate hypertrophy [30]. This suggests that ELR-positive CXC chemokines may play an important role in mediating prostate cancer-derived angiogenesis that supports tumorigenesis and metastasis. This observation in patients has been substantiated in human/SCID mice chimeras of human prostate cancer tumorigenesis [31]. Human prostate cancer cell lines were examined for constitutive production of angiogenic ELR-positive CXC chemokines [31]. Tumorigenesis of the human prostate cancer cell line, PC-3, was shown to be attributable, in part, to the production of the angiogenic CXC chemokine, CXCL8. Depletion of endogenous CXCL8 inhibited PC-3 tumor growth in SCID mice that was entirely attributable to inhibition of PC-3 tumor-derived angiogenesis [31]. In contrast, the human prostate cancer cell line, DU145, was found to utilize a different angiogenic ELR positive CXC chemokine, CXCL1, to mediate tumor-derived angiogenesis [31]. Depletion of endogenous CXCL1, but not CXCL8, reduced tumor growth that was directly related to attenuated tumor-associated angiogenesis [31]. Thus, prostate cancer cell lines can utilize distinct CXC chemokines to mediate their tumorigenic potential. Other studies have confirmed this observation in prostate cancer models [32]. Similar findings have been shown in gastric carcinoma, breast, head and neck cancer, and colon cancer [33–38].

Glioblastoma is a devastating tumor of the central nervous system, with mortality approaching 80% in the first year postdiagnosis. The hallmark of these tumors is the marked presence of angiogenesis [39], which suggests that it is a biomarker that is necessary for malignant progression of this tumor. However, the precise molecular mechanisms underlying the regulation of glioblastoma growth and angiogenesis remain to be elucidated. Garkavtsev et al. [39] have recently identified a candidate tumor suppressor gene, ING4, which is involved in regulating glioblastoma tumor growth and angiogenesis. In this study, the expression of ING4 was found to be significantly reduced in glioblastomas, as compared with normal human brain tissue, and the extent of reduction correlated with the progression from lower to higher grades of these tumors [39]. Human glioblastomas that exhibit decreased expression of ING4 when engrafted into immuno-incompetent mice grew markedly faster and displayed greater angiogenesis than control tumors [39]. The mechanism for increased tumorigenicity in glioblastomas that express lower levels of ING4 was related to ING4's physical ability to interact with the p65 (RelA) subunit of NF-κB, which normally impairs nuclear translocation of NF-κB and transactivation of NF-κB-dependent

genes, such as ELR positive CXC chemokines [39]. In fact, the mechanism for the angiogenic activity of glioblastomas that expressed low levels of ING4 was directly related to CXCL8, as inhibition of CXCL8 in vivo markedly reduced their glioblastoma growth and tumor-associated angiogenesis [39]. These results indicate that ING4 has an important role in brain tumor pathogenesis related to ELR positive CXC chemokines. Furthermore, the above findings of redundancy in the expression of angiogenic ELR-positive CXC chemokines in human tumors, provides a unique opportunity to target ELR-positive CXC chemokine-mediated angiogenesis via targeting CXCR2.

In General, Non-ELR Positive CXC Chemokines Are Inhibitors of Angiogenesis

The angiostatic members of the CXC chemokine family include CXCL4, CXCL4L1, CXCL9, CXCL10, CXCL11, and CXCL14 [2, 5, 40–42] (table 1). Platelet factor-4 (PF-4)/CXCL4 was the first chemokine described to inhibit neovascularization [43]. However, the product of the nonallelic variant gene of CXCL4, PF-4var1/PF-4alt, designated CXCL4L1, was recently isolated from thrombin-stimulated human platelets and purified to homogeneity [42]. Although secreted CXCL4 and CXCL4L1 differ in only three amino acids, CXCL4L1 is more potent for inhibiting angiogenesis in response to angiogenic factors in both in vitro and in vivo models of angiogenesis [42]. CXCL9, CXCL10, and CXCL11 are induced by both type I and II interferons [3, 44–47]. CXCL9 and CXCL11 inhibit neovascularization in response to either ELR positive CXC chemokines, bFGF, or VEGF [40]. These findings suggest that all IFN-inducible non-ELR positive CXC chemokines are potent inhibitors of angiogenesis.

Recently another non-ELR-positive CXC chemokine has been found to inhibit angiogenesis. Breast and kidney-expressed chemokine (BRAK)/CXCL14 is a non-ELR-positive CXC chemokine, which inhibits angiogenesis in vitro and in vivo [41]. CXCL14 was first identified by differential display of normal oral epithelial cells and head and neck squamous cell carcinoma [48]. CXCL14 was found to be down-regulated in tumor specimens [48]. The biological significance of the absence of CXCL14 in these tumors remained to be elucidated until Shellenberger et al. [41] discovered that CXCL14 inhibited microvascular endothelial cell chemotaxis in response to CXCL8, bFGF, and VEGF. Furthermore, CXCL14 inhibited neovascularization in vivo in response to the same angiogenic agonists. These findings support the notion that the loss of CXCL14 constitutive expression during tumorigenesis is associated with the transformation of normal epithelial cells to cancer and the promotion of a pro-angiogenic microenvironment. This supports the concept that CXCL14 may be acting as a tumor suppressor gene.

CXCR3 Appears to Be the Major Receptor for Non-ELR-Positive CXC Chemokine-Mediated Inhibition of Angiogenesis

The major receptor that has been identified for angiostatic non-ELR-positive CXC chemokines is CXCR3. To date, three alternative splice variants of CXCR3 are known – designated as CXCR3A, CXCR3B, and CXCR3-alt – that are involved in mediating recruitment of Th1 cells and acts as the receptor for inhibition of angiogenesis [44–46, 49, 50]. CXCR3A is the major chemokine receptor found on Th1 effector T cells, cytotoxic CD8+ T cells, activated B cells, and NK cells [44, 49, 51–55]. IL-2 is a major agonist for the expression of CXCR3A on these cells [49, 52, 53]. CXCR3 was originally identified on murine endothelial cells [56]. Subsequent studies demonstrated that CXCR3 ligands could block both human microvascular endothelial cell migration and proliferation in response to a variety of angiogenic factors [15, 57].

Yang and Richmond [58] have recently determined that CXCR3 ligands that use CXCR3 on endothelial cells mediate their angiostatic effect via specific binding to endothelial cell-derived CXCR3. They demonstrated that CXCL10 mediates its angiostatic activity in vivo by binding to CXCR3, and not via binding to glycosaminoglycans. To clarify this issue, they created expression constructs for mutants of CXCL10 that exhibit partial (IP-10C) or total (IP-10C22) loss of binding to CXCR3 or loss of binding to glycosaminoglycans (IP-10H and IP-10C22H). They transfected a human melanoma cell line with these expression vectors, and stable clones were selected and inoculated into immuno-incompetent mice [58]. Tumor cells expressing wild-type CXCL10 showed remarkable reduction in tumor growth compared to control vector-transfected tumor cells. Surprisingly, mutation of CXCL10 resulting in partial loss of receptor binding (IP-10C), or loss of glycosaminoglycans binding (IP-10H), did not significantly alter the ability to inhibit tumor growth. The reduction in tumor growth was associated with a reduction in tumor-associated angiogenesis, leading to the observed increase in both tumor cell apoptosis and necrosis [58]. In contrast, expression of the CXCL10 mutant that failed to bind to CXCR3, failed to inhibit tumor growth [58]. The above study has been confirmed in another in vivo angiogenesis-dependent model. Burdick and associates have found that CXCL11 in a CXCR3-dependent manner inhibits angiogenesis in a murine model of pulmonary fibrosis [59]. These data suggest that CXCR3 receptor binding, but not glycosaminoglycan binding, is essential for the tumor angiostatic activity of non-ELR-positive CXC chemokines.

While CXCL12 is a non-ELR-positive CXC chemokine, CXCL12 through its receptor CXCR4 has been implicated in angiogenesis [60–63]. The role of the CXCL12/CXCR4 ligand-receptor pair in tumorigenesis has, however, been shown to be through its ability to mediate metastasis rather than its ability to

promote angiogenic activity. Phillips et al. [64] demonstrated that CXCR4 is predominantly expressed on tumor cells and does not mediate angiogenesis in an in vivo model of heterotopic and orthotopic human NSCLC tumor growth and metastasis. When CXCL12 was blocked in vivo during tumorigenesis and metastases, there was no change in the size of the primary tumor, nor was there any evidence of a decrease in primary tumor-associated angiogenesis. However, there was a marked attenuation of the metastatic capability of these tumors [64], suggesting that the CXCL12/CXCR4 axis mediates metastases of the tumor cells in an angiogenesis-independent manner. One explanation for this dichotomy is that CXCL12 mediates metastases through direct effects on tumor cell migration, whereas ELR-positive chemokines, VEGF, and bFGF mediate metastases through their stimulatory effect on angiogenesis.

Non-ELR Positive CXC Chemokines Attenuate Angiogenesis and Reduce Tumorigenesis

Non-ELR-positive CXC chemokines have been shown to inhibit angiogenesis in several model systems such as Burkitt's lymphoma cell lines form tumors in nude mice [65]. Angiogenesis is essential for tumorigenesis of these lymphomas, analogous to carcinomas. The expression of CXCL10 and CXCL9 were found to be higher in tumors that demonstrated spontaneous regression, and were directly related to impaired angiogenesis [66]. To determine whether this effect was attributable to CXCL10 or CXCL9, more virulent Burkitt's lymphoma cell lines were grown in nude mice and subjected to intratumor inoculation with either CXCL10 or CXCL9. Both conditions resulted in marked reduction in tumor-associated angiogenesis [66–69]. Although these CXCR3 ligands have been shown to bind to CXCR3 on mononuclear cells [44, 49, 51–55], the ability of these non-ELR positive CXC chemokines to inhibit angiogenesis and induce lymphoma regression in immuno-incompetent mice supports the notion that these chemokines mediate their effects in a T lymphocyte independent manner.

The level of CXCL10 in human NSCLC tumor specimens was found to be higher in the tumor specimens than in normal adjacent lung tissue [70]. The increase in CXCL10 from human NSCLC tissue was entirely attributable to the higher levels of CXCL10 present in squamous cell carcinoma (SCCA), as compared to adenocarcinoma [70]. Moreover, depletion of CXCL10 from SCCA surgical specimens resulted in augmented angiogenic activity [70]. The marked difference in the levels and bioactivity of CXCL10 in SCCA and adenocarcinoma is both clinically and pathophysiologically relevant, and represents a possible mechanism for the biologic differences of these two cell-types of NSCLC. Patient survival is lower, metastatic potential is higher, and evidence of angiogenesis is greater for adenocarcinoma, as compared to SCCA of the lung

[71–73]. A SCID mouse system was applied to examine the effect of CXCL10 on human NSCLC cell line tumor growth in a T and B cell independent manner [70]. The production of CXCL10 from adenocarcinoma and SCCA tumors was inversely correlated with tumor growth [70]. However, CXCL10 levels were significantly higher in the SCCA, as compared to adenocarcinoma tumors [70]. The appearance of spontaneous lung metastases in SCID mice bearing adenocarcinoma tumors occurred after CXCL10 levels from either the primary tumor or plasma reached a nadir [70]. In subsequent experiments, SCID mice bearing SCCA tumors were depleted of CXCL10, whereas, animals bearing adenocarcinoma tumors were treated with intratumor CXCL10 [70]. Depletion of CXCL10 in SCCA tumors resulted in a 2-fold increase in their size [70]. In contrast, reconstitution of intratumor CXCL10 in adenocarcinoma tumors reduced both their size and metastatic potential that was unrelated to infiltrating neutrophils or mononuclear cells such as macrophages or NK cells, and was directly attributable to a reduction in tumor-associated angiogenesis [70]. Similar strategies have been found for CXCL10 in melanoma using a gene therapeutic strategy [74].

Similar to CXCL10, CXCL9 also plays a significant role in regulating angiogenesis of NSCLC. CXCL9 levels in human specimens of NSCLC were found to be not significantly different from that found in normal lung tissue [75]. However, these results suggested that the increased expression of ELR-positive CXC chemokines and other angiogenic factors found in these tumors were not counterregulated by a concomitant increase in the expression of the angiostatic CXC chemokine, CXCL9. Thus, this imbalance could promote a micro-environment that promotes angiogenesis. To alter this imbalance, studies were performed to overexpress CXCL9 by three different strategies including gene transfer [75]. These experiments resulted in the inhibition of NSCLC tumor growth and metastasis via a decrease in tumor-derived vessel density [75]. These findings support the importance of the interferon-inducible angiostatic non-ELR positive CXC chemokines in inhibiting NSCLC tumor growth by attenuation of tumor-derived angiogenesis.

Evidence that Chemokines Are Involved in Tumor Cell Invasion

Tumor cell invasion through ECM and entry into the circulation is dependent on cellular loss of adherence, cellular motility, and ECM/basement membrane degradation. Invasive tumors cells have the ability to secrete a variety of enzymes that include matrix metalloproteinases (MMPs) as well as serine and cysteine proteinases. The generation of these proteinases allows for the migration of tumor cells through the ECM and penetration through the basement membrane followed by entry into the circulation. CXC chemokine activation of

tumor cells is important in this process. The expression of CXCL8 by human melanoma cells up-regulates MMP-2 activity and increases tumor growth and metastasis [76]. Luca et al. [76] have demonstrated that the expression of CXCL8 by human melanoma cell lines directly correlates with their metastatic potential. They further substantiated their findings by using non-metastatic melanoma cells with negligible levels of CXCL8 that were subsequently transfected to overexpress CXCL8 [76]. The overexpression of CXCL8 enhanced the tumorigenicity and metastatic potential of the melanoma cells in vivo [76]. In conjunction with this change in biological behavior, the CXCL8-transfected cells displayed upregulation in MMP-2 [76]. This expression was accompanied by heightened collagenase activity and increased tumor cell invasiveness in vitro [76]. Moreover, they found that the effect of CXCL8 activation was at the level of induction of the promoter of the MMP-2 gene, suggesting that CXCL8 was involved in MMP-2 gene transcription [76]. These finding support that CXCL8 can activate tumor cell-derived collagenase activity that can lead to enhanced tumor cell invasion into the host stroma and increased metastatic potential.

These findings have been further substantiated in prostate cancer where CXCL8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer [77]. Inoue et al. [77] have found that prostate cancer cell lines that overexpress CXCL8 are associated with a highly metastatic phenotype. They further evaluated this biology in vivo by implanting these human tumor cells into immuno-incompetent mice [77]. They transfected the overexpressing CXCL8 cells lines with full-sequence antisense CXCL8 cDNA, and transfected the under-expressing CXCL8 cell lines to over-express CXCL8 and engrafted these cells in immuno-incompetent mice [77]. The over-expression of CXCL8 in vitro resulted in the up-regulation of MMP-9 in these cells [77]. The effect was at the levels of mRNA, protein, and biological function with heightened collagenase activity resulting in increased invasiveness of the prostate cancer cell lines in vitro [77]. Orthotopic implantation of the human prostate cancer cells over-expressing CXCL8 that normally in of themselves are not tumorigenic or metastatic in behavior, became highly tumorigenic and metastatic with associated increased angiogenesis, whereas the cells transfected with anti-sense CXCL8 were inhibited in their growth and metastatic potential [77]. These findings have been further corroborated by Kim et al. [32] who found that the expression of CXCL8 correlated with angiogenesis, tumorigenicity, and metastasis of human prostate cancer cells implanted orthotopically in immuno-incompetent mice. These findings suggest that angiogenic ELR positive CXC chemokines, like CXCL8, play a multifunctional role in aiding tumor cell invasion by augmenting their local angiogenic environment and up-regulating the expression of MMPs to aid tumor cell invasion and entry into the circulation.

Evidence that Chemokines Regulate the Pattern of Organ-Specific Metastasis of Cancer

Paget's theory of tumor cell metastasis based on the concept of 'seed' (tumor cell) and 'soil' (specific organ) was first described for the nonrandom visceral metastases of breast cancer [1]. While this theory has been debated, experimental data has demonstrated that sites of metastasis are determined not only by the characteristics of neoplastic cells but also by the microenvironment of the specific organ [78]. However, the specific mechanisms that actually promote organ-specific metastasis have not been fully determined. Breast cancer, as characterized by the original observation of Paget [1], has a distinct metastatic pattern preferentially involving the regional lymph nodes, bone marrow, lung, and liver. This distinctive metastatic pattern is seen in a number of other cancers. Müller et al. [79] have provided new insight into potential mechanisms for chemokines in relation to the organ-specific metastasis of breast cancer cells. They found that of all known chemokine receptors, specifically CXCR4 and CCR7 are highly expressed in human breast cancer cells, malignant breast tumors and metastases [79]. The ligands for these receptors, CXCL12 for CXCR4 and CCL21 for CCR7 exhibited peak levels of expression in organs that are preferential destinations for breast cancer metastasis [79]. In breast cancer cells, signalling through CXCR4 or CCR7 mediated actin polymerization and pseudopodia formation and, subsequently, induced chemotactic and invasive responses at the local level [79]. Moreover, neutralization of CXCL12/CXCR4 interactions significantly inhibited metastasis of breast cancer cells to regional lymph nodes and lung [79]. While this study suggested that the CXCL12/CXCR4 biological axis was important in mediating organ-specific metastases of breast cancer, this concept has remained somewhat controversial, as the findings did not take into consideration other potential biological effects of CXCL12/CXCR4 in the tumor itself.

For example, CXCL12 is a non-ELR-positive CXC chemokine, CXCL12 via CXCR4 has been implicated in promoting angiogenesis [60–63]. This has led to the speculation that the predominant function of this ligand/receptor pair in tumorigenesis is due to its angiogenic effect, not necessarily due to its potential of mediating organ-specific metastases. However, in order for the biological axis of CXCL12/CXCR4 to mediate tumor-associated angiogenesis, both the ligand and receptor should be temporally and spatially present within the tumor. Schrader et al. [80] demonstrated in both renal cell carcinoma cell lines and actual patient specimens of renal cell carcinoma that CXCR4 is expressed predominately by the tumor cells, and its ligand CXCL12 is essentially absent within the tumor. These findings have been further substantiated in human breast cancer and NSCLC tumor specimens, in which CXCR4 is found expressed on the tumor cells (fig. 2),

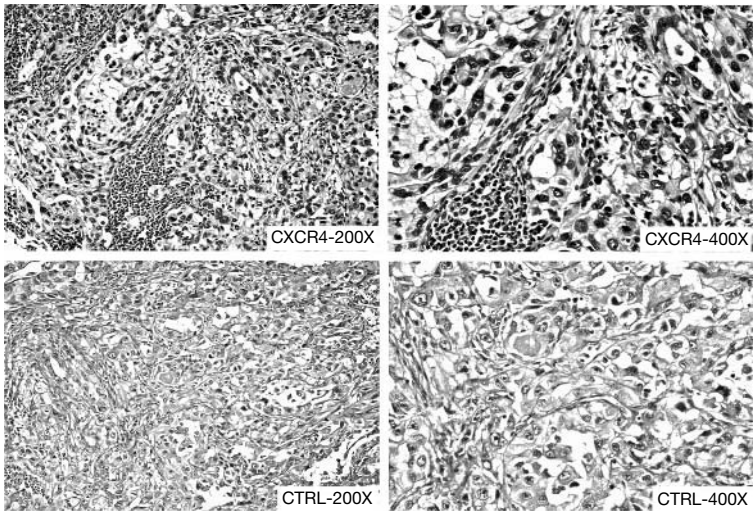

Fig. 2. CXCR4 expression on large cell NSCLC. CXCR4 is highly expressed on cells of the primary tumor of large cell NSCLC.

and does not mediate tumor-associated angiogenesis in vivo [64, 79]. These studies demonstrate that when animals with breast or NSCLC tumors are treated with either neutralizing anti-CXCL12 or anti-CXCR4 antibodies, there is no change in the size of the primary tumor nor is there any evidence for a decline in primary tumor-associated angiogenesis [64; Albert Zlotnik pers. commun.]. However, there is a marked attenuation of tumor metastases in an organ-specific manner [64, 79]. These studies support the notion that CXCL12/CXCR4 biology mediates metastases of the tumor cells in an angiogenesis-independent manner.

An explanation for the disparity of the tumor studies in vivo from in vitro studies of CXCL12/CXCR4 mediated angiogenesis, is that tumor cells expressing CXCR4 are themselves able to 'out compete' endothelial cells for CXCL12 if present. In support of this contention, classical angiogenic factors are elevated in human tumors, whereas CXCL12 is not [2, 64, 70, 80–82]. Moreover, the depletion of classical angiogenic factors in vivo results in a net reduction of angiogenesis, and a consequent reduction in primary tumor size and metastatic potential [2, 70, 81, 82]. These findings suggest a dichotomy in the function of CXCL12 vs. classical angiogenic factors. For instance, angiogenic factors promote metastasis through their effect in mediating angiogenesis, whereas CXCL12 promotes metastasis in an angiogenesis-independent manner via CXCR4-dependent tumor cell migration. This concept supports the notion that expression of CXCR4 on tumor cells may represent a critical biomarker for their propensity to metastasize. Therefore, further understanding of the molecular

mechanisms that are involved in the regulation of CXCR4 expression on tumor cells could lead to targets to modify expression of this receptor that impact on tumor metastases.

Studies on a number of different human cancer types have shown the importance of CXCR4 expression for increased tumor cell survival, tumor cell proliferation and metastasis of cancer cells. In human pancreatic and prostate tumor cell lines CXCR4 is frequently expressed on metastatic cells, and stimulates cell motility and invasion, as well as proliferation and survival [83–85]. In addition, CXCR4 gene expression in primary colorectal cancer is associated with tumor recurrence, survival, and liver metastasis [85], and use of a CXCR4 antagonist prevented lung metastases in a mouse model of osteosarcoma [86].

