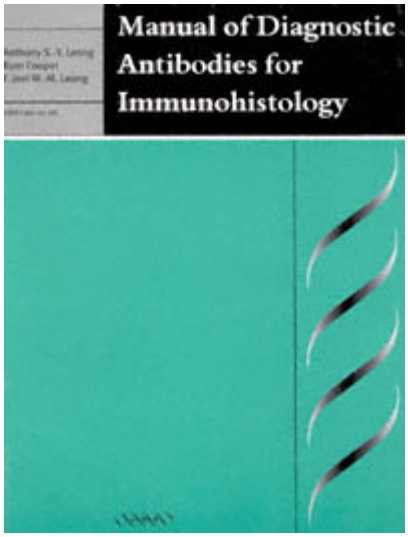
Manual of Diagnostic
Antibodies for
Immunohistology

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Manual of Diagnostic Antibodies for Immunohistology

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To Wendy and Nimmie, for their love, strength and support

PREFACE

The rapid acceptance and entrenchment of immunohistochemistry as an important and, in some cases, indispensable adjunct to morphological examination and diagnosis has imposed the necessity for anatomical pathology laboratories to be proficient in immuno-staining procedures. However, for immunohistochemistry stains to be meaningful, technical competence must be accompanied by a familiarity with the characteristics and specificities of the reagents employed. In particular, the medical technologist and pathologist must have knowledge of the sensitivity and specificity of the primary antibody employed, the nature of the epitope demonstrated by each antibody and its sensitivity to common fixatives. They should be equally conversant with protocols for tissue processing as well as the various methods of antigen/epitope retrieval which are appropriate for the demonstration of the specific protein sought in the tissue section or cell preparation.

The versatility and contributions of immunohistochemistry to diagnostic pathology, particularly in the areas of tumor diagnosis, lineage identification, prognostication and therapy are largely dependent on the ever-increasing range of antisera and monoclonal antibodies which are commercially available. However, this latter feature is a two-edged sword. While the extensive spectrum of antibodies allows the identification of a wider and wider range of cellular antigens, the user must also be familiar with the properties and characteristics of each of these many antibodies.

This book provides a comprehensive list of antisera and monoclonal antibodies that have useful diagnostic applications in tissue sections and cell preparations. Various clones, which are commercially available to detect the same antigen, are listed and the sensitivities and specificities of the antibodies are discussed. Importantly, our own experience with these reagents is provided, together with pertinent references. While as many available sources of antibodies are provided as possible, it is acknowledged that the listing cannot be exhaustive and only major sources are covered. A brief coverage of the diagnostic approach to the general categories of the poorly differentiated round cell and spindle cell tumors in various anatomical sites using panels of selected antibodies is provided in the form of tables. Staining protocols and antigen/epitope retrieval procedures, including those employing enzymes, microwaves and heat, are also given in detail.

It is hoped that this compendium will provide a source of useful and practical information to both the diagnostic and research laboratory.

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INTRODUCTION

This book discusses diagnostic antibodies and antisera in alphabetical order and provides the background and applications of each reagent, together with pertinent references. Common clones of diagnostic relevance and their sources are listed but this is not presented as exhaustive; furthermore, mainly antibodies immunoreactive in fixed paraffin-embedded tissue sections are discussed, as these remain the mainstay of diagnostic histopathology.