Recently, hypoxia and more specifically hypoxia-inducible factor-1α (HIF-1α) has been found to be a critical transcription factor for gene expression of CXCR4 on a variety of cells, including tumor cells [87, 88]. Moreover, the von Hippel-Lindau (VHL) tumor suppressor gene can negatively regulate the expression of CXCR4, owing to its capacity to target HIF-1α for degradation under normoxic conditions [87, 88]. This process may be suppressed under hypoxic conditions in cells allowing HIF-1α-dependent induction of CXCR4 expression [87, 88]. In contrast, under normoxic conditions, RTK activated PI3kinase/AKT/mTor and ERK1/2/MAPkinase pathways can augment the expression of HIF-1α in a post-transcriptional manner [89–91]. Phillips et al. [92] have shown that the combination of hypoxia and epidermal growth factor receptor (EGFR) activation markedly upregulates the expression of CXCR4 on NSCLC cells. This increase in CXCR4 expression is regulated through the PI3-K/PTEN/AKT/mTOR pathway, which activates HIF-1α and results in HIF-1α dependent transcription of the CXCR4 gene [92]. This link between hypoxia-induced HIF-1α and CXCR4 expression provides a novel mechanism to reduce metastases in a variety of cancers (fig. 3).

Conclusion

Although chemokine biology was originally felt to be restricted to recruitment of subpopulations of leukocytes, it has become increasingly clear that these cytokines can exhibit many varied effects in mediating biology that goes beyond their originally described function. Tumor biology provides an excellent system to study this diversity of function. Chemokines have an autocrine, paracrine, and hormonal role at every level related to primary tumor growth, invasion, and organ-specific metastases. The understanding of this expanded role of chemokines in tumor biology will open new doors for novel therapeutic interventions.

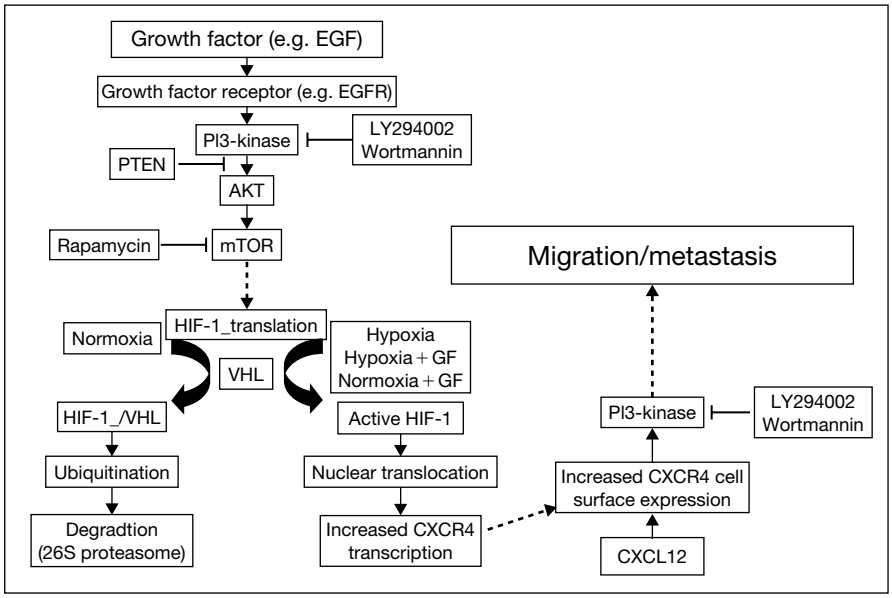

Fig. 3. The signaling pathways linking EGF/EGFR, HIF-1α, and CXCR4 expression. The combination of hypoxia and EGFR signalling markedly upregulates the expression of CXCR4 on NSCLC cells. This pathway demonstrates a mechanism for the development of a metastatic phenotype of NSCLC cells.

Acknowledgements

This work was supported, in part, by NIH grants HL074229 (B.N.G.) and HL66027, CA87879, P50 CA90388, and P50 HL67665 (R.M.S.).

References

1 Paget SL: The distribution of secondary growths in cancer of the breast. Lancet 1889;i:571.
2 Belperio JA, Keane MP, Arenberg DA, Addison CL, Ehlert JE, Burdick MD, Strieter RM: CXC chemokines in angiogenesis. J Leukoc Biol 2000;68:1–8.
3 Strieter RM, Belperio JA, Phillips RJ, Keane MP: CXC chemokines in angiogenesis of cancer. Semin Cancer Biol 2004;14:195–200.
4 Romagnani P, Lasagni L, Annunziato F, Serio M, Romagnani S: CXC chemokines: the regulatory link between inflammation and angiogenesis. Trends Immunol 2004;25:201–209.
5 Strieter RM, Polverini PJ, Kunkel SL, Arenberg DA, Burdick MD, Kasper J, Dzuiba J, Van Damme J, Walz A, Marriott D, Chan SY, Roczniak S, Shanafelt AB: The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem 1995;270:27348–27357.
6 Strieter RM, Polverini PJ, Arenberg DA, Walz A, Opdenakker G, van Damme J, Kunkel SL: Role of C-X-C chemokines as regulators of angiogenesis in lung cancer. J Leukoc Biol 1995;57: 752–762.

7 Nor JE, Christensen J, Liu J, Peters M, Mooney DJ, Strieter RM, Polverini PJ: Up-regulation of Bcl-2 in microvascular endothelial cells enhances intratumoral angiogenesis and accelerates tumor growth. Cancer Res 2001;61:2183–2188.
8 Schruefer R, Lutze N, Schymeinsky J, Walzog B: Human neutrophils promote angiogenesis by a paracrine feedforward mechanism involving endothelial interleukin-8. Am J Physiol 2005;288: H1186–H1192.
9 Dong G, Chen Z, Li ZY, Yeh NT, Bancroft CC, van Waes C: Hepatocyte growth factor/scatter factor-induced activation of MEK and PI3K signal pathways contributes to expression of proangiogenic cytokines interleukin-8 and vascular endothelial growth factor in head and neck squamous cell carcinoma. Cancer Res 2001;61:5911–5918.
10 Hirata A, Ogawa S, Kometani T, Kuwano T, Naito S, Kuwano M, Ono M: ZD1839 (Iressa) induces antiangiogenic effects through inhibition of epidermal growth factor receptor tyrosine kinase. Cancer Res 2002;62:2554–2560.
11 Levine L, Lucci JA 3rd, Pazdrak B, Cheng JZ, Guo YS, Townsend CM Jr, Hellmich MR: Bombesin stimulates nuclear factor kappa B activation and expression of proangiogenic factors in prostate cancer cells. Cancer Res 2003;63:3495–3502.
12 Richmond A: NF-kappa B, chemokine gene transcription and tumor growth. Nat Rev Immunol 2002;2:664–674.
13 Addison CL, Daniel TO, Burdick MD, Liu H, Ehlert JE, Xue YY, Buechi L, Walz A, Richmond A, Strieter RM: The CXC chemokine receptor 2, CXCR2, is the putative receptor for ELR(+) CXC chemokine-induced angiogenic activity. J Immunol 2000;165:5269–5277.
14 Murdoch C, Monk PN, Finn A: Cxc chemokine receptor expression on human endothelial cells. Cytokine 1999;11:704–712.
15 Salcedo R, Resau JH, Halverson D, Hudson EA, Dambach M, Powell D, Wassermann K, Oppenheim JJ: Differential expression and responsiveness of chemokine receptors (CXCR1–3) by human microvascular endothelial cells and umbilical vein endothelial cells. Faseb J 2000;14: 2055–2064.
16 Heidemann J, Ogawa H, Dwinell MB, Rafiee P, Maaser C, Gockel HR, Ottersen MF, Ota DM, Lugering N, Domschke W, Binion DG: Angiogenic effects of interleukin 8 (CXCL8) in human intestinal microvascular endothelial cells are mediated by CXCR2. J Biol Chem 2003;278: 8508–8515.
17 Keane MP, Burdick MD, Xue YY, Lutz M, Belperio JA, Strieter RM: The chemokine receptor, CXCR2, mediates the tumorigenic effects of ELR+ CXC chemokines. Chest 2004;125(suppl 5): 133S.
18 Luan J, Shattuck-Brandt R, Haghnegahdar H, Owen JD, Strieter R, Burdick M, Nirodi C, Beauchamp D, Johnson KN, Richmond A: Mechanism and biological significance of constitutive expression of MGSA/GRO chemokines in malignant melanoma tumor progression. J Leukoc Biol 1997;62:588–597.
19 Owen JD, Strieter R, Burdick M, Haghnegahdar H, Nanney L, Shattuck-Brandt R, Richmond A: Enhanced tumor-forming capacity for immortalised melanocytes expressing melanoma growth stimulatory activity/growth-regulated cytokine beta and gamma proteins. Int J Cancer 1997;73:94–103.
20 Yoneda J, Kuniyasu H, Crispens MA, Price JE, Bucana CD, Fidler IJ: Expression of angiogenesis-related genes and progression of human ovarian carcinomas in nude mice. J Natl Cancer Inst 1998;90:447–454.
21 Gawrychowski K, Skopinska-Rozewska E, Barcz E, Sommer E, Szaniawska B, Roszkowska-Purska K, Janik P, Zielinski J: Angiogenic activity and interleukin-8 content of human ovarian cancer ascites. Eur J Gynaecol Oncol 1998;19:262–264.
22 Smith DR, Polverini PJ, Kunkel SL, Orringer MB, Whyte RI, Burdick MD, Wilke CA, Strieter RM: IL-8 mediated angiogenesis in human bronchogenic carcinoma. J Exp Med 1994;179: 1409–1415.
23 Arenberg DA, Kunkel SL, Burdick MD, Polverini PJ, Strieter RM: Treatment with anti-IL-8 inhibits non-small cell lung cancer tumor growth. J Invest Med 1995;43(suppl 3):479A.
24 Yatsunami J, Tsuruta N, Ogata K, Wakamatsu K, Takayama K, Kawasaki M, Nakanishi Y, Hara N, Hayasi S: Interleukin-8 participates in angiogenesis in non-small cell, but not small cell carcinoma of the lung. Cancer Lett 1997;120:101–108.

25 Arenberg DA, Keane MP, DiGiovine B, Kunkel SL, Morris SB, Xue YY, Burdick MD, Glass MC, Iannettoni MD, Strieter RM: Epithelial-neutrophil activating peptide (ENA-78) is an important angiogenic factor in non-small cell lung cancer. J Clin Invest 1998;102:465–472.
26 White ES, Flaherty KR, Carskadon S, Brant A, Iannettoni MD, Yee J, Orringer MB, Arenberg DA: Macrophage migration inhibitory factor and CXC chemokine expression in non-small cell lung cancer: role in angiogenesis and prognosis. Clin Cancer Res 2003;9:853–860.
27 Chen JJ, Yao PL, Yuan A, Hong TM, Shun CT, Kuo ML, Lee YC, Yang PC: Up-regulation of tumor interleukin-8 expression by infiltrating macrophages: its correlation with tumor angiogenesis and patient survival in non-small cell lung cancer. Clin Cancer Res 2003;9:729–737.
28 Pold M, Zhu LX, Sharma S, Burdick MD, Lin Y, Lee PP, Pold A, Luo J, Krysan K, Dohadwala M, Mao JT, Batra RK, Strieter RM, Dubinett SM: Cyclooxygenase-2-dependent expression of angiogenic CXC chemokines ENA-78/CXC Ligand (CXCL) 5 and interleukin-8/CXCL8 in human non-small cell lung cancer. Cancer Res 2004;64:1853–1860.
29 Bostwick DG, Iczkowski KA: Microvessel density in prostate cancer: prognostic and therapeutic utility. Semin Urol Oncol 1998;16:118–123.
30 Fregene TA, Khanuja PS, Noto AC, Gehani SK, Van Egmont EM, Luz DA, Pienta KJ: Tumor-associated angiogenesis in prostate cancer. Anticancer Res 1993;13:2377–2381.
31 Moore BB, Arenberg DA, Stoy K, Morgan T, Addison CL, Morris SB, Glass M, Wilke C, Xue YY, Sitterding S, Kunkel SL, Burdick MD, Strieter RM: Distinct CXC chemokines mediate tumorigenicity of prostate cancer cells. Am J Pathol 1999;154:1503–1512.
32 Kim SJ, Uehara H, Karashima T, McCarty M, Shih N, Fidler IJ: Expression of interleukin-8 correlates with angiogenesis, tumorigenicity, and metastasis of human prostate cancer cells implanted orthotopically in nude mice. Neoplasia 2001;3:33–42.
33 Miller LJ, Kurtzman SH, Wang Y, Anderson KH, Lindquist RR, Kreutzer DL: Expression of interleukin-8 receptors on tumor cells and vascular endothelial cells in human breast cancer tissue. Anticancer Res 1998;18:77–81.
34 Richards BL, Eisma RJ, Spiro JD, Lindquist RL, Kreutzer DL: Coexpression of interleukin-8 receptors in head and neck squamous cell carcinoma. Am J Surg 1997;174:507–512.
35 Kitadai Y, Haruma K, Sumii K, Yamamoto S, Ue T, Yokozaki H, Yasui W, Ohmoto Y, Kajiyama G, Fidler IJ, Tahara E: Expression of interleukin-8 correlates with vascularity in human gastric carcinomas. Am J Pathol 1998;152:93–100.
36 Singh RK, Gutman M, Radinsky R, Bucana CD, Fidler IJ: Expression of interleukin 8 correlates with the metastatic potential of human melanoma cells in nude mice. Cancer Res 1994;54:3242–3247.
37 Cohen RF, Contrino J, Spiro JD, Mann EA, Chen LL, Kreutzer DL: Interleukin-8 expression by head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 1995;121:202–209.
38 Chen Z, Malhotra PS, Thomas GR, Ondrey FG, Duffey DC, Smith CW, Enamorado I, Yeh NT, Kroog GS, Rudy S, McCullagh L, Mousa S, Quezado M, Herscher LL, Van Waes C: Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. Clin Cancer Res 1999;5:1369–1379.
39 Garkavtsev I, Kozin SV, Chernova O, Xu L, Winkler F, Brown E, Barnett GH, Jain RK: The candidate tumor suppressor protein ING4 regulates brain tumor growth and angiogenesis. Nature 2004;428:328–332.
40 Strieter RM, Belperio JA, Arenberg DA, Smith MI, Burdick MD, Keane MP: CXC chemokine in angiogenesis; in Ransohoff RM, Suzuki K, Proudfoot AEI, Hickey WF (eds): Universities in Delicate Balance: Chemokines and the Nervous System. Amsterdam, Elsevier, 2002, pp 129–148.
41 Shellenberger TD, Wang M, Gujrati M, Jayakumar A, Strieter RM, Ioannides C, Efferson CL, El-Naggar AK, Roberts D, Clayman GL, Frederick MJ: BRAK/CXCL14 is a potent inhibitor of angiogenesis and is a chemotactic factor for immature dendritic cells. Cancer Res 2004;64:8262–8279. Erratum in: Cancer Res 2004;64:9230.
42 Struyf S, Burdick MD, Proost P, Van Damme J, Strieter RM: Platelets release CXCL4L1, a nonallelic variant of the chemokine platelet factor-4/CXCL4 and potent inhibitor of angiogenesis. Circ Res 2004;95:855–857.
43 Maione TE, Gray GS, Petro J, Hunt AJ, Donner AL, Bauer SI, Carson HF, Sharpe RJ: Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. Science 1990;247:77–79.

44 Luster AD: Chemokines-chemotactic cytokines that mediate inflammation. N Engl J Med 1998; 338:436–445.
45 Rollins BJ: Chemokines. Blood 1997;90:909–928.
46 Balkwill F: The molecular and cellular biology of the chemokines. J Viral Hepat 1998;5:1–14.
47 Strieter RM, Belperio JA, Phillips RJ, Keane MP: Chemokines: angiogenesis and metastases in lung cancer. Novartis Found Symp 2004;256:173–184; discussion 84–88, 259–269.
48 Frederick MJ, Henderson Y, Xu X, Deavers MT, Sahin AA, Wu H, Lewis DE, El-Naggar AK, Clayman GL: In vivo expression of the novel CXC chemokine BRAK in normal and cancerous human tissue. Am J Pathol 2000;156:1937–1950.
49 Loetscher M, Loetscher P, Brass N, Meese E, Moser B: Lymphocyte-specific chemokine receptor CXCR3: regulation, chemokine binding and gene localisation. Eur J Immunol 1998;28:3696–3705.
50 Ehlert JE, Addison CA, Burdick MD, Kunkel SL, Strieter RM: Identification and partial characterisation of a variant of human CXCR3 generated by posttranscriptional exon skipping. J Immunol 2004;173:6234–6240.
51 Moser B, Loetscher P: Lymphocyte traffic control by chemokines. Nat Immunol 2001;2:123–128.
52 Loetscher M, Gerber B, Loetscher P, Jones SA, Piali L, Clark-Lewis I, Baggiolini M, Moser B: Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes. J Exp Med 1996;184:963–969.
53 Rabin RL, Park MK, Liao F, Swofford R, Stephany D, Farber JM: Chemokine receptor responses on T cells are achieved through regulation of both receptor expression and signalling. J Immunol 1999;162:3840–3850.
54 Qin S, Rottman JB, Myers P, Kassam N, Weinblatt M, Loetscher M, Koch AE, Moser B, Mackay CR: The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. J Clin Invest 1998;101:746–754.
55 Beider K, Nagler A, Wald O, Franitza S, Dagan-Berger M, Wald H, Giladi H, Brocke S, Hanna J, Mandelboim O, Darash-Yahana M, Galun E, Peled A: Involvement of CXCR4 and IL-2 in the homing and retention of human NK and NK T cells to the bone marrow and spleen of NOD/SCID mice. Blood 2003;102:1951–1958.
56 Soto H, Wang W, Strieter RM, Copeland NG, Gilbert DJ, Jenkins NA, Hedrick J, Zlotnik A: The CC chemokine 6Ckine binds the CXC chemokine receptor CXCR3. Proc Natl Acad Sci USA 1998;95:8205–8210.
57 Romagnani P, Annunziato F, Lasagni L, Lazzeri E, Beltrame C, Francalanci M, Uguccioni M, Galli G, Cosmi L, Maurenzig L, Baggiolini M, Maggi E, Romagnani S, Serio M: Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. J Clin Invest 2001;107:53–63.
58 Yang J, Richmond A: The angiostatic activity of interferon-inducible protein-10/CXCL10 in human melanoma depends on binding to CXCR3 but not to glycosaminoglycan. Mol Ther 2004;9:846–855.
59 Burdick MD, Murray LA, Keane MP, Xue YY, Zisman DA, Belperio JA, Strieter RM: CXCL11 attenuates bleomycin-induced pulmonary fibrosis via inhibition of vascular remodelling. Am J Respir Crit Care Med 2005;171:261–268.
60 Bachelder RE, Wendt MA, Mercurio AM: Vascular endothelial growth factor promotes breast carcinoma invasion in an autocrine manner by regulating the chemokine receptor CXCR4. Cancer Res 2002;62:7203–7206.
61 Salcedo R, Oppenheim JJ: Role of chemokines in angiogenesis: CXCL12/SDF-1 and CXCR4 interaction, a key regulator of endothelial cell responses. Microcirculation 2003;10:359–370.
62 Kijowski J, Baj-Krzyworzeka M, Majka M, Reca R, Marquez LA, Christofidou-Solomidou M, Janowska-Wieczorek A, Ratajczak MZ: The SDF-1-CXCR4 axis stimulates VEGF secretion and activates integrins but does not affect proliferation and survival in lymphohematopoietic cells. Stem Cells 2001;19:453–466.
63 Salcedo R, Wasserman K, Young HA, Grimm MC, Howard OM, Anver MR, Kleinman HK, Murphy WJ, Oppenheim JJ: Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: in vivo neovascularisation induced by stromal-derived factor-1alpha. Am J Pathol 1999;154:1125–1135.

64 Phillips RJ, Burdick MD, Lutz M, Belperio JA, Keane MP, Strieter RM: The stromal derived factor-1/CXCL12-CXC chemokine receptor 4 biological axis in non-small cell lung cancer metastases. Am J Respir Crit Care Med 2003;167:1676–1686.
65 Gurtsevitch VE, O'Conor GT, Lenoir GM: Burkitt's lymphoma cell lines reveal different degrees of tumorigenicity in nude mice. Int J Cancer 1988;41:87–95.
66 Sgadari C, Angiolillo AL, Tosato G: Inhibition of angiogenesis by interleukin-12 is mediated by the interferon-inducible protein 10. Blood 1996;87:3877–3882.
67 Sgadari C, Angiolillo AL, Cherney BW, Pike SE, Farber JM, Koniaris LG, Vanguri P, Burd PR, Sheikh N, Gupta G, Teruya-Feldstein J, Tosato G: Interferon-inducible protein-10 identified as a mediator of tumor necrosis in vivo. Proc Natl Acad Sci USA 1996;93:13791–13796.
68 Sgadari C, Farber JM, Angiolillo AL, Liao F, Teruya-Feldstein J, Burd PR, Yao L, Gupta G, Kanegane C, Tosato G: Mig, the monokine induced by interferon-gamma, promotes tumor necrosis in vivo. Blood 1997;89:2635–2643.
69 Teruya-Feldstein J, Jaffe ES, Burd PR, Kanegane H, Kingma DW, Wilson WH, Longo DL, Tosato G: The role of Mig, the monokine induced by interferon-gamma, and IP-10, the interferon-gamma-inducible protein-10, in tissue necrosis and vascular damage associated with Epstein-Barr virus-positive lymphoproliferative disease. Blood 1997;90:4099–4105.
70 Arenberg DA, Kunkel SL, Polverini PJ, Morris SB, Burdick MD, Glass MC, Taub DT, Iannettoni MD, Whyte RI, Strieter RM: Interferon-gamma-inducible protein 10 (IP-10) is an angiostatic factor that inhibits human non-small cell lung cancer (NSCLC) tumorigenesis and spontaneous metastases. J Exp Med 1996;184:981–992.
71 Minna JD: Neoplasms if the lung; in Isselbacher KJ (ed): Principles of Internal Medicine, ed 12. New York, McGraw-Hill, 1991, pp 1102–1110.
72 Carney DN: Cancers of the lungs; in Fishman AP (ed): Pulmonary Diseases and Disorders, ed 2. New York, McGraw-Hill, 1988, pp 1885–2068.
73 Yuan A, Yang PC, Yu CJ, Lee YC, Yao YT, Chen CL, Lee LN, Kuo SH, Luh KT: Tumor angiogenesis correlates with histologic type and metastasis in non-small cell lung cancer. Am J Resp Crit Care Med 1995;152:2157–2162.
74 Feldman AL, Friedl J, Lans TE, Libutti SK, Lorang D, Miller MS, Turner EM, Hewitt SM, Alexander HR: Retroviral gene transfer of interferon-inducible protein 10 inhibits growth of human melanoma xenografts. Int J Cancer 2002;99:149–153.
75 Addison CL, Arenberg DA, Morris SB, Xue YY, Burdick MD, Mulligan MS, Iannettoni MD, Strieter RM: The CXC chemokine, monokine induced by interferon-gamma, inhibits non-small cell lung carcinoma tumor growth and metastasis. Hum Gene Ther 2000;11:247–261.
76 Luca M, Huang S, Gershenwald JE, Singh RK, Reich R, Bar-Eli M: Expression of interleukin-8 by human melanoma cells up-regulates MMP-2 activity and increases tumor growth and metastasis. Am J Pathol 1997;151:1105–1113.
77 Inoue K, Slaton JW, Eve BY, Kim SJ, Perrotte P, Balbay MD, Yano S, Bar-Eli M, Radinsky R, Pettaway CA, Dinney CP: Interleukin 8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer. Clin Cancer Res 2000;6:2104–2119.
78 Hart IR, Fidler IJ: Role of organ selectivity in the determination of metastatic patterns of B16 melanoma. Cancer Res 1980;40:2281–2287.
79 Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A: Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50–56.
80 Schrader AJ, Lechner O, Templin M, Dittmar KE, Machtens S, Mengel M, Probst-Kepper M, Franzke A, Wollensak T, Gatzlaff P, Atzpodien J, Buer J, Lauber J: CXCR4/CXCL12 expression and signalling in kidney cancer. Br J Cancer 2002;86:1250–1256.
81 Smith DR, Polverini PJ, Kunkel SL, Orringer MB, Whyte RI, Burdick MD, Wilke CA, Strieter RM: Inhibition of interleukin 8 attenuates angiogenesis in bronchogenic carcinoma. J Exp Med 1994;179:1409–1415.
82 Arenberg DA, Kunkel SL, Polverini PJ, Glass M, Burdick MD, Strieter RM: Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. J Clin Invest 1996;97:2792–2802.

83 Marchesi F, Monti P, Leone BE, Zerbi A, Vecchi A, Piemonti L, Mantovani A, Allavena P: Increased survival, proliferation, and migration in metastatic human pancreatic tumor cells expressing functional CXCR4. Cancer Res 2004;64:8420–8427.
84 Arya M, Patel HR, McGurk C, Tatoud R, Klocker H, Masters J, Williamson M: The importance of the CXCL12-CXCR4 chemokine ligand-receptor interaction in prostate cancer metastasis. J Exp Ther Oncol 2004;4:291–303.
85 Kim J, Takeuchi H, Lam ST, Turner RR, Wang HJ, Kuo C, Foshag L, Bilchik AJ, Hoon DS: Chemokine receptor CXCR4 expression in colorectal cancer patients increases the risk for recurrence and for poor survival. J Clin Oncol 2005;23:2744–2753.
86 Perissinotto E, Cavalloni G, Leone F, Fonsato V, Mitola S, Grignani G, Surrenti N, Sangiolo D, Bussolino F, Piacibello W, Aglietta M: Involvement of chemokine receptor 4/stromal cell-derived factor 1 system during osteosarcoma tumor progression. Clin Cancer Res 2005;11:490–497.
87 Schioppa T, Uranchimeg B, Saccani A, Biswas SK, Doni A, Rapisarda A, Bernasconi S, Saccani S, Nebuloni M, Vago L, Mantovani A, Melillo G, Sica A: Regulation of the chemokine receptor CXCR4 by hypoxia. J Exp Med 2003;198:1391–1402.
88 Staller P, Sulitkova J, Lisztwan J, Moch H, Oakeley EJ, Krek W: Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumor suppressor pVHL. Nature 2003;425:307–311.
89 Semenza GL: Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003;3:721–732.
90 Semenza GL: Signal transduction to hypoxia-inducible factor 1. Biochem Pharmacol 2002;64: 993–998.
91 Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, Simons JW, Semenza GL: Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. Cancer Res 2000;60:1541–1545.
92 Phillips RJ, Mestas J, Gharaee-Kermani M, Burdick MD, Sica A, Belperio JA, Keane MP, Strieter RM: Epidermal growth factor and hypoxia-induced expression of CXC chemokine receptor 4 on non-small cell lung cancer cells is regulated by the phosphatidylinositol 3-kinase/PTEN/AKT/mammalian target of rapamycin signalling pathway and activation of hypoxia inducible factor-1alpha. J Biol Chem 2005;280:22473–22481.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 191–199

Involvement of Chemokine Receptors in Organ-Specific Metastasis

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Abstract

The chemokines are a family of small proteins known for their ability to control cell migration in the body. Their receptors belong to the class A subfamily of G protein-coupled receptors. In recent years, chemokines have grown in importance, because they are involved in inflammation and autoimmune disease. Some of them are also involved in infectious disease, since two chemokine receptors, CXCR4 and CCR5, are used by the human immunodeficiency virus (HIV) to gain entry to cells. Several years ago it also became clear that chemokines can also influence tumor cells. Specifically, tumor cells express chemokine receptors in a nonrandom manner, and this suggested a role for chemokines in the metastatic destination of tumor cells. By far the most common chemokine receptor expressed by many cancer cells is CXCR4. Its ligand, CXCL12, is strongly expressed in lung, liver, bone marrow and lymph nodes, places that represent common metastatic destinations in many cancers. Many studies have now validated the concept that chemokines and their receptors influence metastasis. The potential therapeutic importance of these observations depends on the role that each metastatic destination such as liver, lung, bone marrow, etc., plays in the prognosis of a cancer patient.

Cancer represents a number of diseases that are characterized by the uncontrolled proliferation of various cells of the body. Some cells are notably more likely to undergo this ‘transformation’ to the cancer phenotype than others. Examples of some of these cell types and the diseases they cause include breast mammary epithelium (breast cancer), melanocytes (melanoma), and microglia (glioblastoma). A critical characteristic of cancer, and one that indeed defines the ‘malignant’ vs. ‘benign’ nature of a given tumor is their ability to metastasize, that is, to give rise to secondary tumors in other parts of the body. Thus, benign tumors do not metastasize, and depending on their anatomical

location they can usually be removed surgically with excellent prognosis. An example of these benign tumors are lipomas, tumors of fatty cells that are very common but are not a cause for concern. Serious cancers metastasize, and it is this characteristic that makes them so dangerous. While the primary tumor(s) can usually be removed surgically, it is usually very difficult to remove all the transformed cells and the leftovers may in time grow again and form secondary foci elsewhere in the body. This is why early diagnosis is so important; in cancer, early diagnosis can literally be the difference between a complete cure – by removing the nascent primary tumor – and a very poor prognosis if metastasis has already taken place.

There are several tumor development facts that deserve comment: firstly, any tumor detected macroscopically has likely been there and growing for a long time (depending on the tumor type, this may be years). Second, at some point, which we do not understand well, some tumor cells will escape from the primary tumor and somehow reach either the lymphatic or circulatory systems. The tumor cells then travel and will enter target organs where they begin to proliferate and develop into secondary tumors. Importantly, not all organs of the body develop metastases at the same rate. In fact, the 'target' organs for metastatic development depend on the type of primary tumor, and often exhibit significant specificity. However, we can generalize that certain organs are much more likely to be metastatic destinations than others. The common ones include bone marrow, lung, liver, and lymph nodes. Rare metastatic destinations include the stomach, kidney, or pancreas. These organs are typically only involved when the primary tumor arises in these organs, and therefore are more likely to be sources of cancer cells than metastatic destinations.

Several years ago metastasis was considered to be a mostly mechanical process, that is, if a tumor cell escaped from a tumor, reached the circulation and finally got lodged in a small blood vessel, that would become the center of a new metastatic focus. This process can occur, however, this usually happens only at more advanced stages of the disease. The metastatic patterns described above eventually break down and multiple metastases can develop in many organs. However, at early stages of cancer development, the mechanisms that control metastasis are controlled by a number of specific molecular interactions. We have now identified several of these molecular interactions. Here, I will discuss the role of a family of small molecules called the chemokines in cancer metastasis.

Chemokines

The chemokines are a superfamily of small molecules, produced by many different cell types of the body. To date, there are 45 known human chemokines

and 18 receptors [1]. There are likely to be more receptors since at least 2 human chemokines do not yet have known receptors (CXCL14 and CCL18). The chemokines typically include a conserved structure with four cysteines that form two disulfide bonds; there is only one chemokine that makes it with two cysteines (instead of four) and therefore only one instead of two disulfide bonds (XCL1/lymphotactin). Chemokines were originally characterized through their ability to chemoattract cells. There are two main subfamilies of chemokines. The first one has an amino acid between the first two cysteines (CXC) and the second one has these two first cysteines together (CC). Given the rapid pace of discovery of ligands in the chemokine superfamily we now use a systematic nomenclature that reflects these structures [2]. There are two other types of chemokines that are not families but instead represent single types: XCL1/lymphotactin and CX3CL1/fractalkine, which do not fulfill the criteria (CXC/CC) of the other two large subfamilies.

As mentioned above chemokines were originally characterized by their ability to chemotax different cells, especially those of the immune system. Since then, chemokines have been implicated in a variety of fields including infectious disease such as HIV (the chemokine receptors CXCR4 and CCR5 are coreceptors for the AIDS virus); control of immune responses, and many other areas including organ development. Furthermore, they are produced by a variety of cells and organs in the body. They have been divided into 'homeostatic' and 'inflammatory' depending on their expression patterns. The inflammatory ones tend to be those whose genes are located in certain chromosomal locations (the CC are in human chromosome 17q11.2 while the CXC cluster is in chromosome 4q12–13). In contrast, the genes for the homeostatic chemokines are located in discrete chromosomal locations, and they also tend to have a single ligand/receptor relationship. A typical homeostatic chemokine is CXCL12, which has a single receptor, CXCR4. This is an important ligand/receptor pair that we will discuss in detail below.

We knew that chemokines can control cell migration, but it was not clear to what extent they did so in vivo. Nakano et al. [3] described a natural mouse mutant, the plt (for paucity of lymph node T cells) mouse, which had very few T cells in the lymph nodes. The defect of this mouse is the inability to express two chemokines (CCL21 and CCL19) in the lymph nodes. These chemokines bind CCR7 and knockout mice of this receptor also had a phenotype resembling the plt mouse [4]. This observation underscored the importance of chemokines in normal lymphocyte recirculation. Not only could chemokines influence migration of leukocytes – as in inflammatory responses – but indeed, they were completely necessary for, in this case, T cells to enter lymph nodes. Importantly, it did not matter that thousands of lymphocytes were in circulation in lymphatic or blood vessels. Without the adequate molecular signal – the

interaction of CCL21 with its receptor CCR7 – the T cells could not enter lymph nodes. Similar observations were also made for CXCL13/CXCR5, a relationship that controls B cell migration to the lymph nodes [4].

Chemokines and Metastasis

Against this backdrop we hypothesized that, if chemokines were in fact so important in determining the migration and mobility of leukocytes in the body, could they also do it for tumor cells? In order to test this hypothesis, we measured the expression of chemokine receptors in breast cancer tumor cell lines. What we observed was that the expression of chemokine receptors in these lines was not random [5]. This was an absolute requirement for the hypothesis to be correct, since random chemokine receptor expression would make it impossible for chemokines to account for the specificity observed in metastatic tumor cell patterns. Furthermore, two receptors appeared specially expressed – and interesting – in breast cancer cells: CXCR4 and CCR7. I have already discussed that CCR7 is of critical importance to enter lymph nodes. This suggested that this receptor was important in breast cancer metastasis to the lymph nodes. However, the ligand of CXCR4 was expressed in various tissues that collectively represent very common metastatic destinations of breast cancer including lung, liver, and bone marrow. We used a mouse model of a the breast cancer cell line MDA-MB-231 that metastasizes to the lung, and showed that blocking CXCR4 prevented metastasis of these breast cancer cells to the lung [5]. Taken together, these studies demonstrated a role for CXCL12/CXCR4 in an animal model of breast cancer metastasis.

Since then, many reports have appeared documenting the expression of chemokine receptors in many cancers. At this point, these are too numerous to list here. But we can generalize the findings and some studies deserve further comment. Firstly, some general findings are as follows: the expression of chemokine receptors in cancer cells is not random. Second, the most common chemokine receptor expressed in most types of cancer cells is CXCR4. A distinguished second place may go to CCR7, which is likely important for lymph node metastasis. For example, a retrospective clinical study by Takanami [6] found that expression of CCR7 in non-small-cell lung cancer showed excellent correlation with lymph node metastasis.

But there are several reasons to conclude that the CXCL12/CXCR4 interaction is of critical importance in cancer. As mentioned above, CXCR4 is by far the most widely expressed receptor in many cancers [7]. In some, like ovarian, it is the dominant chemokine receptor expressed [8]. Moreover, its ligand, CXCL12, is strongly expressed in liver, lung, bone marrow (and lymph nodes)

and all common sites of metastasis in many cancers. A series of studies have shown that CXCR4 neutralization has impressive effects in cancer development and metastasis in animal models [7]. Many studies have independently reached the same conclusion in many different cancers [9–14]. Finally, other known mechanisms that influence cancer growth and development now have been recognized to be acting through the control of CXCR4 expression [15].

All these observations have transformed our understanding of the role of chemokines in cancer. But they also raised many questions. We do not know the mechanism(s) how, for example, CXCR4 signaling influences tumor cells. Our progress, however, does show that the interaction CXCL12/CXCR4 is of particular importance in cancer, and this gives us the advantage to focus on understanding this interaction in order to obtain practical information that may lead to cancer therapeutics.

Mechanism of Action of CXCR4 in Cancer

One discrepancy in the studies that have described effects of CXCR4 in cancer development is that in some cases, the interaction CXCL12/CXCR4 does not influence tumor cell growth directly, e.g. in breast cancer cells [5], whereas other studies have observed direct growth effects of CXCL12 in tumor cells, e.g., in cells from the central nervous system [9]. However, even in the nervous system, evidence for CXCL12/CXCR4 influence in metastasis of, e.g., breast cancer cells, has been documented in detail [16, 17].

One concept we should consider in interpreting these data is that tumor cells, while transformed, still have many of the characteristics of their normal counterparts. This means that we may learn from the functions of CXCL12/CXCR4 in normal development. The interaction CXCL12/CXCR4 has profound effects on the development of various organs including the heart and central nervous system (CNS). In fact, the CXCR4 knockout mouse is lethal, but the developing embryo showed distinct defects in CNS development [18]. Neurobiologists now consider CXCL12/CXCR4 as one of the most interesting chemokines because of its involvement in CNS development [19]. This points to the involvement of CXCL12/CXCR4 in a process we can call organogenesis. A developing organ has multiple challenges to conquer in order to produce a fully functioning organ. These include angiogenesis, and a ‘minimum’ level of structural organization at the cellular level. The fine specificity of homeostatic chemokine expression in many organs suggests that they play an important role in this process. Similarly, metastasis can be viewed as a similar process. What the developing tumor must achieve is, again, a minimum of cellular organization, angiogenesis, and protection from attacks from the host’s immune system.

In all of these areas chemokines, and more specifically CXCL12/CXCR4, are likely to play pivotal roles, and they may involve direct effects such as growth and differentiation, as well as indirect effects, e.g. the regulation of gene expression in the tumor cells. I consider that these questions represent the next frontiers in this field. We now have many studies that have validated the involvement of chemokines in tumor biology both in animal models as well as in retrospective studies. The next challenge is to obtain practical information that may allow us to exploit these findings for therapeutic purposes.

CXCR4 in Breast Cancer

In the particular case of breast cancer CXCL12/CXCR4 are more likely to be involved in the metastatic process by defining the metastatic destination. Some practical aspects arise in this disease: while the metastatic destinations in breast cancer include lymph nodes, lung, liver, and bone marrow (and to a lesser extent brain), the critical metastatic destination in this disease that will most likely impact the survival of the patient will be the lung. Metastasis to the lymph nodes may affect the immune system and may even compromise it at later stages but it is not likely to be the cause of death. A similar argument may be made for bone marrow. In the case of liver, it can still function even with large metastatic foci. But the main function of the lung is gas exchange and this will be seriously compromised by disseminated metastases from the breast cancer cells, which lack the capacity to do this function and will also seriously compromise the elasticity of the organ necessary for its main function. For this reason, if disrupting the CXCL12/CXCR4 interaction could slow down the progress of metastasis in the lung it could have a significant impact on life expectancy, a critical clinical goal that new drugs must fulfill in cancer. Unfortunately, breast cancer is, at a certain level, a bad disease in which to test the value of CXCR4 antagonists in cancer. The reason is that most patients get diagnosed at early stages, get treated with neo-adjuvant therapy and if they respond, will be declared in remission and will just be monitored carefully in the future for signs of recurring disease. This would be the patient cohort that could potentially benefit from an anti-metastatic agent, since the future lesions could show up in the lung. However, CXCL12/CXCR4 also has significant effects in the immune system. CXCR4 is expressed in T cell subsets and many other cells and it is not clear what effects long-term CXCR4 antagonism could have in the immune system. Moreover, the length of time required for such a clinical trial would make this project impractical for most pharmaceutical companies.

A significantly different scenario would apply for cancers where the effects of CXCR4 included direct growth and organogenesis. One of these

could be ovarian cancer. In this disease, patients are typically first treated surgically to remove the main tumors and then with chemotherapy to try to kill most leftover cells. Unfortunately this is a very aggressive disease and most patients will recur within a year and their prognosis is very poor. This creates an opportunity for more focused clinical trials of CXCR4 antagonists that may significantly influence the course of the disease. For this reason ovarian cancer may provide a better opportunity for proof of principle studies.

Conclusion and Future Directions

The concept that chemokines influence organ-specific metastatic destinations is now well established. This conclusion is best supported by some of the retrospective clinical studies that have been reported [6, 20]. The picture that emerges is that chemokine receptor expression by tumor cells is a potentially important aspect of tumorigenesis and metastasis. Not all chemokine receptors are equally important in this mechanism. Clearly the most widely expressed chemokine receptor in most cancers is CXCR4 and it is likely to be involved in metastasis to lung, liver, and bone marrow. CCR7 has emerged as the chemokine receptor most likely to mediate metastasis to the lymph nodes. The clinical importance of the latter, however, may be more questionable as this process – lymph node metastasis – is not likely to result in significant mortality, as discussed above for breast cancer. This is a point that deserves further comment. The morbidity and cause of death of different cancers differ significantly. For example, for ovarian cancer patients bowel obstruction may be life-threatening, while in prostate cancer bone marrow metastasis is a significant problem. If the overall hypothesis is correct – that organ-specific metastasis involves molecular signals – then each of these diseases must be considered separately and the causes of its morbidity considered independently. Even if a single therapeutic was being considered, for instance CXCR4 antagonists, the conditions surrounding each disease should be analyzed carefully to design the correct clinical trials. Furthermore, as mentioned above, different cancers may be more or less susceptible to immune system alterations. Since many chemokines are involved in immune response regulation, this is another factor to consider. However, in the real world, any such therapeutic would be unlikely to be used alone, and would instead be used in combination with established chemotherapy protocols, and therefore immune system alterations would already be occurring in the patient.

From the basic science perspective, we still have much to learn about this area. We do not know, for example, what are the effects of a chemokine ligand such as CXCL12 on tumor cells are. This is unlikely to simply be a matter of migration regulation, indeed, a whole gene expression program must be induced. This

program may differ depending on the cancer cell. CXCR4 has been found to be involved in other cancer metastasis mechanisms, for example, hypoxia [20]. Instead of trying to antagonize this receptor, it may also be possible to prevent or modulate its expression in tumor cells. There are many studies already indicating that transfecting CXCR4 into a tumor cell greatly enhances its metastatic potential [21].

Notably, while CXCR4 is by far the most widely expressed chemokine receptor in most cancers, there are other examples of chemokine receptors associated with some specific cancers. One is CCR10 which is expressed by normal melanocytes [22] but is also present in melanoma [23]. Another is CX3CR1 which is present in microglia and also present in glioblastoma [24]. The latter receptor may participate in the unusual invasive ability of glioblastoma to invade normal brain tissue. These examples, moreover, have another underlying message: tumor cells by and large tend to express the chemokine receptors that their normal counterparts are already programmed to express. Thus, inhibitors of these receptors may affect specific functions of the tumor cells in these cases, but, in contrast to CXCR4 inhibitors, they are likely to be restricted to these specific cancers.

Finally, the central question remains of the nature of organogenesis. There are now many examples, some of them from model organisms such as drosophila or zebrafish, where genes specifically control the development of certain organs. As this field advances, eventually we may get a much better picture of the role of chemokine receptors, and especially of CXCR4, in this process. This may give us the tools to identify potential secondary targets induced by CXCR4 that control specific aspects of this process. I conclude that this is a field that should see strong interest in the next few years.