Diagnostic Approach

Diagnostic antibodies should not be employed in isolation but always as part of a panel of antibodies directed to the entities considered in differential diagnosis. As the latter is derived from the cytomorphologic appearances of the tumor, it is clearly evident that immunohistochemical diagnosis is morphology based. Indeed, we favor "immunohistology" over the better established term "immunohistochemistry" as it emphasizes the relationship of immunostaining to morphology. To assist with the diagnosis, antibodies to markers recognized to be expressed by the tumor in question, as well as those associated with the entities considered in differential diagnoses, should make up the panel. As markers are almost never tissue specific, the application of a panel of antibodies will generate an immunologic profile comprising both positive and negative findings, which in combination will produce the most accurate results. By obtaining an immunophenotypic profile of the tissue tested, the errors of false-positive and false-negative staining will be reduced and the highest diagnostic yield obtained (Gown & Leong, 1993; Leong & Gown, 1993). For example, anaplastic large cell lymphoma has carcinoma and melanoma as morphologic mimics. Anaplastic large cell lymphoma may express epithelial membrane antigen in about 45% of cases and may fail to stain for CD 45 (leukocyte common antigen) in as many cases. These findings taken in isolation, may be mistaken for that of a carcinoma. However, if antibodies to vimentin, broad-spectrum cytokeratin, S100 and HMB45 (melanoma-associated antigen) are also employed, the error will be averted as the profile of EMA+, CD45-,VIM+, CK-, S100-, HMB45- fits best with that of anaplastic large cell lymphoma. In some situations it may be

necessary to perform the immunostaining in two stages. A primary panel of antibodies provides the major categorization of the tumor and a secondary panel allows further subtyping. For example, positivity for CD30 will be useful for the confirmation of the diagnosis of anaplastic large cell lymphoma and lineage typing can be further performed.

As an alternative, the algorithmic approach may be adopted (Leong et al, 1997) but whichever approach is favored, it is important that antibodies directed to all entities considered in differential diagnosis be employed. The few exceptions to this rule would include the application of immunostaining for prognostic markers and the identification of infectious agents in tissue sections.

Standardization and Optimization of Immunostaining

Much has been discussed in recent times about standardization in immunohistology (Taylor, 1993), but this goal is difficult or impossible to achieve simply because fixatives, durations of fixation and methods of tissue processing employed by laboratories are different. The ability to demonstrate various tissue antigens is very much dependent on their preservation and therefore on the method of fixation and processing employed. With the vastly different practices in laboratories throughout the world, is clear that standardization as a goal may be impossible to achieve.

It would be more appropriate to aim for optimization of immunostaining within the individual laboratory. This means consistency, reproducibility and the ability to obtain the optimal results with the method of fixation and processing employed. To this end, it is necessary for each laboratory to adopt a method of fixation and tissue processing which will allow the optimal antigen preservation and yet not compromise cytomorphological preservation. It may be appropriate to examine each fixation and processing step and adjust for optimization, remembering that antigen preservation may also be influenced by the surgeon or physician who has responsibility for fixing the excised specimen.

It is imperative to test every new antibody on tissue blocks processed in your own laboratory. While reagent dilutions and tissue preparation instructions provided by the manufacturers are useful guides, they are universal recommendations and not individualized. It is necessary to evaluate various methods of antigen retrieval and to determine, by titration, antibody concentrations that are optimal for tissue processed in your laboratory. The introduction of the heat-induced epitope retrieval (HIER) procedure (Shi et al, 1991) has contributed significantly to our ability to optimize immunostaining procedures and HIER must be evaluated for each new antibody used. With very rare exceptions, we have not found HIER to be deleterious to the majority of diagnostic antigens and recommend that it be performed as a routine before any immunostaining (Gown et al, 1993; Leong & Milles, 1993). The combination of HIER with enzymatic digestion should also be explored for some antigens. A protocol for HIER employing microwaves is provided in Appendix II.

The diagnostic interpretation in immunohistology includes the assessment of internal positive control cells or tissues. Although not every tested antigen may have a normal internal counterpart which can be used for this purpose, test sections do contain such determinants. Positive controls should also be used routinely in each antibody staining run, remembering that it is more appropriate to employ neoplastic tissues known to express equivalent amounts of the antigen tested rather than non-lesional tissues which may express much higher levels of antigen. A negative control of tissues known not to express the antigen should also be employed. In addition a nonspecific negative reagent control should be employed in place of the primary antibody to evaluate non-specific staining. Ideally a negative reagent control contains the same isotype as the primary antibody but exhibits no specific reactivity with human tissues in the same matrix or solutions as the primary antibody. All control tissues should be fixed, processed and embedded in a manner identical to the test example.