References

1 Bacon K, Baggiolini M, Broxmeyer H, Horuk R, Lindley I, Mantovani A, Maysushima K, Murphy P, Nomiyama H, Oppenheim J, Rot A, Schall T, Tsang M, Thorpe R, Van Damme J, Wadhwa M, Yoshie O, Zlotnik A, Zoon K: Chemokine/chemokine receptor nomenclature. J Interferon Cytokine Res 2002;22:1067–1068.

2 Zlotnik A, Yoshie O: Chemokines: a new classification system and their role in immunity. Immunity 2000;12:121–127.

3 Nakano H, Gunn MD: Gene duplications at the chemokine locus on mouse chromosome 4: multiple strain-specific haplotypes and the deletion of secondary lymphoid-organ chemokine and EBI-1 ligand chemokine genes in the plt mutation. J Immunol 2001;166:361–369.

4 Muller G, Hopken UE, Lipp M: The impact of CCR7 and CXCR5 on lymphoid organ development and systemic immunity. Immunol Rev 2003;195:117–135.

5 Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A: Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50–56.

6 Takanami I: Overexpression of CCR7 mRNA in non-small-cell lung cancer: correlation with lymph node metastasis. Int J Cancer 2003;105:186–189.

7 Balkwill F: The significance of cancer cell expression of the chemokine receptor CXCR4. Semin Cancer Biol 2004;14:171–179.
8 Balkwill F: Chemokine biology in cancer. Semin Immunol 2003;15:49–55.
9 Barbero S, Bajetto A, Bonavia R, Porcile C, Piccioli P, Pirani P, Ravetti JL, Zona G, Spaziante R, Florio T, Schettini G: Expression of the chemokine receptor CXCR4 and its ligand stromal cell-derived factor 1 in human brain tumors and their involvement in glial proliferation in vitro. Ann NY Acad Sci 2002;973:60–69.
10 Eisenhardt A, Frey U, Tack M, Rosskopf D, Lummen G, Rubben H, Siffert W: Expression analysis and potential functional role of the CXCR4 chemokine receptor in bladder cancer. Eur Urol 2005;47:111–117.
11 Hartmann TN, Burger M, Burger JA: The role of adhesion molecules and chemokine receptor CXCR4 (CD184) in small cell lung cancer. J Biol Regul Homeost Agents 2004;18:126–130.
12 Hartmann TN, Burger JA, Glodek A, Fujii N, Burger M: CXCR4 chemokine receptor and integrin signaling co-operate in mediating adhesion and chemoresistance in small cell lung cancer (SCLC) cells. Oncogene 2005;24:4462–4471.
13 Hwang JH, Chung HK, Kim DW, Hwang ES, Suh JM, Kim H, You KH, Kwon OY, Ro HK, Jo DY, Shong M: CXC chemokine receptor 4 expression and function in human anaplastic thyroid cancer cells. J Clin Endocrinol Metab 2003;88:408–416.
14 Kim J, Takeuchi H, Lam ST, Turner RR, Wang HJ, Kuo C, Foshag L, Bilchik AJ, Hoon DS: Chemokine receptor CXCR4 expression in colorectal cancer patients increases the risk for recurrence and for poor survival. J Clin Oncol 2005;23:2744–2753.
15 Helbig G, Christopherson KW 2nd, Bhat-Nakshatri P, Kumar S, Kishimoto H, Miller KD, Broxmeyer HE, Nakshatri H: NF-kappaB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. J Biol Chem 2003;278:21631–21638.
16 Lee BC, Lee TH, Avraham S, Avraham HK: Involvement of the chemokine receptor CXCR4 and its ligand stromal cell-derived factor 1alpha in breast cancer cell migration through human brain microvascular endothelial cells. Mol Cancer Res 2004;2:327–338.
17 Altundag K, Morandi P, Altundag O, Gunduz M: Possible role of CXCR4-mediated chemotaxis in breast cancer patients with central nervous system metastases. Breast Cancer Res Treat 2005;89:317.
18 Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR: Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 1998;393:595–599.
19 Tissir F, Wang CE, Goffinet AM: Expression of the chemokine receptor CXCR4 mRNA during mouse brain development. Brain Res Dev Brain Res 2004;149:63–71.
20 Staller P, Sulitkova J, Lisztwan J, Moch H, Oakeley EJ, Krek W: Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumor suppressor pVHL. Nature 2003;425:307–311.
21 Zeelenberg IS, Ruuls-Van Stalle L, Roos E: Retention of CXCR4 in the endoplasmic reticulum blocks dissemination of a T cell hybridoma. J Clin Invest 2001;108:269–277.
22 Morales J, Homey B, Vicari AP, Hudak S, Oldham E, Hedrick J, Orozco R, Copeland NG, Jenkins NA, McEvoy LM, Zlotnik A: CTACK, a skin-associated chemokine that preferentially attracts skin-homing memory T cells. Proc Natl Acad Sci USA 1999;96:14470–14475.
23 Murakami T, Cardones AR, Hwang ST: Chemokine receptors and melanoma metastasis. J Dermatol Sci 2004;36:71–78.
24 Hulshof S, van Haastert ES, Kuipers HF, van den Elsen PJ, De Groot CJ, van der Valk P, Ravid R, Biber K: CX3CL1 and CX3CR1 expression in human brain tissue: noninflammatory control versus multiple sclerosis. J Neuropathol Exp Neurol 2003;62:899–907.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 200–208

Visualization of Tumor Cell Extravasation

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Abstract

In cancer the blood-borne spread of tumor cells leads to the formation of secondary tumors at distant loci, whereby the extravasation of tumor cells is a prerequisite step during hematogenous metastasis. In regard to the fate of endothelial cells located at the site of tumor cell infiltration, tumor cell-endothelial interactions were analyzed using an in vitro real-time model. This model shows the complete sequence of the transmigration process and gave new insights into the complex and dynamic cell-cell and cell-matrix interactions which occur during tumor cell transmigration across the endothelial barrier. An in vitro real-time apoptosis assay permits the distinction between apoptotic cell death from necrotic cell death. This model indicates that transmigration of tumor cell clusters derived from the invasive human bladder carcinoma cell line T24 irreversibly damages the endothelial cells by inducing apoptosis at the site of tumor cell infiltration. It is postulated here that apoptosis induction facilitates the removal of detached endothelial cells, thereby forestalling a local inflammatory response which might be detrimental to extravasating tumor cells.

Cancer as a disease has already been described in the earliest medical records found in the history of mankind, dating back to ancient Egypt. The term ‘cancer’ is attributed to the Greek physician Hippocrates and is derived from bizarre ‘crablike’ growth forms of tumors – ‘karkinoma’ is the Greek word for ‘crab’. Today, cancer is the second most prevalent cause of death after heart disease in the industrialized world. The transformation of a normal somatic cell to a malignant phenotype is generally perceived to proceed in a series of sequential

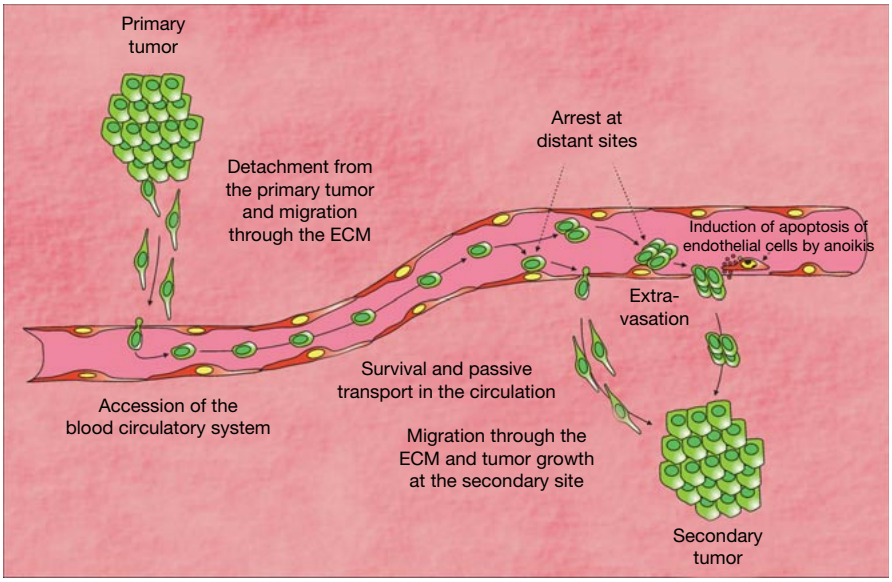

Fig. 1. The metastasis cascade. The events leading to metastasis can be summarized as follows: (1) detachment from the primary tumor; (2) accession of the lymphatic or blood circulatory system; (3) survival in the circulation; (4) arrest at distant sites; (5) transfer of cancer cells, both single cells and cell clusters, across the vessel wall into the parenchymal tissue; and (6) tumor growth at the secondary site. Transendothelial migration of tumor cell clusters is likely associated with an irreversible disruption of the endothelium.

steps, including gene mutations, deletions, and chromosome aberrations. The hallmark in cancer disease is the progression towards unrestricted proliferation of cancer cells as well as dedifferentiation, which implies loss of growth control and tissue specific function in tumor tissue.

The formation of metastasis is the primary cause of death in cancer [1, 2]. Metastasizing tumor cells (fig. 1) must traverse natural barriers, such as connective tissue components and organ epithelia at multiple stages of the metastatic process [3]. Thereby, a variety of cellular events are mandatory for secondary tumor formation far away from the primary tumor site. For instance, multiple cell-cell and cell-matrix interactions are necessary to allow the spread of tumor cells and growth of secondary tumors [4]. During this process, tumor cells may alternatively demonstrate increased or decreased adhesive properties depending on the metastatic stage [5, 6]. The way by which cancer cells spread, i.e. by the lymphatic or blood circulatory system, depends on the tumor type. In this chapter, the hematogenous spread of cancer cells will be discussed.

Tumor Cell Extravasation

Tissue or organ compartments in the body are separated by two types of extracellular matrix, basement membranes and interstitial stroma. Connective tissue components, as well as epithelia which are localized on basement membranes, form natural barriers which malignant cells have to traverse at multiple stages of the metastatic process [3]. Cancer cells must detach from the primary tumor in order to metastasize. This requires the disruption of existing homophilic cell-cell contacts at the primary site. For example, downregulation of E-cadherin, an adhesion molecule, which is found preferentially at cell-to-cell junctions in epithelial tissues, correlates with a higher metastatic potential [7–9]. Additionally, Dittmar et al. [10] have recently shown that upregulation of the growth factor receptor tyrosine kinase c-erbB-2 in EGFR overexpressing cells contributed to the EGF-induced migration of such cells due to a c-erbB-2-dependent modulation of the kinetics of the adaptor protein phospholipase C-γ1 (PLC-γ1).

Malignant cells must gain access to the blood capillary vessels (intravasation) to spread with the blood circulation to distant organs. Thereby, single tumor cells, as well as multi-cellular aggregates, escape into blood vessels from a primary solid tumor. It is well established that only a small fraction of tumor cells which are released from the primary tumor actually form lesions at distant sites [11]. For instance, experimental observations show that only about 0.01% of tumor cells injected into mice survive the passage through the blood circulation [12]. It has been suggested that cancer cells are rapidly destroyed either by the immune system [13, 14] or by hemodynamic forces in the microvasculature [15]. The formation of multi-cellular aggregates is perceived to enhance tumor cell survival in the vasculature by providing a suitable micro-environment [16]. However, other studies suggest that tumor cells survive the circulation primarily by rapid adhesion and escape from the vasculature [17, 18].

During their passage through the blood circulation, tumor cells arrest in the capillary bed of distant organs. Tumor cell extravasation can be briefly defined as the process, which translocates tumor cells from the blood circulation across the vascular endothelium to the surrounding tissue. Extravasation has been described as a rate-regulating event in metastasis [19, 20]. Thereby, it can be subdivided into the following steps: (1) adhesion of tumor cells to the vascular endothelium; (2) transmigration across the endothelial lining and the underlying basement membrane; and (3) invasion of the surrounding tissue [4]. It has been demonstrated in situ that single tumor cells adhere to vascular endothelium and form microcolonies within the vasculature prior to extravasation [21]. In addition, it has been proposed that tumor cell clusters detach from the primary tumor and are transported through the blood vasculature as

multi-cellular aggregates [22], and are subsequently arrested in the micro-capillary system where they form tight interactions with the endothelial cell lining [23].

The initial site of tumor cell arrest is effectively determined by size constraints, depending on the relative size of the tumor cells and the capillaries. The observation that some tumors preferentially metastasize to certain organs may be explained by (1) anatomical criteria, such as the location of the next capillary bed, where the spreading cancer cells are entrapped after entrance into the vasculature and transport by the blood circulation. Furthermore, (2) by the 'seed and soil' hypothesis, which was originally postulated by Paget [24], where both 'seed' (cancer cells) and 'soil' (organ environment) contribute to an organ-specific pattern of secondary tumor formation [25]. Recent findings suggest an important role of chemokines in the direction of organ-specific metastasis formation. Muller et al. [26] were able to show that breast cancer metastasis into regional lymph nodes, bone marrow, liver, and lung is directed by the interplay of the stromal cell-derived factor-1α (SDF-1α; CXCL12), which is predominantly expressed in these tissues, and its receptor CXCR4 found to be expressed on breast cancer cells. Neutralizing the interaction between SDF-1α/CXCR4 using a specific SDF-1α-antibody significantly impaired the metastasis of breast cancer cells into regional lymph nodes and lung in vivo. A similar mechanism was reported for the metastasis pattern of prostate cancer [27, 28].

Tumor Cell-Endothelial Cell Interactions during Extravasation

Endothelial cells were once believed to be a passive barrier for blood cells. However, it is now well-established that the endothelial lining of the blood circulation assumes an active role in many physiological processes, such as the extravasation of leukocytes during inflammation and homing, e.g. by well-regulated expression of cell adhesion molecules [29]. It is generally accepted that the extravasation of tumor cells is preceded by the adhesion to vascular endothelium [5, 30]. The subsequent extravasation event occurs within 24–48 h for more than 80% of cancer cells after their initial arrest in the microvasculature [18]. However, the exact mechanism by which tumor cells leave the blood circulation remains controversial. Nicolson [31] observed that melanoma cell extravasation induces endothelial cell retraction, with subsequent reformation of the endothelial monolayer and closing of the gap at the site of extravasation. In vivo observations of mouse liver and chick chorioallantoic membrane suggest that individual tumor cells may extravasate without observable disruption of the

microvasculature [32, 33]. Alternatively, morphological studies of early lung metastases show disintegration of endothelial cells adjacent to attached tumor cells [34]. Also, it has been shown in vitro that pancreatic tumor cells are able to impair endothelium at the site of extravasation [35]. A study by Kebers et al. [36] demonstrated that tumor cell lines derived from mammary epithelial tissue (MCF-7), as well as a fibrosarcoma cell line (HT-1080) were able to induce apoptosis in endothelial cells.

The findings of Kebers et al. [36] are in view with recent data we obtained by studying the transendothelial migration of tumor spheroids. In this study, the extravasation of T24 tumor spheroids was investigated in a three-dimensional extravasation assay. This method allows for live cell imaging of tumor spheroid transmigration events from a perpendicular point of view and an observation of endothelial cell/tumor cell interactions in real-time. Moreover, this assay helps to overcome those limitations when using modified Boyden chamber/transwell assay systems [37–41]. Due to the design of modified Boyden chamber/transwell assays, the mechanism of transmigration can only be viewed in a plane parallel to the endothelial monolayer. However, this can be overcome if several optical sections of one sample are taken and the three-dimensional process of transmigration is reconstructed from a stack of two-dimensional images using three-dimensional imaging/deconvolution software. Nonetheless, since mostly fixed samples are used the time dimension of transmigration can not be resolved by such an assay.

Therefore, we analyzed the infiltration of the endothelial monolayer by tumor spheroids in real-time by confocal laser scanning microscopy. Endothelial cells and tumor spheroids were stained with fluorescent dyes (endothelial cells: calcein AM, T24 tumor spheroids: PKH-26) prior to analysis. The image sequences in figure 2 show the transmigration process of a single T24 tumor spheroid across the endothelial monolayer. In figure 2A, only the endothelial cells were stained with Calcein green AM, whereas in figure 2B both endothelial cells and tumor spheroids were stained with Calcein green AM and PKH-26, respectively. Both image sequences clearly indicate that the complete T24 tumor spheroid transmigrates across the endothelial monolayer within a time frame of 4 h. Approximately 90 min after addition of the T24 spheroid, tumor cells established a close contact to the apical surface of the endothelial cells. One hour later, the endothelial cells showed a contracted morphology (fig. 2A, B, white arrows) at the site of contact to the T24 tumor cell cluster, finally assuming an almost rounded shape after 3.5 h (fig. 2A, B, yellow arrows). During this time period, the T24 tumor spheroid completely invaded the collagen matrix beneath the endothelium, concomitant with a complete destruction of the endothelial layer at the site of infiltration. Single endothelial cells showed a rounded morphology with structures resembling membrane

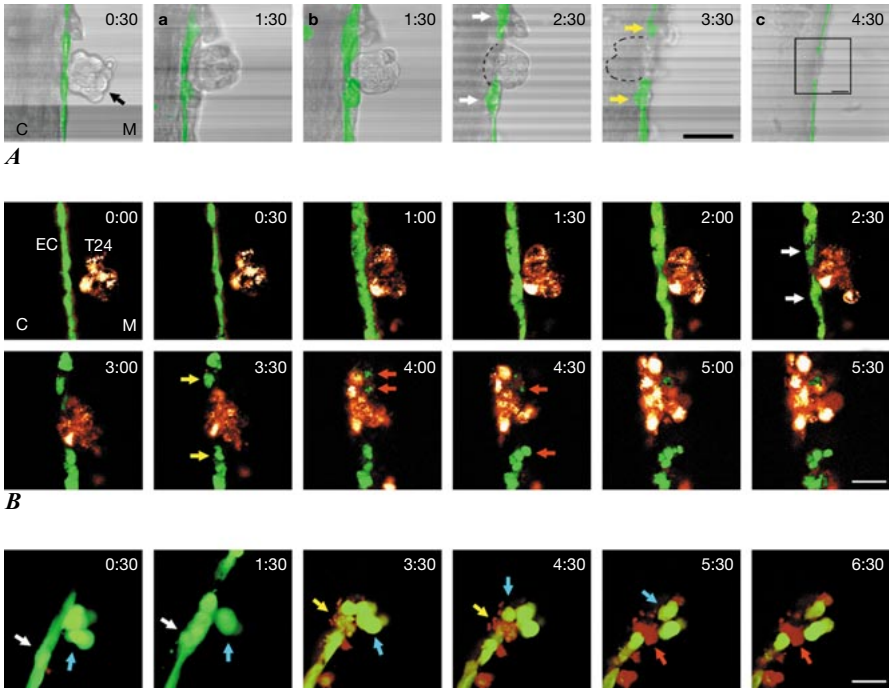

Fig. 2. Confocal laser scanning microscopy analysis of T24 tumor cell extravasation. ***A*** The endothelial monolayer was stained by Calcein AM (green fluorescence) and dissociates irreversibly in the course of tumor spheroid (black arrow) transmigration. The dotted line indicates the cell body mass of the invaded spheroid. The images (**a**) and (**b**) show a different focal plane (step size 4 μm) at 1:30 h. **c** Lower magnification view at 4:30 h showing that disruption of the HUVEC monolayer integrity is restricted to the extravasation site. ***B*** T24 cell spheroids were stained with the lipophilic dye PKH-26 (red fluorescence), whereas HUVEC were labeled by using Calcein AM (green fluorescence). The image sequences clearly indicate that within a time period of 4 h, a complete tumor spheroid transmigrates across the endothelium. White arrows indicate the retraction process of endothelial cells at the invasion front of the tumor spheroid. Yellow and red arrows indicate rounded morphologies on endothelial cells with structures resembling membrane blebbing. ***C*** HUVEC (white arrows) and T24 tumor spheroid (blue arrow) were stained by Calcein AM. Annexin-V-Cy3 labeling (red fluorescence) in conjunction with a strong green fluorescent signal from Calcein AM after 3.30 h indicates that endothelial cells are triggered to undergo apoptosis (yellow and red arrows) at the site of tumor spheroid infiltration. ***A–C*** Time is shown in hours, the bar represents 50 μm. Movie files (Quicktime) of the complete image sequences can be viewed at: http://www.uni-wh.de/immunology

blebbing (fig. 2B, red arrows). The cell-to-cell contacts between the endothelial cells were completely destroyed at the site of transmigration. A lower magnification view verified that the destruction of the endothelial monolayer was solely restricted to the site of transmigration.

For a better definition of the processes involved in the destruction of the endothelial monolayer, an annexin-V based assay was conducted to test if endothelial cells undergo apoptosis or necrosis at the site of transmigration (fig. 2C). After 3.5 h, the tumor cell cluster was in the process of transmigrating across the endothelium into the underlying collagen matrix, whereby the endothelium at the site of invasion was disintegrated and showed strong annexin-V staining (red fluorescence). The retention of Calcein green AM within the cytosol indicates that the plasma membrane of endothelial cells was still intact. In combination with annexin-V staining, this is a typical indication for early apoptotic events (fig. 2C, yellow arrows). Moreover, the formation of membrane blebs is visible, which is a characteristic morphological feature in apoptotic cell death. The finding that disruption of endothelium integrity is restricted to the site of tumor spheroid extravasation supports the hypothesis that apoptosis of endothelial cell by tumor cells is induced via a direct cell-to-cell contact. However, transmigration of T24 spheroids is not influenced by blockade of caspase-dependent pathways through caspase inhibitors leading to the assumption that endothelial cell apoptosis is caused by anoikis [42, 43] due to disengagement of endothelial adhesion receptors from the extracellular matrix and loss of endothelial homophilic cell-to-cell interactions [23].

Our data strongly suggest that the real-time in vitro extravasation model will give new insights into the complex and dynamic cell-to-cell and cell-to-matrix interactions during transendothelial migration of tumor cells.

Acknowledgements

The authors are grateful for the technical assistance of Silvia Keil and Gabi Troost. This work was supported by the Fritz-Bender-Foundation, Munich, Germany.

References

1 Fidler IJ, Ellis LM: The implications of angiogenesis for the biology and therapy of cancer metastasis. Cell 1994;79:185–188.
2 Weinstat-Saslow D, Steeg PS: Angiogenesis and colonization in the tumor metastatic process: basic and applied advances. FASEB J 1994;8:401–407.
3 Liotta LA, Rao CN, Barsky SH: Tumor invasion and the extracellular matrix. Lab Invest 1983;49:636–649.
4 Nicolson GL: Metastatic tumor cell interactions with endothelium, basement membrane and tissue. Curr Opin Cell Biol 1989;1:1009–1019.

5 Pauli BU, Augustin-Voss HG, el-Sabban ME, Johnson RC, Hammer DA: Organ-preference of metastasis: the role of endothelial cell adhesion molecules. Cancer Metastasis Rev 1990;9: 175–189.
6 Stetler-Stevenson WG, Liotta LA, Kleiner DE Jr: Extracellular matrix 6: role of matrix metalloproteinases in tumor invasion and metastasis. FASEB J 1993;7:1434–1441.
7 Mareel M, Vleminckx K, Vermeulen S, Bracke M, Van Roy F: E-cadherin expression: a counterbalance for cancer cell invasion. Bull Cancer 1992;79:347–355.
8 Masur K, Lang K, Niggemann B, Zaenker KS, Entschladen F: High PKC alpha and low E-cadherin expression contribute to high migratory activity of colon carcinoma cells. Mol Biol Cell 2001;12: 1973–1982.
9 Contreras RG, Shoshani L, Flores-Maldonado C, Lazaro A, Monroy AO, Roldan ML, Fiorentino R, Cereijido M: E-Cadherin and tight junctions between epithelial cells of different animal species. Pflügers Arch 2002;444:467–475.
10 Dittmar T, Husemann A, Schewe Y, Nofer JR, Niggemann B, Zaenker KS, Brandt BH: Induction of cancer cell migration by epidermal growth factor is initiated by specific phosphorylation of tyrosine 1248 of c-erbB-2 receptor via EGFR. FASEB J 2002;16:1823–1825.
11 Weiss L: Cancer cell traffic from the lungs to the liver: an example of metastatic inefficiency. Int J Cancer 1980;25:385–392.
12 Fidler IJ: Metastasis: quantitative analysis of distribution and fate of tumor emboli labelled with ^{125}I-5-iodo-2'-deoxyuridine. J Natl Cancer Inst 1970;45:773–782.
13 Key ME: Macrophages in cancer metastases and their relevance to metastatic growth. Cancer Metastasis Rev 1983;2:75–88.
14 Hanna N: Role of natural killer cells in control of cancer metastasis. Cancer Metastasis Rev 1982;1:45–64.
15 Weiss L, Dimitrov DS, Angelova M: The hemodynamic destruction of intravascular cancer cells in relation to myocardial metastasis. Proc Natl Acad Sci USA 1985;82:5737–5741.
16 Ruiter DJ, van Krieken JH, Van Muijen GN, de Waal RM: Tumor metastasis: is tissue an issue? Lancet Oncol 2001;ii:109–112.
17 Luzzi KJ, MacDonald IC, Schmidt EE, Kerkvliet N, Morris VL, Chambers AF, Groom AC: Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. Am J Pathol 1998;153:865–873.
18 Chambers AF, Naumov GN, Varghese HJ, Nadkarni KV, MacDonald IC, Groom AC: Critical steps in hematogenous metastasis: an overview. Surg Oncol Clin N Am 2001;10:243–255, vii.
19 Haier J, Korb T, Hotz B, Spiegel HU, Senninger N: An intravital model to monitor steps of metastatic tumor cell adhesion within the hepatic microcirculation. J Gastrointest Surg 2003;7: 507–514; discussion 514–515.
20 Orr FW, Wang HH, Lafrenie RM, Scherbarth S, Nance DM: Interactions between cancer cells and the endothelium in metastasis. J Pathol 2000;190:310–329.
21 Al-Mehdi AB, Tozawa K, Fisher AB, Shientag L, Lee A, Muschel RJ: Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: a new model for metastasis. Nat Med 2000;6:100–102.
22 Friedl P, Noble PB, Walton PA, Laird DW, Chauvin PJ, Tabah RJ, Black M, Zaenker KS: Migration of coordinated cell clusters in mesenchymal and epithelial cancer explants in vitro. Cancer Res 1995;55:4557–4560.
23 Heyder C, Gloria-Maercker E, Entschladen F, Hatzmann W, Niggemann B, Zaenker KS, Dittmar T: Realtime visualization of tumor cell/endothelial cell interactions during transmigration across the endothelial barrier. J Cancer Res Clin Oncol 2002;128:533–538.
24 Paget S: The distribution of secondary growths in cancer of the breast: 1989. Cancer Metastasis Rev 1989;8:98–101.
25 Chambers AF, Groom AC, MacDonald IC: Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer 2002;2:563–572.
26 Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A: Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50–56.

27 Vaday GG, Hua SB, Peehl DM, Pauling MH, Lin YH, Zhu L, Lawrence DM, Foda HD, Zucker S: CXCR4 and CXCL12 (SDF-1) in prostate cancer: inhibitory effects of human single chain Fv antibodies. Clin Cancer Res 2004;10:5630–5639.
28 Mochizuki H, Matsubara A, Teishima J, Mutaguchi K, Yasumoto H, Dahiya R, Usui T, Kamiya K: Interaction of ligand-receptor system between stromal-cell-derived factor-1 and CXC chemokine receptor 4 in human prostate cancer: a possible predictor of metastasis. Biochem Biophys Res Commun 2004;320:656–663.
29 Jahroudi N, Greenberger JS: The role of endothelial cells in tumor invasion and metastasis. J Neurooncol 1995;23:99–108.
30 Honn KV, Tang DG: Adhesion molecules and tumor cell interaction with endothelium and subendothelial matrix. Cancer Metastasis Rev 1992;11:353–375.
31 Nicolson GL: Cancer metastasis: tumor cell and host organ properties important in metastasis to specific secondary sites. Biochim Biophys Acta 1988;948:175–224.
32 Morris VL, Koop S, MacDonald IC, Schmidt EE, Grattan M, Percy D, Chambers AF, Groom AC: Mammary carcinoma cell lines of high and low metastatic potential differ not in extravasation but in subsequent migration and growth. Clin Exp Metastasis 1994;12:357–367.
33 Chambers AF, MacDonald IC, Schmidt EE, Koop S, Morris VL, Khokha R, Groom AC: Steps in tumor metastasis: new concepts from intravital videomicroscopy. Cancer Metastasis Rev 1995;14: 279–301.
34 Crissman JD, Hatfield JS, Menter DG, Sloane B, Honn KV: Morphological study of the interaction of intravascular tumor cells with endothelial cells and subendothelial matrix. Cancer Res 1988;48:4065–4072.
35 Shaughnessy SG, Lafrenie RM, Buchanan MR, Podor TJ, Orr FW: Endothelial cell damage by Walker carcinosarcoma cells is dependent on vitronectin receptor-mediated tumor cell adhesion. Am J Pathol 1991;138:1535–1543.
36 Kebers F, Lewalle JM, Desreux J, Munaut C, Devy L, Foidart JM, Noel A: Induction of endothelial cell apoptosis by solid tumor cells. Exp Cell Res 1998;240:197–205.
37 Boyden S: The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J Exp Med 1962;115:453–466.
38 Akedo H, Shinkai K, Mukai M, Komatsu K: Potentiation and inhibition of tumor cell invasion by host cells and mediators. Invasion Metastasis 1989;9:134–148.
39 Gehlsen KR, Davis GE, Sriramarao P: Integrin expression in human melanoma cells with differing invasive and metastatic properties. Clin Exp Metastasis 1992;10:111–120.
40 Kramer RH, Nicolson GL: Interactions of tumor cells with vascular endothelial cell monolayers: a model for metastatic invasion. Proc Natl Acad Sci USA 1979;76:5704–5708.
41 Rice GE, Gimbrone MA Jr, Bevilacqua MP: Tumor cell-endothelial interactions. Increased adhesion of human melanoma cells to activated vascular endothelium. Am J Pathol 1988;133:204–210.
42 Meredith JE Jr, Fazeli B, Schwartz MA: The extracellular matrix as a cell survival factor. Mol Biol Cell 1993;4:953–961.
43 Frisch SM, Francis H: Disruption of epithelial cell-matrix interactions induces apoptosis. J Cell Biol 1994;124:619–626.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 209–231

Options for Visualizing Metastatic Disease in the Living Body

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Abstract

Detection and observation of primary tumor growth and metastasis in living subjects is an important task in clinical and basic cancer research. Recently several approaches and techniques emerged which offer a huge variety of options with respect to the specific objectives and questions of a given study. Recent developments in the field of in vivo imaging not only allow the assessment of anatomic information but also functional processes with cellular resolution and molecular sensitivity. This chapter will provide an overview of the most common imaging techniques which are currently available for the detection and observation of metastasizing tumor cells. General capacities, advantages, limitations and drawbacks will be discussed. These techniques include computed tomography (CT), molecular resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), fluorescence imaging (FI), and bioluminescent imaging (BLI). The objective is to provide the cancer researcher with information that will help solve the dilemma of how best to apply the latest imaging tools for studying biological questions in the context of the living body.

Since W.C. Roentgen's discovery of 'a new form of rays' in 1895, we have been advancing our ability to gain insights into the bodies of humans and animals noninvasively [1]. Since this time a multitude of new techniques has emerged, and the current revolution includes assessing functional changes with cellular resolution and molecular sensitivity in addition to anatomic information. What was inconceivable only a decade ago, imaging gene expression patterns and cell movement in the living body, is rapidly becoming common place in today's biomedical laboratories and soon will emerge as opportunities in the clinic. These incredible advances present researchers, however, with a new

dilemma, what modality to use. This demands that we understand the advantages and drawbacks of each new technique given the specific objectives and questions of the study.

The first part of this chapter will give a short overview of the best established in vivo imaging techniques and where they offer potential for revealing cellular and molecular changes, with special attention to the usefulness of these modalities for imaging metastasis and secondary sites of tumor growth post therapy. The second part will focus on those techniques, which have had the greatest impact on metastasis associated cancer research. These will then be highlighted in studies presented in the most recent literature. The objective here is to provide the cancer biologist with information that will help solve the dilemma of how to best apply the latest imaging tools studying biological question in the context of the living body. Studying these processes noninvasively where the influences of organ systems, a functional immune system and active circulation are intact the outcome will provide greater insights into disease mechanisms and improve our ability to intervene metastatic disease.

The new tools of molecular imaging rely on advances in established modalities and development of several novel modalities. Based on Roentgen's classical X-ray imaging, computed X-ray tomography (CT) provides excellent anatomic imaging, especially of bone. In contrast to imaging using external radiation sources, positron emission tomography (PET) and single photon emission computed tomography (SPECT) utilize energies emitted by radionuclides that are injected into the body. Another modality that has been developed for anatomic imaging is magnetic resonance imaging (MRI), which is based on the measurement of the relaxation dynamics of magnetic dipoles in a strong magnetic field. The oldest modality for whole body imaging is based on optics, and physicians still rely on the 'optical' appearance of the patient in their diagnosis, but optical methods are in a renaissance with a number of new developments and advances. Although not yet well established for clinical applications, optical imaging techniques, based on the detection of endogenous or exogenous reporter molecules within the body are beginning to revolutionize preclinical studies in oncology. The emerging optical modalities rely on photon emission either from fluorescent proteins or dyes, or light-emitting enzymes, called luciferases that produce biological light (bioluminescence).

Extension of imaging modalities beyond obtaining only anatomic information toward representation, characterization and quantification of biological processes with cellular resolution und molecular sensitivity comprise the emerging field of molecular imaging [2–6]. To achieve measurements of this type, signal amplification strategies based on novel molecular reporter designs are necessary. For this purpose, there are three basic design strategies. First, there are reporter probes, which are initially evenly distributed in the body and

then their design enables them to be trapped in certain tissues or cells by metabolic conversion or internalization by cell surface proteins. This trapping might either occur naturally in the cells/tissue of interest by targeting intrinsic molecules, or alternatively additional specificity can be achieved through the expression of reporter molecules such as herpes simplex virus 1 thymidine kinase (HSV-TK), dopamine 2 receptor (D2R), and transferrin receptor (TfR) [7–11]. This approach has been used to concentrate radioactive tracers and for magnetic particles in MRI. To reduce background signals an activation step can be used with modalities other than PET and SPECT to improve detection capabilities. The second strategy, therefore, is one where the molecular probe is silenced but targets an activation process to yield a detectable signal. This is the underlying concept for many optical imaging approaches using exogenous expression of reporter genes; the goal is to also use this approach to target intrinsic enzymatic activity [12–16]. This approach is also the focus of considerable efforts in the development of novel MRI contrast agents [17, 18]. The third strategy is the use of regulated expression of exogenous reporter proteins such as green fluorescent protein, β-galactosidase, or luciferases, with some of these requiring chemical substrates and others external excitation light in the case of fluorescence detection [19, 20]. For a general overview of imaging techniques see table 1.

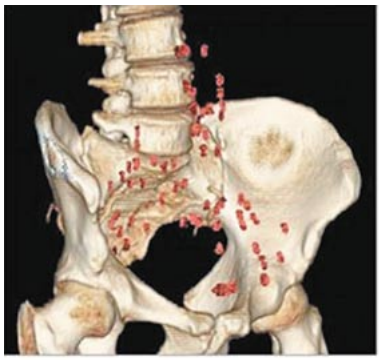
Applications of Whole-Body in vivo Imaging Techniques

Computed Tomography

CT imaging is based on differential absorption of X-rays by tissues of different density and properties. Images are acquired by a rotating X-ray source and detectors, classically consisting of a scintillator for conversion of X-rays to photons and CCD detectors for the collection of the photons. The resulting images, taken from different angles, are transformed into three-dimensional information by a computer. CT imaging is characterized by low contrast of soft tissues, relative to MRI, and considerable efforts have been directed at the development of improved contrast agents with successes in the literature for some organ systems [21, 22]. A standard approach is the administration of iodinated contrast media, which allows a more clear visualization of organs and tumors. While there is some development of molecular probes for CT imaging, the technique is typically used for morphological analyses and not specifically for molecular imaging. CT serves as an excellent second modality for combinations where structural data can be used to enhance functional data sets.

A significant contribution to the field is the combination of CT with other imaging techniques like PET and SPECT, as a means of complementing anatomic information with functional data [23–25] (cf. fig. 1). Multimodality imaging

a



b

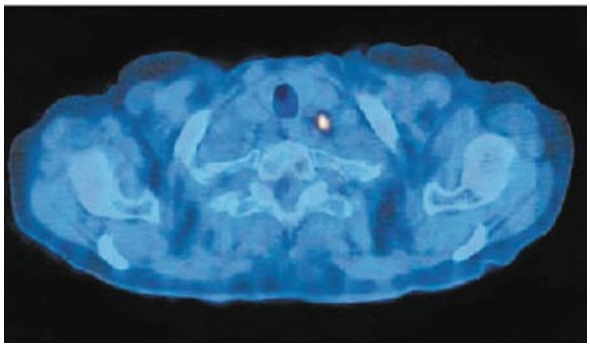


Fig. 1. Clinical imaging of metastases. ***a*** Fusion of a monocrystalline iron oxide nanoparticle-enhanced MRI image with a CT image (MION-CT). Preoperatively detected pelvic lymph node metastases of a prostate cancer patient are colored red. They were later confirmed histologically [71, 103]. Figure taken from Jaffer and Weissleder [3]. Copyright © 2005, American Medical Association. All rights reserved. ***b*** PET-CT-Fusion image of the apical thorax of a patient with non-small-cell lung cancer. Application of [^{18}F]fluoro-2-deoxy-*D*-glucose before PET scan led to the identification of a focus of radionuclide uptake which turned out to be a lymph node by CT imaging. After surgical excision histological analysis confirmed the existence of a 5-mm lymph-node metastasis [70]. Copyright © 2003, Massachusetts Medical Society. All rights reserved.

using hybrid instruments, or probes with dual, or multiple, signals is being realized as necessary for maximizing image quality and localization of signals, and greatly improving the diagnostic capability of imaging. Imaging of small animals by CT is possible with special μ-CT scanners [26], and combined PET/CT and SPECT/CT scanners for small animals are in development and being used in some imaging centers [27–29]. The availability of these instruments for small animal imaging will undoubtedly lead to advances in contrast agents that will have a significant impact on clinical imaging, as well as lead to image processing advances that will improve image quality.

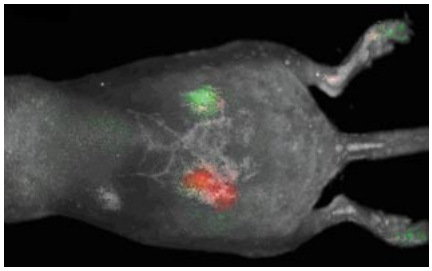


Fig. 2. Fluorescence imaging. In vivo fluorescence imaging of A375M human melanoma tumor cells growing subcutaneously of a nude mouse using CRI Maestro. Cells were labelled by lentiviral transduction and are stably expressing the hrluc-DsRed2-ttk tri-fusion reporter gene on left and Fluc-IRES-GFP reporter on right flank of the mouse [Image kindly provided by De A, Keren S, Gambhir SS, unpubl. data].

Magnetic Resonance Imaging

Like in CT, MRI scans are transformed computationally into three-dimensional information, leading to cross-sectional images of the body. However, the method of data acquisition is completely different. MRI imaging is based on the magnetic properties of unpaired nuclear spins. These magnetic dipoles align within a very strong magnetic field, which is applied in MRI scanners. For the production of image data, temporary radiofrequency pulses are produced by coils in the scanner, which lead to alteration of the spin alignment. Following a pulse, the orientation will return to the original status, in a way which strongly depends on the surrounding physicochemical conditions. The relaxation of the dipoles such as hydrogen in water and carbohydrates, can be detected by the same radiofrequency coil, which produces the pulses, as a change in electromagnetic flux. Since tissues vary in composition and content of all kinds of molecule classes with and without dipole character, the signals collected this way can be computed into three-dimensional images of the body [30].

In contrast to CT, MR images provide very good contrast in soft tissue, which may even be improved by contrast agents, namely paramagnetic cations, chelated gadolinium, ferric ammonium citrate and manganese chloride or super paramagnetic iron oxide particles. Most of these have specific distribution patterns and require careful selection for a given application [31]. Additionally, the emerging technique of MR ^{1}H and ^{31}P spectroscopic imaging (MRS) allows detection of a number of metabolites, which aids in identifying tissue types, determining the composition of malignant tissue and can assist in tumor grading [32]. While MRI is characterized by high spatial resolution (about 10 μm), greater than that of other imaging modalities, it is generally less sensitive to molecular species compared to radionuclide based and optical imaging. For this reason several studies have reported combined data that take advantage of the high sensitivity of optical or radionuclide imaging with the high soft-tissue resolution of MRI [33, 34]. A number of attempts have been made to obtain gene expression information by MRI using the activity of tyrosinase or TfR, as

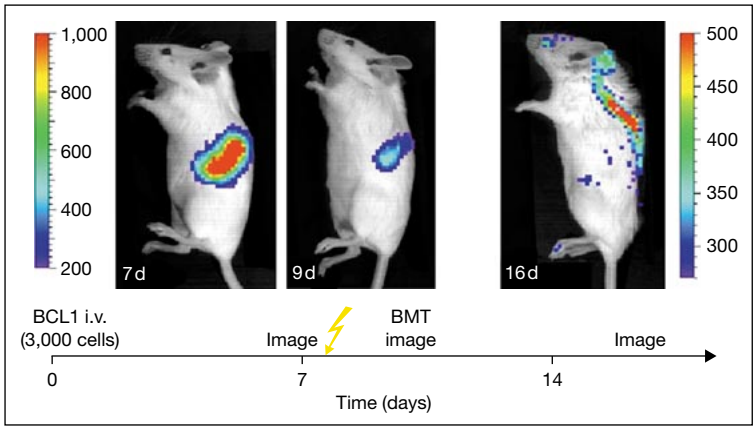


Fig. 3. Bioluminescence Imaging. Use of in vivo bioluminescence imaging to reveal the patterns of disease progression following radiation therapy and bone marrow transplant for the treatment of lymphoma. The images of disease burden are shown on top and the time line of treatment and imaging is shown below. BCL1 lymphoma cells that had been labeled via retrovirus transduction were delivered i.v. to mice (3,000 cells per mouse) to generate an orthotopic model, and the animals were imaged seven days later. This image shows signal primarily from the spleen. Following the imaging mice were treated with radiation and given a syngeneic bone marrow transplant and imaged 2 days later (9 days). The response to therapy is apparent in the 9-day image with a noticeable reduction in tumor burden. After a total of 16 days, the animals were imaged again. The cells that persisted following treatment were apparent at 16 days with signals largely from the spinal column. All of the animals in this study showed this pattern and all of these animals eventually relapsed. Imaging enables the entire disease course to be monitored including primary and secondary sites of tumor growth, response to therapy, metastasis and relapse. These approaches refine the animal models and accelerate their analyses such that treatment regimens can be tested and refined. This research was originally published in Edinger et al. [57]. Copyright © 2003, American Society of Hematology.

reporter genes, to concentrate molecules with magnetic properties in cells [9, 35, 36]. These strategies allow for detection of labeled cell populations in vivo by MRI. Imaging of small animals by MRI is well established [37, 38] and the development of novel molecular agents will benefit from the number of scanners that are available to the scientific community.