In addition to these technical aspects, consideration should also be given to the nature of the diagnostic specificity of the antibodies used and the properties of the target antigen. Much of this information is

theoretical and beyond the control of the diagnostic laboratory. Nonetheless, you should have some familiarity with this aspect of the reagents and the information is often available in the literature and may be covered in the product profiles

provided by the manufacturer.

It is clear from the foregoing that immunohistology is not a simple matter of a positive or negative stain. While it is a powerful diagnostic tool, immunostaining is only an adjunct to histologic examination and requires careful optimization if it is intended to produce the highest diagnostic yield (Leong, 1992).

This book contains antibodies and antisera which we consider to be of diagnostic relevance. Except for the cytokeratins, pituitary and pancreatic hormones, the antibodies are discussed separately and listed in alphabetical order for easy reference. The antibodies are indexed by their main and alternative names but specific clone numbers are not indexed.

How to Use this Book

This book is written by three experts who provide a unique coverage of monoclonal antibodies and antisera applicable to the field of immunohistology. Each diagnostic antibody is dealt with separately in a succinct fashion. A comprehensive range of available antibody clones and their sources are listed and a brief background is given before the applications of the reagents are discussed. In addition, there is an up-to-date list of important references together with practical tips on immunostaining and interpretation.

The text is cross referenced to an Appendix that contains an extensive assembly of panels of antibodies for application to a wide variety of diagnostic situations, providing an easy-to-use practical guide. The addresses of worldwide suppliers of these reagents are included. Also contained in the appendix is a detailed discussion of antigen retrieval techniques with a detailed protocol of the procedure.

This book will provide essential information for pathologists, technologists and clinicians who employ antibodies and antisera in their work. It will be an important reference and contains much information for all those interested in cancer research and biology.

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SECTION 1
ANTIBODIES

α-Smooth Muscle Actin (α-SMA)

Sources/Clones

Accurate (1A4), Biodesign (asm-1, A4), Biogenex (1A4), Cymbus Bioscience (asm-1), Dako (1A4), Enzo (CGA7), ICN (1A4), Immunotech (1A4), Medac (TCS), Novocastra (asm1), RDI (asm-1), Sigma (1A4) and Zymed (Z060).

Fixation/Preparation

Several of the antibody clones toα-smooth muscle actin (α-SMA) are immunoreactive in fixed paraffin-embedded sections. HIER does not significantly enhance staining.

Background

Cytoplasmic actins vary in amino acid sequences and can be separated by electrophoresis into six different isotopes, all having the same molecular weight of 42 kDα Actins are found in muscle cells. While β and γ actins may be present in muscle cells as well as most other cell types in the body, including non-muscle cells. Both striated and smooth muscle fibers differ in their expression of actin isotypes and this has formed the basis for the generation of antibodies directed at musclespecific actin subtypes. HHF35 (muscle-specific actin) identifies all four actin isoforms present in smooth muscle as well as skeletal muscle cells, pericytes, myoepithelial cells and myofibroblasts. In contrast, antibodies to α-SMA specifically identify the singleα isoform characteristic of smooth muscle cells and those cells with myofibroblastic differentiation.