Nuclear Medicine Modalities (PET/SPECT)

In contrast to MRI and CT, which typically utilize intrinsic differences of tissue compartments for generation of an image, radionuclide imaging depends on the administration of a high energy emitting molecule, accumulating differentially in tissues based on the unique cellular physiology in the organ or tissue.

Table 1. Overview of IVI imaging techniques suitable for whole body imaging

Approach/ imaging technique	EM radiation utilized	Depth limit mm	Spatial resolution mm	Time/scan	Sensitivity mol/l	Main field of use	Pros	Cons
Magnetic resonance imaging (MRI)	Radiowaves (1–100 MHz)	none	0.025–0.1	Minutes to hours	$>10^{-5}$	anatomic, gene expression	highest spatial resolution, combination of functional and morphological imaging	very expensive apparatus, long imaging/ processing time, medium-low sensitivity
Computed tomography (CT)	X-rays	none	0.05–0.2	minutes	n/a	anatomic	medium cost solution for anatomical lung/ bone and tumor imaging	radiation exposure, low soft tissue resolution, limited functional applications
Radionuclide imaging								
Positron emission tomography (PET)	γ-rays (high energy), γ-decay	none	1–2	seconds to minutes	10^{-11}–10^{-12}	metabolic, gene expression, reporter tracking	tagging of natural compounds, quantitative, high sensitivity, clinical applications	radiation exposure, cyclotron or generator needed, medium-low spatial resolution, very cost-intensive
Single photon emission computed tomography (SPECT)	γ-rays (lower energy), alpha decay	none	1–2	minutes	10^{-10}–10^{-11}	gene expression, probe tracking (antibodies, peptides etc.)	simultaneous imaging of multiple probes, multitude of available probes, clinical applications	radiation exposure, collimation, medium to low spatial resolution

Table 1. (continued)

Approach/ imaging technique	EM radiation utilized	Depth limit mm	Spatial resolution mm	Time/scan	Sensitivity mol/l	Main field of use	Pros	Cons
Optical imaging								
Fluorescent imaging	visible/near infrared light	<10	>10% of depth	seconds to minutes	10^{-12}	cell tracking, gene expression	cost-efficient	medium to low spatial resolution, surface weighted
Bioluminescent imaging (BLI)	visible light	<30	minimum resolution = depth	seconds to minutes	$<10^{-12}$	cell tracking, gene expression	cost-efficient, detection in live max sensitivity	low spatial resolution, substrate injection, surface weighted, restricted clinical application

Since PET and SPECT imaging are based on the emission of high-energy photons, produced during nuclear decay of instable isotopes, penetration of tissues is generally good and thus the sensitivity of detection is high. The basic difference between the two nuclear medicine methods is that SPECT utilizes isotopes which generate a single photon, e.g. ^{99m}Tc, ^{111}In, ^{123}I, ^{131}I, while decay of the isotopes used in PET leads to emission of a positron, which emits two gamma rays at a 180° angle upon annihilation with an electron. The most commonly used isotopes in PET are ^{15}O, ^{13}N, ^{11}C, ^{18}F, and the less frequently used are ^{14}O, ^{62}Cu, ^{64}Cu, ^{124}I, ^{76}Br, ^{82}Rb, ^{68}Ga. ^{18}F is typically used for hydrogen replacement. The simultaneous emission of two gamma rays at a known angle makes it possible to localize the source of the signal. For SPECT imaging it is necessary to use collimators for identifying the origin of the signal; although collimators enable 3D localization of the source, they exclude a majority of the signal resulting in a significant loss in signal. Collimators with pinhole arrays have been used to capture some of this data and this approach results in more rapid data acquisition [39, 40]. The basic differences in PET and SPECT results in SPECT being generally less sensitive than PET by at least one order of magnitude. In addition, many of the positron-emitting isotopes for PET can be used as substitutes for naturally occurring atoms in bioorganic molecules, which opens up a multitude of possibilities in living organisms. However, the decay of all positron-emitting isotopes results in the production of two gamma rays of the same energy, which hinders the simultaneous imaging of two individual probes. In contrast, it is theoretically possible in SPECT to detect two isotopes of different energies, but is not practicable with current technologies. In practice, the main drawback of PET is its requirement for a nearby cyclotron to generate imaging agents [41–44], while the isotopes used in SPECT are generally longer lived and can be obtained from off site sources.

Linking molecular biology and imaging has been accomplished for both SPECT and PET. Several PET strategies are available for visualizing gene expression patterns, which mostly rely on a trapping effect, keeping the tagged molecules inside the expressing cells. A prominent example is the dopamine D2R receptor which triggers internalization and subsequent sequestration of the probe FESP (3-(2′(-[^{18}F]-fluoroethyl)spiperone). More recently a D2R mutant was developed, which acts independent of signal transduction. Another well established reporter gene for both, PET and SPECT visualization is HSV-TK. This enzyme catalyses the phosphorylation of a number of ^{18}F (for PET) or ^{131}I (for SPECT) labeled reporter molecules such as ^{18}F-fluoropenciclovir (^{18}F-FPCV), 9-(4-[^{18}F]fluoro-3-(hydroxymethyl)butyl)guanine (^{18}F-FHBG) and 2′fluoro-2′deoxy-*D*-arabinofuranosyl-5-[^{131}I]iodo-uracil (^{131}I-FAIU), respectively) and thus leads to intracellular trapping of the processed and tagged molecules [7, 8, 10, 11]. The somatostatin receptor has been similarly used for SPECT imaging [45]. The techniques have

been extensively used in small animal models [46–48], and two clinical studies have been reported that use reporter genes for assessing DNA transfer to tumors [49, 50]. Extremely low spatial resolution of radionuclide imaging can, in part, be overcome by combining it with CT [24, 25] (cf. fig. 1).

Optical Imaging

Generally speaking, optical imaging techniques, based on either bioluminescence or fluorescence, were specifically developed for obtaining functional data and are not particularly well suited for anatomic information. Both of these optical methods have been developed around the use of reporter genes for studies of gene expression, tumor burden and cell trafficking in vivo [13, 51–53] (cf. fig. 2 and fig. 3). Moreover, there are dyes that can be used with fluorescent approaches and these have had utility in developing in vivo enzyme assays [14]. The range of optical imaging approaches offers a number of complementary opportunities for studying biology in living animals [54]. Detection of optical reporters in vivo depends on the optical properties of tissues and the penetration of light through mammalian tissues is controlled by both absorbance and scatter, which is largely wavelength dependent [55]. The primary absorber in the body is hemoglobin; this pigment absorbs blue and green wavelengths of light. In addition to reduced absorption of light at wavelengths greater than 600 nm, there is less autofluorescence from mammalian tissues at these longer wavelengths. Taken together these optical properties of tissues has led investigators to develop dyes and reporter proteins that excite and emit at longer wavelengths.

An advantage of using fluorescent proteins, relative to bioluminescent, is that they can be used in fluorescence microscopy and in flow cytometry, which can greatly contribute to interpretation of the in vivo data by providing validation with other methods [56, 57]. Furthermore, it has been noted in the literature that an advantage to fluorescent proteins is that generation of signal does not require exogenous addition of chemical substrates; however, the pharmacology of these substrates has been studied and levels in given tissues can be predicted. The excitation of fluorescent proteins using external light sources requires understanding tissue optics, and predicting the amount of light that reaches the fluorophor for quantification of emitted signals.

The recent description of a wide range of colors of fluorescent proteins suggests that multiplexing may be possible [58] and that proteins with longer wavelengths of excitation and emission will lead to more sensitive in vivo assays. However, this is only an advantage if the excitation wavelengths are long enough to penetrate mammalian tissues and excite the fluorophor. The amount of excitation light that hits the fluorophor determines the brightness of the signal and assessing the levels of excitation can be challenging. The need

for light to cross the tissue twice is both an advantage and a disadvantage. Since the excitation intensity and subsequence emission intensity can be controlled and can be pulsed there are opportunities for quantitation. However, efficient delivery of excitation light of short wavelengths can be limiting. The availability of a multitude of fluorescence proteins such as the green fluorescent protein (GFP), the enhanced GFP (EGFP), or the red fluorescent protein (RFP), and fluorescent dyes, which can be coupled to ligands and antibodies that can be distinguished by their excitation and emission spectra may enable parallel imaging of several reporters. Furthermore the recent development of near infrared fluorochromes might overcome some of the problems caused by tissue absorption of light [59, 60]. Recent studies suggest the use of fluorescence-tagged Quantum dots to overcome several problems of classical fluorophores such as photobleaching and the need for individual excitation at different wavelengths for parallel imaging of multiple fluorophores [61, 62].

Bioluminescence imaging (BLI) utilizes the expression of enzymes called, as a class, luciferases. These emit light upon oxidation of a substrate. To date several of these have been used in vivo and two have been widely used for in vivo applications. These include firefly luciferase (Fluc), which is derived from the North American firefly *Photinus pyralis,* and *Renilla* luciferase (Rluc), which was isolated from the sea pansy *Renilla reniformis* [12, 51, 63–66]. While Fluc catalyses the oxidation of luciferin, Rluc is highly specific for its substrate coelenterazine. Since the substrates are not utilized by the respective other enzyme, imaging of both reporters in a given subject is possible. Two luciferase enzymes derived from the click beetle *Pyrophorus plagiophalam* have been engineered to emit red, CBred, and green light, CBgreen, and these have also been used in vivo [67]. Although these are not yet widely used for in vivo imaging, their coding sequences have been extensively modified for expression in mammalian cells and hold promise to improve expression levels. Furthermore, their expression patterns may more accurately reflect that of the native gene that they have been engineered to emulate. Although the reported wavelength of Fluc is 560 nm, its emission peak shifts from 560 nm at room temperature to 618 nm at 37°C. Thus, the potential advantage of CBred – emission peak at 620 nm at 22 and 37°C – having a longer wavelength and potentially more efficient penetration has not been realized [67]. For use of luciferases in vivo the chemical substrates need to be injected into the animals and this has been done both via intraperitoneal and intravenous routes. Understanding the biodistribution of the chemical substrates in animals is important for their application and can be used to the advantage of the investigator. Luciferin has a comparatively long circulation time while ceolenterazine is rapidly cleared from the body such that its conversion should be analyzed first in a sequence of images. In contrast to luciferin, which is relatively

inexpensive, ceolenterazine is, at present, too expensive to use in concentrations that saturate the enzyme in many models and this affects quantitative measurements of this reporter. The rapid clearance of coelenterazine also presents the problem of not being able to acquire the data prior to clearance of the substrate.

The red light of Fluc and CBred penetrates tissues to greater depths than the blue light of Rluc and thus the sensitivity of detection of the red emitters may be greater than that of luciferase that emit blue light. However, for the detection of two biological processes in a given animal, it is necessary to use two luciferases that use different substrates and all coelenterazine-utilizing enzymes at present emit blue light.

In both, bioluminescence and fluorescence techniques emitted photons can be detected by charge-coupled device (CCD) cameras, which are ideally cooled down to –105°C for reduction of thermal noise leading to minimal background signals. Tissue autofluorescence can generate significant noise and thus efficient means of separating signal from noise are beneficial for in vivo detection of fluorophores. Unfortunately, the need for exogenous expression of fluorescent and bioluminescent reporters and the injection of luciferase substrates or delivery of excitation wavelengths can be limiting. These features will have a dramatic impact on possible translation to the clinic. Furthermore, light absorbance by tissue is a significant barrier to sensitivity, and absorbance by melanin in dark skin and fur limits the choice of animals that can be effectively imaged with maximum sensitivity. Nevertheless, optical techniques are characterized by very high sensitivity and easy handling, which allows the detection of hundreds to thousands cells in whole body imaging in vivo, without cutting tissue windows or otherwise exposing the tissues. In addition, the methods are of relatively high throughput with parallel imaging of up to five animals in time frames of a few seconds to a few minutes.

Tomographic reconstruction methods have been reported for both fluorescence and bioluminescence [68, 69]. For BLI, a system using a rotating CCD camera is currently under development, which would allow CT for more detailed information on the exact position and size of the light emitting source. For fluorescence imaging (FI) similar systems are currently being tested, which might allow routine fluorescence tomography in small animals in the near future. Future developments promise to increase the utility of each of these optical methods.

Clinical Imaging of Metastases

Generally speaking, the attempt of imaging of metastases in living patients may be compared to their treatment. Usually there is only little information

available about the properties of putative metastases, which makes it hard to apply probes for specifically detecting them. While characterization of a primary tumor after surgery gives hints about probable characteristics of metastases the characteristics of the primary lesion should be interpreted relative to metastasis since metastasizing tumor cells may undergo several evolutionary steps, which may more or less change their properties. At the moment all imaging techniques that are available clinically fail to detect extremely small-size metastases.

Current techniques are able to detect metastases of about several millimeters in size. Most useful in this task seems either a combination of PET-CT or MRI. Using PET-CT imaging, it was shown recently that by intravenous injection of [^{18}F]fluoro-2-deoxy-*D*-glucose (^{18}F-FDG) a remarkable improvement in identification of metastases could be achieved compared to conventional PET or CT analysis. The approach is based on an accumulation of the radiolabeled glucose analogue in metabolically highly active tumor cells and combines the sensitivity of PET with the high spatial resolution of CT [70] (cf. fig. 1b). Even higher sensitivity, however, is reached, when highly lymphotropic super-paramagnetic iron oxide nanoparticles are administered intravenously before imaging by MRI. This technique is able to detect occult nodal prostate cancer metastasis as small as 2 mm, which is one third to one fifth the size of the detection limit of conventional MRI [71]. Another promising approach of metastasis detection in patients implies the use of marked antibodies, or antibody fragments, directed against tumor-specific or tumor-associated antigens. This possibility is especially alluring because many therapeutic antibodies are under clinical investigations at present, which could be utilized in lower concentrations, or by using derivatives of these antibodies, for imaging purposes, and even combined imaging and therapy. However, advances are still necessary to overcome the limitation of the minimum detectable size of 2 mm when combined with PET imaging. At present a significant number of metastasizing tumor cells can be missed; nonetheless, it is still considered to be useful in cancer staging [5, 72, 73].

Despite the progress that has been made in the clinical imaging of metastases, none of the techniques to date is suitable for reliable detection of minimal residual disease following therapy. This topic is reviewed in more detail elsewhere [3, 42].

In vivo Imaging of Metastases in Animal Models

Previously, metastases in animal models could only be identified after sacrifice of the animals and examination of excised tissues. For this reason, preclinical studies of metastatic disease had required a huge number of

animals with serial sacrifices for data analyses. Moreover, it was not possible to follow the development of metastasis in a certain group of animals and only relatively large metastases could be identified using these time-consuming approaches.

In vivo imaging techniques have overcome most of these problems, although the approach to a given study and the modalities used for analysis should be considered carefully when planning a project. There are two basic strategies for preclinical models of metastasis. The first consists of mouse models with conditionally or chemically induced spontaneous tumors and metastases, while the second approach is based on tumors cells implanted in animals; this is primarily performed in rodents. While several models of sporadic tumors are available, spontaneously metastasizing cancers are rare in all animals commonly used in biomedical research. Moreover, these models face the same problems as clinical imaging of metastases, since only large metastases can be found without killing and time-consuming microscopic analysis of the animal [74–76]. Nevertheless, sporadic metastases of xenografted prostate cancer cells have been successfully detected in a mouse model with the help of a reporter gene delivered by an adenoviral vector in vivo. This vector contained the Fluc gene under control of an enhanced prostate-specific antigen promoter, enabling the detection of metastases originating from the implanted prostate tumor [42]. Utilizing additional tissue-specific promoters, e.g. whey acidic protein (WAP) or mouse mammary tumor virus (MMTV) for breast cancer cells, this strategy might prove successful for the detection of sporadic metastases of other origins as well. Another approach for the visualization of tumors and metastases not labeled ex vivo was described by Yu et al. [77]. This group reports the detection of tumors and metastases by injecting bacteria and vaccinia virus, expressing luciferase and GFP. These pathogens preferentially settle and replicate in the tumor microenvironment where they are protected from the host's immune system. Although this system facilitates the search for spontaneous, nonlabeled tumors and metastases, it implicates the drawback of yielding only endpoint results.

While the general usefulness of these approaches and ideal applications remain to be elucidated, a multitude of studies has been published examining the behavior of tumor cells marked with a reporter gene before implantation.

Models of Metastasis Utilizing Implanted Tumor Cells

Tumor cell implantation models can be divided into two groups: syngeneic and xenograft models. Syngeneic transplantation describes the implantation of cells and tumors, which were originally derived from the same species, which is

used as a recipient. This has several important implications regarding an intact interaction between tumor cells, their microenvironment and all cell-cell and cell-stroma interactions, which play a role during the complex process of metastasis [78, 79]. Xenograft models usually consist of human cells implanted in immunocompromized rats or mice. While this approach is often inevitable there are several aspects to keep in mind, when working with such models. One of these are the already mentioned cell-cell and cell-stroma interactions which are considered to be crucial in carcinogenesis and metastasis and which are often impaired in xenografts because essential molecular interactions do not occur across species boundaries. The use of immunocompromized animals is of similar importance because it impedes the study of the role of the immune system during cancer progression. Furthermore, it should be considered that immunocompromized mice often have additional unintentional properties. Nude mice – characterized by depletion of T cells and an impaired function of T and B cells – are for example described to have impaired angiogenesis, whereas severe combined immunodeficiency (SCID) mice – characterized by deficits in number and function of B and T cells – display an age-dependent leakage of their immune functions, which might interfere with long-term studies of metastasis [80–83]. With respect to the important process of tumor angiogenesis it should be mentioned that substantial differences were observed in mice and men, which strongly influences the development of xenografted human tumors and metastasis [84, 85].

Furthermore, an important decision to be made in the planning of metastasis models is the site of implantation/injection of the tumor cells. Several strategies are well established to date. Orthotopic tumor cell engraftment is preferentially done to visualize the whole process of metastasis originating from the original organ tissue, while bloodstream injection or direct implantation is more suitable to examine processes involved in later steps of metastasis like tumor cell homing, attachment and vascularization [86]. Thus far, orthotopic implantation models leading to metastasis have been described for all of the most common types of cancer, including breast, prostate and lung cancer [19, 52, 87]. A frequently used, though rather unspecific approach is subcutaneous implantation, which is commonly used for the assessment of tumor cell behavior [42, 88]. Bloodstream injections are common to study circulating tumor cells of different tumor origins. Depending on the site of injection and the choice of cells this approach is suitable to simulate the second phase of metastasis of a number of different metastatic cancers. The most common injection sites are the tail vein, the portal vein and the left ventricle. Portal vein injections are likely to result in liver metastasis, which was shown for colon cancer and pancreatic cancer cells [19, 89]. Cells injected into the tail vein mainly get trapped in the lung [88, 90–92] and cells injected into the left heart ventricle

most commonly form bone marrow metastases even though the formation of metastases in other organs has also been described [93–95]. Direct implantation of tumor cells into the target tissue has been described for examination of bone metastasis [93, 95].

Choice of Imaging Techniques for Visualization of Metastasizing Tumor Cells in Animal Models

To date, reporter genes suitable for the in vivo imaging of metastasizing tumor cells are available for radionuclide imaging (PET/SPECT), MRI and optical imaging (BLI/FI). The general strategy in most of these approaches is to label cells ex vivo with a reporter gene of which the expression can be visualized, with or without help of a specific probe. The reporter gene is put under the control of a strong, constitutive and non-tissue-specific promoter. Most frequently used for this task is the cytomegalovirus promoter (CMV) or SV-40 promoter [96–98]. Though it is highly recommended to use cells with a stable expression of the marker gene, coupled to a selection marker, temporary transfected cells might be used for short term studies (<7 days). Several conditions should be met by a suitable reporter: The reporter and its probe, if necessary, should be nontoxic and not trigger an immune response. The reporter gene product should furthermore not leave the cells/tissue where it is expressed. It should have a short clearance time, while its probe, if applicable, should be stable until it reaches its target. The image signal should quantitatively reflect the reporter gene expression.

Even though suitable reporter genes are available for MRI, PET, and SPECT such as HSV-TK, D2R, and TfR [9, 99–102], which allow imaging with deep tissue penetration, studies examining metastasis in animal models in vivo, as published recently, utilize one of the two optical imaging techniques. The main reasons are probably the ease of use, small expenditure of animals and time and cost effectiveness. Thus, optical imaging allows sensitive screening of large numbers of mice at several time points and leads to strong, statistically significant data with reasonable effort. For most studies these points are obviously more important than spatial resolution, which is highest in MRI and PET-CT imaging, respectively. Routine application of PET-CT so far is limited to imaging of humans [3, 70, 71, 103, 104], although feasibility studies have been carried out in small animals [27, 105]. MRI has been used for obtaining anatomic information of metastases detected by BLI or FI [90, 106].

Even though the detection of single tumor cells, labeled with GFP has been reported in microscope assisted studies, these require removal of overlying tissues and skin flap windows [54, 107]. BLI has largely been used and tested

without cutting into the animals and has been shown to have good sensitivity of detection. Studies published to date support the idea that it is useful to combine the expression of luciferase and a fluorescent protein for the labeling of tumor cells. By this means it is possible to sort successfully transfected cells by flow cytometry using the fluorescent protein, perform whole-body screening for tumors and metastasis pattern at high sensitivity with luciferase and identify minimum amounts of tumor cells in situ after sacrifice of the animals or using intravital fluorescence microscopy. By combining optical imaging with X-ray analysis, this elaborated approach was used in the identification and gene expression profiling of breast cancer cells with a bone-specific metastatic phenotype, utilizing a triple-modality reporter gene vector, whereby HSV-TK was used as the additional reporter gene [34, 108].

Taken together, the imaging of metastasis with help of reporter gene based optical imaging has become routine during recent years and is already an integral part of cancer research utilizing animal models [91, 92, 107–110], while similar approaches for MRI, PET, and SPECT are still in a more experimental phase. Even though MRI, PET, and CT analyses are used in combination with optical imaging, their general distribution is still rather limited due to their high cost and the need for sophisticated equipment. MRI, PET, SPECT, and CT are, however, the modalities of choice for clinical translation, at least at the present time.

Future Outlook

The use of imaging in preclinical models of human biology and disease holds great promise for revealing new biology, and for greatly accelerating and refining the study of these models. As the number of reporters and dyes increases and new chemistries are described there will be opportunities for multiplexed assays and more detailed analyses of regulatory pathways and networks. This will greatly aid in the development of new therapeutics and lead to a greatly improved understanding of disease mechanisms, specifically in metastatic disease. Micrometastatic lesions can be revealed more easily and with greater sensitivity than ever before and as such the course of disease from initiation, through progression, regression and relapse can be studied. As the tools improve the lessons learned will increase. We only need to be careful not to overinterpret the data. However, although a picture is worth a thousand words it is imperative that the correct words are gleaned from an image. Image data are subject to interpretation and it is incumbent on us all to take objective views of the new data and to validate the new assays with as much supporting data from more conventional approaches as possible. We have an opportunity to

open a window into mammalian biology and let the light shine out. We are on the verge of a powerful set of new tools and we have the potential to learn substantially from these approaches.

References

1 Roentgen WC: On a new kind of rays. Nature 1896;53:274.
2 Gross S, Piwnica-Worms D: Spying on cancer: molecular imaging in vivo with genetically encoded reporters. Cancer Cell 2005;7:5–15.
3 Jaffer FA, Weissleder R: Molecular imaging in the clinical arena. JAMA 2005;293:855–862.
4 Massoud TF, Gambhir SS: Molecular imaging in living subjects: seeing fundamental biological processes in a new light. Genes Dev 2003;17:545–580.
5 Benaron DA: The future of cancer imaging. Cancer Metastasis Rev 2002;21:45–78.
6 Lyons SK: Advances in imaging mouse tumor models in vivo. J Pathol 2005;205:194–205.
7 MacLaren DC, Gambhir SS, Satyamurthy N, Barrio JR, Sharfstein S, Toyokuni T, Wu L, Berk AJ, Cherry SR, Phelps ME, Herschman HR: Repetitive, non-invasive imaging of the dopamine D-2 receptor as a reporter gene in living animals. Gene Ther 1999;6:785–791.
8 Liang Q, Satyamurthy N, Barrio JR, Toyokuni T, Phelps MP, Gambhir SS, Herschman HR: Noninvasive, quantitative imaging in living animals of a mutant dopamine D2 receptor reporter gene in which ligand binding is uncoupled from signal transduction. Gene Ther 2001;8:1490–1498.
9 Weissleder R, Moore A, Mahmood U, Bhorade R, Benveniste H, Chiocca EA, Basilion JP: In vivo magnetic resonance imaging of transgene expression. Nat Med 2000;6:351–355.
10 Tjuvajev JG, Avril N, Oku T, Sasajima T, Miyagawa T, Joshi R, Safer M, Beattie B, DiResta G, Daghighian F, Augensen F, Koutcher J, Zweit J, Humm J, Larson SM, Finn R, Blasberg R: Imaging herpes virus thymidine kinase gene transfer and expression by positron emission tomography. Cancer Res 1998;58:4333–4341.
11 Gambhir SS, Bauer E, Black ME, Liang QW, Kokoris MS, Barrio JR, Iyer M, Namavari M, Phelps ME, Herschman HR: A mutant herpes simplex virus type 1 thymidine kinase reporter gene shows improved sensitivity for imaging reporter gene expression with positron emission tomography. Proc Natl Acad Sci USA 2000;97:2785–2790.
12 Zhao H, Doyle TC, Wong RJ, Cao Y, Stevenson DK, Piwnica-Worms D, Contag CH: Characterisation of coelenterazine analogs for measurements of *Renilla luciferase* activity in live cells and living animals. Mol Imaging 2004;3:43–54.
13 Contag CH, Spilman SD, Contag PR, Oshiro M, Eames B, Dennery P, Stevenson DK, Benaron DA: Visualising gene expression in living mammals using a bioluminescent reporter. Photochem Photobiol 1997;66:523–531.
14 Tung CH, Mahmood U, Bredow S, Weissleder R: In vivo imaging of proteolytic enzyme activity using a novel molecular reporter. Cancer Res 2000;60:4953–4958.
15 Weissleder R, Tung CH, Mahmood U, Bogdanov A: In vivo imaging of tumors with protease-activated near-infrared fluorescent probes. Nat Biotechnol 1999;17:375–378.
16 Ntziachristos V, Tung CH, Bremer C, Weissleder R: Fluorescence molecular tomography resolves protease activity in vivo. Nat Med 2002;8:757–760.
17 Allen MJ, MacRenaris KW, Venkatasubramanian PN, Meade TJ: Cellular delivery of MRI contrast agents. Chem Biol 2004;11:301–307.
18 Meade TJ, Taylor AK, Bull SR: New magnetic resonance contrast agents as biochemical reporters. Curr Opin Neurobiol 2003;13:597–602.
19 Yang M, Jiang P, Yamamoto N, Li LN, Geller J, Moossa AR, Hoffman RM: Real-time whole-body imaging of an orthotopic metastatic prostate cancer model expressing red fluorescent protein. Prostate 2005;62:374–379.
20 Yang M, Baranov E, Jiang P, Sun FX, Li XM, Li LN, Hasegawa S, Bouvet M, Al Tuwaijri M, Chishima T, Shimada H, Moossa AR, Penman S, Hoffman RM: Whole-body optical imaging of

green fluorescent protein-expressing tumors and metastases. Proc Natl Acad Sci USA 2000;97: 1206–1211.
21 Pickhardt PJ, Halberg RB, Taylor AJ, Durkee BY, Fine J, Lee FT Jr, Weichert JP: Microcomputed tomography colonography for polyp detection in an in vivo mouse tumor model. Proc Natl Acad Sci USA 2005;102:3419–3422.
22 Weber SM, Peterson KA, Durkee B, Qi C, Longino M, Warner T, Lee FT Jr, Weichert JP: Imaging of murine liver tumor using micro-CT with a hepatocyte-selective contrast agent: accuracy is dependent on adequate contrast enhancement. J Surg Res 2004;119:41–45.
23 Dendy P, Heaton B: Tomographic Imaging; in Dendy P, Heaton B (eds): Physics for Diagnostic Radiology. Bristol, Institute of Physics, 1999, pp 249–278.
24 Townsend DW: A combined PET/CT scanner: the choices. J Nucl Med 2001;42:533–534.
25 Townsend DW, Cherry SR: Combining anatomy and function: the path to true image fusion. Eur Radiol 2001;11:1968–1974.
26 Paulus MJ, Gleason SS, Easterly ME, Foltz CJ: A review of high resolution X-ray computed tomography and other imaging modalities for small animal research. Lab Anim 2001;30:36–45.
27 Tatsumi M, Nakamoto Y, Traughber B, Marshall LT, Geschwind JFH, Wahl RL: Initial experience in small animal tumor imaging with a clinical positron emission tomography/computed tomography scanner using 2-[F-18]fluoro-2-deoxy-*D*-glucose. Cancer Res 2003;63:6252–6257.
28 Goertzen AL, Meadors AK, Silverman RW, Cherry SR: Simultaneous molecular and anatomical imaging of the mouse in vivo. Phys Med Biol 2002;47:4315–4328.
29 Hamilton RJ, Blend MJ, Pelizzari CA, Milliken BD, Vijayakumar S: Using vascular structure for CT-SPECT registration in the pelvis. J Nucl Med 1999;40:347–351.
30 Hornak JP: The basics of MRI. 2005;http://www.cis.rit.edu/htbooks/mri/index.html
31 Jackson EF: Principles of magnetic resonance imaging and magnetic resonance spectroscopy; in Kim EE, Yang DJ (eds): Targeted Molecular Imaging in Oncology. Springer, New York, 2005, pp 30–61.
32 Kurhanewicz J, Vigneron DB, Nelson SJ: Three-dimensional magnetic resonance spectroscopic imaging of brain and prostate cancer. Neoplasia 2000;2:166–189.
33 Rehemtulla A, Hall DE, Stegman LD, Prasad U, Chen G, Bhojani MS, Chenevert TL, Ross BD: Molecular imaging of gene expression and efficacy following adenoviral-mediated brain tumor gene therapy. Mol Imaging 2002;1:43–55.
34 Ponomarev V, Doubrovin M, Serganova I, Vider J, Shavrin A, Beresten T, Ivanova A, Ageyeva L, Tourkova V, Balatoni J, Bornmann W, Blasberg R, Tjuvajev JG: A novel triple-modality reporter gene for whole-body fluorescent, bioluminescent, and nuclear noninvasive imaging. Eur J Nucl Med Mol Imaging 2004;31:740–751.
35 Louie AY, Huber MM, Ahrens ET, Rothbacher U, Moats R, Jacobs RE, Fraser SE, Meade TJ: In vivo visualization of gene expression using magnetic resonance imaging. Nat Biotechnol 2000;18:321–325.
36 Weissleder R, Simonova M, Bogdanova A, Bredow S, Enochs WS, Bogdanov A: MR imaging and scintigraphy of gene expression through melanin induction. Radiology 1997;204:425–429.
37 Chatham JC, Blackband SJ: Nuclear magnetic resonance spectroscopy and imaging in animal research. ILAR J 2001;42:189–208.
38 Johnson GA, Cofer GP, Gewalt SL, Hedlund LW: Morphologic phenotyping with MR microscopy: the visible mouse. Radiology 2002;222:789–793.
39 Jaszczak RJ, Li J, Wang H, Zalutsky MR, Coleman RE: Pinhole collimation for ultra-high-resolution, small-field-of-view SPECT. Phys Med Biol 1994;39:425–437.
40 Pani R, Pellegrini R, Cinti MN, Trotta C, Bennati P, Betti M, De Vincentis G, Cusanno F, Garibaldi F, Ridolfi S, Majewsky S, Tsui BMW: New devices for imaging in nuclear medicine. Cancer Biother Radiopharm 2004;19:121–128.
41 Price P: PET as a potential tool for imaging molecular mechanisms of oncology in man. Trends Mol Med 2001;7:442–446.
42 Adams JY, Johnson M, Sato M, Berger F, Gambhir SS, Carey M, Iruela-Arispe ML, Wu L: Visualisation of advanced human prostate cancer lesions in living mice by a targeted gene transfer vector and optical imaging. Nat Med 2002;8:891–896.

43 Rosenthal MS, Cullom J, Hawkins W, Moore SC, Tsui BM, Yester M: Quantitative SPECT imaging: a review and recommendations by the Focus Committee of the Society of Nuclear Medicine Computer and Instrumentation Council. J Nucl Med 1995;36:1489–1513.
44 Luker GD, Piwnica-Worms D: Molecular imaging – beyond the genome: molecular imaging in vivo with PET and SPECT. Acad Radiol 2001;8:4–14.
45 Sharma V, Piwnica-Worms D: Molecular imaging of gene expression and protein function in vivo with PET and SPECT. J Magn Reson Imaging 2002;16:336–351.
46 Chatziioannou AF: Molecular imaging of small animals with dedicated PET tomographs. Eur J Nucl Med 2002;29:98–114.
47 Cherry SR, Gambhir SS: Use of positron emission tomography in animal research. ILAR J 2001;42:219–232.
48 Acton PD, Kung HF: Small animal imaging with high resolution single photon emission tomography. Nucl Med Biol 2003;30:889–895.
49 Penuelas I, Mazzolini G, Boan JF, Sangro B, Marti-Climent J, Ruiz M, Ruiz J, Satyamurthy N, Qian C, Barrio JR, Phelps ME, Richter JA, Gambhir SS, Prieto J: Positron emission tomography imaging of adenoviral-mediated transgene expression in liver cancer patients. Gastroenterology 2005;128:1787–1795.
50 Voges J, Reszka R, Gossmann A, Dittmar C, Richter R, Garlip G, Kracht L, Coenen HH, Sturm V, Wienhard K, Heiss WD, Jacobs AH: Imaging-guided convection-enhanced delivery and gene therapy of glioblastoma. Ann Neurol 2003;54:479–487.
51 Contag CH, Bachmann MH: Advances in vivo bioluminescence imaging of gene expression. Annu Rev Biomed Eng 2002;4:235–260.
52 Hoffman RM: Green fluorescent protein imaging of tumor growth, metastasis, and angiogenesis in mouse models. Lancet Oncol 2002;3:546–556.
53 Ikawa M, Kominami K, Yoshimura Y, Tanaka K, Nishimune Y, Okabe M: Green fluorescent protein as a marker in transgenic mice. Dev Growth Differ 1995;37:455–459.
54 Choy G, O'Connor S, Diehn FE, Costouros N, Alexander HR, Choyke P, Libutti SK: Comparison of noninvasive fluorescent and bioluminescent small animal optical imaging. BioTechniques 2003;35:1022–1026, 1028–1030.
55 Jobsis FF: Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. Science 1977;198:1264–1267.
56 Cao YA, Wagers AJ, Beilhack A, Dusich J, Bachmann MH, Negrin RS, Weissman IL, Contag CH: Shifting foci of hematopoiesis during reconstitution from single stem cells. Proc Natl Acad Sci USA 2004;101:221–226.
57 Edinger M, Cao YA, Verneris MR, Bachmann MH, Contag CH, Negrin RS: Revealing lymphoma growth and the efficacy of immune cell therapies using in vivo bioluminescence imaging. Blood 2003;101:640–648.
58 Tsien RY: Building and breeding molecules to spy on cells and tumors. FEBS Lett 2005;579:927–932.
59 Ballou B, Ernst LA, Waggoner AS: Fluorescence imaging of tumors in vivo. Curr Med Chem 2005;12:795–805.
60 Frangioni JV: In vivo near-infrared fluorescence imaging. Curr Opin Chem Biol 2003;7:626–634.
61 Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS: Noninvasive imaging of quantum dots in mice. Bioconjug Chem 2004;15:79–86.
62 Voura EB, Jaiswal JK, Mattoussi H, Simon SM: Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy. Nat Med 2004;10:993–998.
63 Contag PR, Olomu IN, Stevenson DK, Contag CH: Bioluminescent indicators in living mammals. Nat Med 1998;4:245–247.
64 Zhang W, Contag PR, Madan A, Stevenson DK, Contag CH: Bioluminescence for biological sensing in living mammals. Adv Exp Med Biol 1999;471:775–784.
65 Bhaumik S, Lewis XZ, Gambhir SS: Optical imaging of Renilla luciferase, synthetic Renilla luciferase, and firefly luciferase reporter gene expression in living mice. J Biomed Opt 2004;9:578–586.

66 Bhaumik S, Gambhir SS: Optical imaging of *Renilla luciferase* reporter gene expression in living mice. Proc Natl Acad Sci USA 2002;99:377–382.
67 Zhao H, Doyle TC, Coquoz O, Kalish F, Rice BW, Contag CH: Emission spectra of bioluminescent reporters and interaction with mammalian tissue determine sensitivity of detection in vivo. J Biomed Opt 2005;in press.
68 Graves EE, Ripoll J, Weissleder R, Ntziachristos V: A submillimeter resolution fluorescence molecular imaging system for small animal imaging. Med Phys 2003;30:901–911.
69 Wang G, Li Y, Jiang M: Uniqueness theorems in bioluminescence tomography. Med Phys 2004; 31:2289–2299.
70 Lardinois D, Weder W, Hany TF, Kamel EM, Korom S, Seifert B, von Schulthess GK, Steinert HC: Staging of non-small-cell lung cancer with integrated positron-emission tomography and computed tomography. N Engl J Med 2003;348:2500–2507.
71 Harisinghani MG, Barentsz J, Hahn PF, Deserno WM, Tabatabaei S, van de Kaa CH, de la Rosette J, Weissleder R: Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. N Engl J Med 2003;348:2491–2499.
72 Crippa F, Agresti R, Seregni E, Greco M, Pascali C, Bogni A, Chiesa C, De Sanctis V, Delledonne V, Salvadori B, Leutner M, Bombardieri E: Prospective evaluation of fluorine-18-FDG PET in presurgical staging of the axilla in breast cancer. J Nucl Med 1998;39:4–8.
73 De Cicco C, Cremonesi M, Luini A, Bartolomei M, Grana C, Prisco G, Galimberti V, Calza P, Viale G, Veronesi U, Paganelli G: Lymphoscintigraphy and radioguided biopsy of the sentinel axillary node in breast cancer. J Nucl Med 1998;39:2080–2084.
74 Lyons SK, Meuwissen R, Krimpenfort P, Berns A: The generation of a conditional reporter that enables bioluminescence imaging of Cre/loxP-dependent tumorigenesis in mice. Cancer Res 2003;63:7042–7046.
75 Vooijs M, Jonkers J, Lyons S, Berns A: Noninvasive imaging of spontaneous retinoblastoma pathway-dependent tumors in mice. Cancer Res 2002;62:1862–1867.
76 Jonkers J, Berns A: Conditional mouse models of sporadic cancer. Nat Rev Cancer 2002;2: 251–265.
77 Yu YA, Shabahang S, Timiryasova TM, Zhang Q, Beltz R, Gentschev I, Goebel R, Szalay AA: Visualization of tumors and metastases in live animals with bacteria and vaccinia virus encoding light-emitting proteins. Nat Biotechnol 2004;22:313–320.
78 De Wever O, Mareel M: Role of tissue stroma in cancer cell invasion. J Pathol 2003;200:429–447.
79 Cooper CR, Chay CH, Gendernalik JD, Lee HL, Bhatia J, Taichman RS, McCauley LK, Keller ET, Pienta KJ: Stromal factors involved in prostate carcinoma metastasis to bone. Cancer 2003;97:739–747.
80 Clarke R: Human breast cancer cell line xenografts as models of breast cancer – the immunobiologies of recipient mice and the characteristics of several tumorigenic cell lines. Breast Cancer Res Treat 1996;39:69–86.
81 Garofalo A, Chirivi RG, Scanziani E, Mayo JG, Vecchi A, Giavazzi R: Comparative-Study on the metastatic behavior of human tumors in nude, beige/nude/xid and severe combined immunodeficient mice. Invasion Metastasis 1993;13:82–91.
82 Mueller BM, Reisfeld RA: Potential of the scid mouse as a host for human tumors. Cancer Metastasis Rev 1991;10:193–200.
83 Takizawa Y, Saida T, Tokuda Y, Dohi S, Wang YL, Urano K, Hioki K, Ueyama Y: New immunodeficient (nude-scid, beige-scid) mice as excellent recipients of human skin grafts containing intraepidermal neoplasms. Arch Dermatol Res 1997;289:213–218.
84 Sikder H, Huso DL, Zhang H, Wang BH, Ryu B, Hwang ST, Powell JD, Alani RM: Disruption of Id1 reveals major differences in angiogenesis between transplanted and autochthonous tumors. Cancer Cell 2003;4:291–299.
85 Alani RM, Silverthorn CF, Orosz K: Tumor angiogenesis in mice and men. Cancer Biol Ther 2004;3:498–500.
86 Khanna C, Hunter K: Modelling metastasis in vivo. Carcinogenesis 2005;26:513–523.
87 Hiraga T, Williams PJ, Ueda A, Tamura D, Yoneda T: Zoledronic acid inhibits visceral metastases in the 4T1/luc mouse breast cancer model. Clin Cancer Res 2004;10:4559–4567.

88 Jenkins DE, Oei Y, Hornig YS, Yu SF, Dusich J, Purchio T, Contag PR: Bioluminescent imaging (BLI) to improve and refine traditional murine models of tumor growth and metastasis. Clin Exp Metastasis 2003;20:733–744.
89 Bouvet M, Wang JW, Nardin SR, Nassirpour R, Yang M, Baranov E, Jiang P, Moossa AR, Hoffman RM: Real-time optical imaging of primary tumor growth and multiple metastatic events in a pancreatic cancer orthotopic model. Cancer Res 2002;62:1534–1540.
90 Smith MCP, Luker KE, Garbow JR, Prior JL, Jackson E, Piwnica-Worms D, Luker GD: CXCR4 regulates growth of both primary and metastatic breast cancer. Cancer Res 2004;64:8604–8612.
91 Nogawa M, Yuasa T, Kimura S, Kuroda J, Sato K, Segawa H, Yokota A, Maekawa T: Monitoring luciferase-labelled cancer cell growth and metastasis in different in vivo models. Cancer Lett 2005;217:243–253.
92 Douma S, van Laar T, Zevenhoven J, Meuwissen R, van Garderen E, Peeper DS: Suppression of anoikis and induction of metastasis by the neurotrophic receptor TrkB. Nature 2004;430:1034–1040.
93 Rosol TJ, Tannehill-Gregg SH, Leroy BE, Mandl S, Contag CH: Animal models of bone metastasis. Cancer 2003;97:748–757.
94 Yoneda T: Cellular and molecular basis of preferential metastasis of breast cancer to bone. J Orthop Sci 2000;5:75–81.
95 Wetterwald A, van der Pluijm G, Que I, Sijmons B, Buijs J, Karperien M, Lowik CWGM, Gautschi E, Thalmann GN, Cecchini MG: Optical Imaging of cancer metastasis to bone marrow: a mouse model of minimal residual disease. Am J Pathol 2002;160:1143–1153.
96 Wood KV: Marker proteins for gene expression. Curr Opin Biotechnol 1995;6:50–58.
97 Andersson S, Davis DL, Dahlback H, Jornvall H, Russell DW: Cloning, structure, and expression of the mitochondrial cytochrome-P-450 sterol 26-hydroxylase, a bile-acid biosynthetic enzyme. J Biol Chem 1989;264:8222–8229.
98 Schmidt EV, Christoph G, Zeller R, Leder P: The cytomegalovirus enhancer – a pan-active control element in transgenic mice. Mol Cell Biol 1990;10:4406–4411.
99 Koike C, Oku N, Watanabe M, Tsukada H, Kakiuchi T, Irimura T, Okada S: Real-Time pet analysis of metastatic tumor-cell trafficking in vivo and its relation to adhesion properties. Biochim Biophys Acta – Biomembranes 1995;1238:99–106.
100 Ray P, Wu AM, Gambhir SS: Optical bioluminescence and positron emission tomography imaging of a novel fusion reporter gene in tumor xenografts of living mice. Cancer Res 2003;63: 1160–1165.
101 De A, Lewis XZ, Gambhir SS: Noninvasive imaging of lentiviral-mediated reporter gene expression in living mice. Mol Ther 2003;7:681–691.
102 Moore A, Josephson L, Bhorade RM, Basilion JP, Weissleder R: Human transferrin receptor gene as a marker gene for MR imaging. Radiology 2001;221:244–250.
103 Harisinghani MG, Weissleder R: Sensitive, noninvasive detection of lymph node metastases. Plos Med 2004;1:202–209.
104 Antoch G, Vogt FM, Freudenberg LS, Nazaradeh F, Goehde SC, Barkhausen J, Dahmen G, Bockisch A, Debatin JF, Ruehm SG: Whole-body dual-modality PET/CT and whole-body MRI for tumor staging in oncology. JAMA 2003;290:3199–3206.
105 Seemann MD: Human PET/CT scanners: feasibility for oncological in vivo imaging in mice. Eur J Med Res 2004;9:468–472.
106 Lewis BC, Klimstra DS, Socci ND, Xu S, Koutcher JA, Varmus HE: The absence of p53 promotes metastasis in a novel somatic mouse model for hepatocellular carcinoma. Mol Cell Biol 2005;25: 1228–1237.
107 Yang M, Baranov E, Wang JW, Jiang P, Wang X, Sun FX, Bouvet M, Moossa AR, Penman S, Hoffman RM: Direct external imaging of nascent cancer, tumor progression, angiogenesis, and metastasis on internal organs in the fluorescent orthotopic model. Proc Natl Acad Sci USA 2002;99:3824–3829.
108 Minn AJ, Kang YB, Serganova I, Gupta GP, Giri DD, Doubrovin M, Ponomarev V, Gerald WL, Blasberg R, Massague J: Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. J Clin Invest 2005;115:44–55.

109 Edinger M, Cao YA, Hornig YS, Jenkins DE, Verneris MR, Bachmann MH, Negrin RS, Contag CH: Advancing animal models of neoplasia through in vivo bioluminescence imaging. Eur J Cancer 2002;38:2128–2136.
110 Edinger M, Sweeney TJ, Tucker AA, Olomu AB, Negrin RS, Contag CH: Noninvasive assessment of tumor cell proliferation in animal models. Neoplasia 1999;1:303–310.

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Dittmar T, Zaenker KS, Schmidt A (eds): Infection and Inflammation: Impacts on Oncogenesis.
Contrib Microbiol. Basel, Karger, 2006, vol 13, pp 232–239

Infection, Inflammation and Neoplasia: An Interdisciplinary Challenge

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Abstract

During the past two to three decades there has been an exciting revolution in our understanding of the multistage carcinogenic process and of the molecular genetics of cancer. The general principle of multifactor interactions is central to our understanding of cancer causation. The paradigm that persistent infections and chronic inflammation contributes via cytokine- and chemokine-mediated disbalanced immune response to carcinogenesis becomes more and more attractive in cancer research. Besides genetic factors, the epigenetics of impaired cell signaling and signal transduction by proinflammatory cytokines and chemokines are important potentiators of carcinogenesis. The activation of the nuclear factor κB, for example, a hallmark of inflammatory responses that is frequently detected in tumors, might constitute a missing link between inflammation and cancer. It will be a challenge for future therapeutic and preventive cancer research to detect potential targets in chronic inflammatory disease which are essential links to promote inflammation-associated cancer.

Inflammation, Wound Healing, and Carcinogenesis

The association between human carcinogenesis and inflammation is a classic theme of cancer research arising in the late 1970s, when it had become apparent that the growth of normal cells is largely controlled by the interplay between several polypeptide hormones and hormone-like growth factors that are present in tissue fluids. In general, it had been shown that malignant cells required less of these exogenous growth factors than did their normal counterparts for optimal growth and multiplication, and the Nobel laureate, Robert Holley, has suggested that ‘transformed or malignant cells escape from normal growth control by requiring less of such hormones or growth factors’ [1].

Indeed, it had long been hypothesized that there was a functional connection between tumors and wound healing, as manifested in Haddow's [2] famous dictum that 'the wound is a tumor that heals itself', later inverted by Dvorak [3] to 'tumors are wounds that do not heal'. Wound healing by itself is a very complex process, involving the limited proliferation of fibroblasts, the formation of extracellular matrix (ECM) proteins, like various types of collagens, the deposit of hyaluronic acid and the vascularization of the connective tissue in the presence of immune competent cells.

Ultimately, in the world of cellular physiology, one described later on cytokines and chemokines as molecular elements of a complex biological signaling language, which is used for both intercellular and intracellular communication. At the laboratory bench and from the results of translational research by applying cytokines and chemokines in mouse models and from clinical settings, we learned within the last two decades that these molecules are like symbols or letters of an alphabet in a code or in a language, which need to be considered in the context of all other signals present. These signaling molecules should be regarded as cues or cellular switches with multifunctional activities, and that their true function is to provide subtle mechanisms for coupling a cell to its environment, so that the cell has the necessary plasticity to respond appropriately to changes in its environment, or even within its own state.

Multifunctionality of Cytokines and Chemokines

In the past, research in the field of immunology predominantly studied the role of cytokines and chemokines as regulators of inflammatory processes after viral, bacterial or helminthic infections, in allergy and in autoimmune diseases and the results led rapidly to a much more sophisticated appreciation of the role of cytokines and chemokines as multifunctional molecules.

For instance, although the first description of the activity of the cytokine transforming growth factor-β (TGF-β) on both T and B lymphocytes emphasized its inhibitory effects, it was soon found that TGF-β could act as both a stimulator and inhibitor of IgA production in B lymphocytes [4].

At present, we have a more sophisticated understanding of the actions of cytokines as multifunctional molecules in both inflammation and the immune response, summarized as: 'the good, the bad, and the ugly', where the latter distinctive mark might be seriously linked to cancer development.

The chemokine system controls leukocyte trafficking during homeostasis as well as during inflammation and is necessary for the linkage between innate and adaptive immunity. Tissue regulation outside the hematopoietic compartment, for instance, angiogenesis, organogenesis and tumor development, growth and

metastasis, is another important function of the chemokine system [5]. The chemokine-mediated regulation of angiogenesis, e.g. in the process of wound healing, is highly sophisticated and fine tuned, and involves pro-angiogenic chemokines, like CXCL8 (IL-8) interacting with the CXC-receptor 2 (CXCR2), and anti-angiogenic chemokines such as CXCL10 (IP10) interacting with CXC-receptor 3 (CXCR3). Chemokines also regulate angiogenesis in a receptor-independent manner by means of perturbation of basic fibroblast growth factor (bFGF) and VEGF. Examples of the delicate angiogenesis (1) in wound healing – and of the dysregulation; (2) in tumors – considered as 'wounds that do not heal' – are provided with the interesting phenomenon of molecular piracy of host-encoded genes within the chemokine system. Yet, a certain group of herpes virus – the γ2 herpes virus – encode a functional CXCR2 homologue that is activated by angiogenic chemokines and antagonized by angiostatic chemokines, and this particular gene seems to cause the development of a vascular tumor, the Kaposi's sarcoma, in the host.

Chemokines have now emerged as key regulators in the development, differentiation and anatomic distribution of immunocompetent cells. Chemokines orchestrate both the innate immune response and the antigen specific immunity through their coordination of dendritic cells and lymphocytes. Due to their vast functional responsibility, they are linked to the pathogenesis of many seemingly unrelated diseases that include HIV, infection, cancer, atherosclerosis, autoimmune disease, graft rejection and dermatological disorders [6].

Perhaps the biggest lesson we can learn from the multifunctionality of cytokines and chemokines in health and illness is that their physiological interplay keeps on going in a healthy organism. However, if this very complex and regulative system between cells, tissues and organs is perturbated, a pathological status will be determined and multimorbidity might occur, because these molecules, when malfunctioning, bridge different diseases as mentioned above.

'Wounds that Never Heal' and Carcinogenesis

In many solid tumor types the abundance of tumor-associated macrophages (TAMs) or tumor-infiltrating lymphocytes (TILs) is correlated with poor prognosis [7]. Macrophages and leukocytes are recruited through the local expression of chemokines, such as colony stimulating factor-1 (CSF-1) or the inflammatory mediator IL-8. Overexpression of such factors is also correlated with poor prognosis in a variety of tumors. TAMs and TILs are recruited to tumors through the expression of potent chemoattractants and in this site their normal trophic functions are subverted to promote tumor progression and metastasis [8]. In wound healing and immune surveillance, the physiological function of immunocompetent cells is, besides control of pathogens, matrix remodeling,

angiogenesis, stimulation of migration of mesenchymal and endothelial cells through the synthesis of growth and chemotactic factors. However, these functions are also found pathologically during chronic inflammation. This supports the notion that tumors are 'wounds that never heal' and suggests that chronic inflammation through persistent infections or by other means might be important cofactors in the genesis and promotion of tumors [9]. If chemokines are responsible for the excessive recruitment of leukocytes to inflammatory sites and damaged tissues, it is rational to argue that the chemokine system offers many potential entry points for innovative anti-inflammatory therapies in autoimmune diseases, arthritis, and cancer [10].

Chemokine-Mediated Cell Function Inhibition – Where, Which and How?

Unlike cytokines, chemokines signal via seven transmembrane G-protein-coupled receptors (7-helix receptors) and are favored targets by the pharmaceutical industry. For the future in drug development we have to decipher the meaning of (1) the input layer; (2) the signal processing layer; and (3) the output layer of a given cell which is in the therapeutic focus. Understanding the signaling network of targeted cells implicitly opens a window to find inhibitory or stimulating molecules which can be side-directed to different molecules of one of the three layers of the signaling network. Small molecule receptor antagonists have been developed to abrogate competitively incoming signals by natural ligands. Furthermore, chemokines have an in vivo requirement to bind to extracellular glycosaminoglycans (GAGs) in order to mediate cell locomotor directionality. Prevention of the GAG interaction has been shown to be a viable therapeutic approach. Targeting chemokine intracellular signaling pathways at the level of the signal processing layer offers a further promising alternative small molecule approach. Key signaling targets downstream of a variety of chemokines receptors identified to date are the dynamic spatial calcium distribution in a cell, the phosphoinositide 3-kinase γ (PI3Kγ), a member of the class I PI3K family, or members of the protein kinase C (PKC) isotype family [11]. However, as recently shown, serpentine signaling induces in neutrophils dichotomically two signal transduction pathways for the regulation of cellular locomotion [12]. This finding shows that the complexity of the therapeutic approach is drastically increased, because of the different cell types such as tumor cells, immune competent cells, and mesenchymal cells, which have to be considered and because of the putative usage of a salvage pathway which is switched on if pivotal cell signals are disturbed by small molecule approaches. All these features make a rational approach of intracellular signal cascade targeting so

complex and complicated intending to manipulate the cellular read out – growth, differentiation, apoptosis, migration – selectively.

Cancer and Inflammation: From Epidemiological Perspectives to Molecular Mechanisms

The critical role of inappropriate inflammation is becoming accepted in many diseases, including autoimmune disorders, neurodegenerative conditions, and tumor development. It is estimated that approximately 20% of human cancers develop at the background of chronic inflammation [13]. Many chronic inflammatory conditions increase the risk of cancer in affected tissues. The inflammatory bowel diseases, ulcerative colitis and Crohn's disease, predispose to the development of cancers of the large bowel and/or terminal ileum. Chronic cholecystitis and gallstones predispose to cancer of the gallbladder. Epidemiology data have revealed an increased risk of prostate cancer in men with a history of certain sexually transmitted infections or prostatitis [14]. A novel putative prostate cancer precursor lesion, proliferative inflammatory atrophy (PIA), which shares some molecular traits with prostate intraepithelial neoplasia (PIN) and prostate cancer, has been characterized [14]. The expression of cyclooxygenase-2 (COX-2) and lipid mediators of inflammation increases during the multistage progression of these tumors. Nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit COX-2 activity and tumor development in many experimental and clinical settings are inversely associated with certain cancers in epidemiological studies. In an orthotopic mouse model with the human pancreatic carcinoma cell line PANC-1, it has been shown that N-acetyl-salicylic acid repressed tumor formation by PANC-1 cells in vivo in a prophylactic setting, suggesting a possible mechanism for this NSAID to be effective in pancreatic carcinoma through inhibition of NF-κB activation and a mechanistic link between inflammation and tumorigenesis [15]. Despite their promise, however, NSAIDs have to be further evaluated for the risk/benefit ratio before being used in treatment regimens in designated patient populations. Cancer cells upregulate the angiotensin II type 1 (AT1) receptor through systemic oxidative stress and hypoxia mechanisms, thereby triggering chronic inflammatory processes to remodel surrounding tissue and subdue the immune system. It is anticipated that manipulation of the angiotensin system with existing anti-hypertensive drugs could provide a new approach to the treatment of cancer [16].

There is a vast body of evidence that chronic inflammation contributes to carcinogenesis, but the underlying mechanisms are poorly understood, yet. Recently, it was hypothesized that the higher infection burden in developing countries might mean an earlier aging of immune competent cells by telomere

shortening, resulting in decreased efficiency of immune surveillance and, thus, predisposing to cancer at an earlier age than seen in developed countries with lesser infection burden [17]. Very recently, an inflammation-based prognostic score (Glasgow Prognostic Score, GPS) was found to be a significant predictor of survival in patients with inoperable non-small-cell lung cancer [18]. It is likely that the new paradigm of chronic inflammation-associated neoplasms will prove useful in future investigations understanding and drug-targeting the underlying mechanisms.

Infection and Cancer

Since the discovery that *Helicobacter pylori* infection leads to gastric cancer, other chronic bacterial infections have been shown to cause cancer [19]. *Streptococcus bovis* (*S. bovis*)/*infantarius* was traditionally considered a lower grade pathogen frequently involved in bacteremia and endocarditis. This bacterium became important in human health as it was shown that 25–80% of patients who presented with *S. bovis* bacteremia also had a colorectal tumor. It could also be demonstrated that *S. bovis* wall extracted antigens were able to promote carcinogenesis in rats [20]. Tobacco smoking can give a growth advantage to tobacco tas-resistant *Staphylococcus aureus* (*S. aureus*) because tobacco tar-sensitive *S. aureus* would not usually exist in the tumor micro-environment. The tumor promotion stage would be the result of dominant growth of tumor necrosis factor-α (TNF-α) inducing *S. aureus* and probably other bacteria, resulting in focal trauma of the buccal cavity, respiratory tract, and other organs in humans [21].

Many bacteria that cause persistent infections produce toxins that specifically disrupt cellular signaling to perturb the regulation of cell growth or to induce inflammation. Other bacterial toxins directly damage DNA. Such toxins mimic carcinogens and tumor promotors, like TNF-α, and might represent a paradigm for bacterially induced carcinogenesis [22]. Presently, about 100 genotypes of human papilloma virus (HPV) are known and several types have been identified that cause specific types of cancers. The etiology of cancer of the cervix has been linked to several types of HPV, with a high preponderance of HPV 16. A major portion of anal, vulvar and penile cancers appeared to be linked to the same HPV infections. In addition, close to 25% of oropharyngeal cancers contain DNA from the same types of HPV and recent evidence suggests a possible role of HPV infections in squamous cell carcinomas of the skin. There is a viral (hepatitis B and C virus) and a nonviral cause of hepatocellular carcinoma. Chronic necroinflammatory hepatic disease generates oxygen and nitrogen reactive species with may influence cellular gene expression leading to hepatocellular carcinoma [23].

Although there are enormous regional differences, the global frequency of cancer linked to infectious agents is between 17 and 24% with a rising tendency because of increasing research efforts to evaluate the epidemiological, clinical and molecular linkages between infection, inflammation and neoplasia [23].

Interdisciplinary Challenge

There is increasing experimental and clinical evidence that infections either inducing oncogene products, e.g. HPV oncogene products E6 and E7, or producing mutagenic bacterial toxins or maintaining chronic inflammation are causally linked to cancer. These emerging insights into (1) the interplay of cytokines and chemokines between cells, tissues and ECM; (2) the vascular and lymphatic functions; and (3) the processes of chronic inflammation in the etiology of carcinogenesis hold the promise of spawning new diagnostic, preventive or therapeutic modalities for cancer-prone people or men with cancer. However, this holds only true, if and only if, the global scientific community in health, social and economic sciences exchanges their ideas without discrimination, either politically, economically or ideologically and scientists, businessman and patients respect each other; because every human being will become a patient once.

References

1 Holley R: Control of growth of mammalian cells in cell culture. Nature 1975;258:487–490.
2 Haddow A: Molecular repair, wound healing, and carcinogenesis: tumor production a possible overhealing? Adv Cancer Res 1972;16:181–234.
3 Dvorak H: Tumors: wounds that do not heal: similarities between tumor stroma generation and wound healing. N Engl Med 1986;315:1650–1659.
4 Kehrl JH, Roberts AB, Wakerfield LM, Jakowlew S, Sporn MB, Fauci AS: Transforming growth factor beta is an important immunoregulatory protein for human B lymphocytes. J Immunol 1986;137:3855–3860.
5 Rosenkilde MM, Schwartz TW: The chemokine system – a major regulator of angiogenesis in health and disease. APMIS 2004;112:481–495.
6 Dong VM, McDermott DH, Abdi R: Chemokines and diseases. Eur J Dermatol 2003;13:224–230.
7 Lin EY, Pollard JW: Macrophages: modulators of breast cancer progression. Novartis Found Symp 2004;256:158–168.
8 Entschladen F, Zaenker KS: Locomotion of tumor cells: a molecular comparison to migrating pre- and postmitotic leukocytes. J Cancer Res Clin Oncol 2000;126:671–681.
9 De Visser KE, Coussens LM: The interplay between the innate and the adaptive immunity regulates cancer development. Cancer Immunol Immunother 2005;12:(Epub ahead of print).
10 Johnson Z, Power CA, Weiss C, Rintelen F, Ji H, Ruckle T, Camps M, Wells TN, Schwarz MK, Proudfoot AE, Rommel C: Chemokine inhibition – why, when, where, which and how? Biochem Soc Trans 2004;32:366–377.
11 Entschladen F, Drell TL IV, Lang K, Joseph J, Zaenker KS: Tumour-cell migration, invasion, and metastasis: navigation by neurotransmitters. Lancet Oncol 2004;5:254–258.

12 Lang K, Hatt H, Niggemann B, Zaenker KS, Entschladen F: A novel function of chemokines: downregulation of neutrophil migration. Scand J Immunol 2003;57:350–361.
13 Thun MJ, Henley SJ, Gansler T: Inflammation and cancer: an epidemiological perspective. Novartis Found Symp 2004;256:6–21.
14 Palapattu GS, Sutcliffe S, Bastian PJ, Platz EA, De Marzo AM, Isaacs WB, Nelson WG: Prostate carcinogenesis and inflammation: emerging insights. Carcinogenesis 2004;26:1170–1181.
15 Sclabas GM, Uwagawa T, Schmidt C, Hess KR, Evans DB, Abbruzesse JL, Chiao PJ: Nuclear factor kappa B activation is a potential target for preventing pancreatic carcinoma by aspirin. Cancer 2005;103:2485–2490.
16 Smith GR, Missailidis S: Cancer, inflammation and the AT1 and AT2 receptors. J Inflamm (Lond) 2004;30:3.
17 Sastry PS, Parikh P: The earlier age of onset of malignancy in developing world is related to overall infection burden and could be due to the effect on telomere length. Med Hypothesis 2003;60:573–574.
18 Forrest LM, McMillan DC, McArdle CS, Angerson WJ, Dagg K, Scott HR: A prospective longitudinal study of performance status, an inflammation-based score (GPS) and survival in patients with inoperable non-small-cell lung cancer. Br J Cancer 2005;92:1834–1836.
19 Houghton J, Wang TC: *Helicobacter pylori* and gastric cancer: a new paradigm for inflammation-associated epithelial cancers. Gastroenterology 2005;128:1567–1578.
20 Biarc J, Nguyen IS, Pini A, Gosse F, Richert S, Thierse D, Van Dorsselaer A, Leize-Wagner E, Raul F, Klein JP, Scholler-Guinard M: Carcinogenic properties of proteins with pro-inflammatory activity from *Streptococcus infantarius* (formerly *S. bovis*). Carcinogenesis 2004;25:1477–1484.
21 Fujiki H, Takeuchi H, Nishitani N, Yamanaka H, Suzuki K, Kurusu M, Suganuma M: Carcinogenic potential of tobacco tar-resistant *Staphylococcus aureus* in the buccal cavity. J Cancer Res Clin Oncol 2004;130:301–305.
22 Rogers AB, Fox JG: Inflammation and cancer. I. Rodent models of infectious gastrointestinal and liver cancer. Am J Physiol 2004;286:G361–G366.
23 Zaenker KS, Mihich E: International symposium on emerging perspectives in clinical cancer research: meeting report. J Cancer Res Clin Oncol 2004;130:491–495.

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