Applications

Antibodies to α-SMA are used in several diagnostic situations. These include the identification of myoepithelial cells, which are admixed, with epithelial cells in benign proliferative lesions of the breast, allowing their distinction from neoplastic proliferations. Myoepithelial cells also line benign ductules of the breast compared to their absence in neoplastic tubules (Raymond & Leong, 1991)α-SMA is also a useful marker to identify myofibroblastic differentiation and has been used in studies of idiopathic pulmonary fibrosis (Ohta et al, 1995) and of the fibrogenic Ito cells in the liver (Enzan et al, 1994). In diagnostic pathology, α-SMA is used mostly as a discriminator of smooth muscle tumors in the identification of spindled and pleomorphic tumors (Jones et al, 1990). It is important to emphasize that this marker should not be used in isolation (Leong et al, 1997a). Because myogenic determinants are not always synthesized by normal and neoplastic cells simultaneously, the highest diagnostic yield is obtained with a panel of antibodies that includeα-SMA, desmin and muscle-specific actin (Appendices 1.22 and 1.23). In the diagnostic context of the morphologically indeterminate spindle cell tumor, it should also be remembered that myofibroblasts may express these myogenic markers. However, expression of desmin tends to be focal and within scattered cells in myofibroblastic proliferations and these cell types show a thin and fragmented basal lamina compared to the thick, irregular and long runs of basal lamina around smooth muscle tumors (Leong et al, 1997b). Myofibroblastic proliferations may display a characteristic "tram track" pattern of distribution of muscle actins distributed in a subplasmalemmal location. Furthermore, smooth muscle cells may express low molecular weight cytokeratin. α-SMA positivity is also observed in adult and juvenile granulosa cell tumors, and in the theca externa and focally in the cortex-medulla

of the ovary (Santini et al, 1995). The significance of muscle actin expression observed in mesotheliomas is presently unknown (Kung et al, 1995).

Comments

Clone 1A4 produces the best results in our hands. Immunoreactivity appears not to be enhanced by HIER or proteolytic digestion.

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Santini D, Ceccarelli C, Leone O, et al 1995. Smooth muscle differentiation in normal human ovaries, ovarian stromal hyperplasia and ovarian granulosa-stromal cell tumors. Modern Pathology 8:25-30.

α-1-Antichymotrypsin

Sources/Clones

Biodesign (8E6, polyclonal), Biogenesis (polyclonal), Calbiochem/Novocastra (monoclonal), Dako (polyclonal), and Fitzgerald (polyclonal).

Fixation/Preparation

The antibodies are immunoreactive in fixed, paraffin-embedded tissues. Immunoreactivity is increased following proteolytic digestion.

Background

α-1-Antichymotrypsin (AACT), a 68 kD glycoprotein, is a serum protease inhibitor which is synthesized mainly by cells of the mononuclear phagocytic system. AACT was initially employed as a marker of histiocytes (monocytes/macrophages) but the demonstration of this enzyme in a large variety of normal and neoplastic tissues of both epithelial and mesenchymal derivation has resulted in only restricted use in diagnostic immunohistochemistry. It most likely identifies cells that are rich in phagolysosomes and has no tissue specificity. With restricted settings, AACT can be of value in diagnostic immunohistology. (See discussion onα-1-antitrypsin.)

α-1-Antitrypsin

Sources/Clones

Axcel/Accurate (polyclonal), Biodesign (1101, 1103), Biogenesis (polyclonal), Biogenex, Biomeda (polyclonal), Chemicon (monoclonal), Calbiochem/Novocastra, Dako (polyclonal), Fitzgerald (polyclonal), Sanbio (F50.4.1, F43.8.1), Sera Lab (polyclonal), Serotec (polyclonal) and Zymed (ZMAAT3).

Fixation/Preparation

The antigen is preserved in formalin-fixed, paraffin-embedded tissue. Proteolytic digestion increases immunoreactivity but HIER appears unhelpful.

Background

α-1-Antitrypsin (AAT), a 51 kD glycoprotein, is mainly synthesized in the liver, where a pair of at least 24 possible codominant alleles, which belong to the protease inhibitor (Pi) locus on chromosome 2, determine production. It functions as an inhibitor of proteases, especially elastase, collagenase and chymotrypsin. Individual homozygotes of Pi M produce normal quantities of functionally normal AAT whereas, individuals with abnormal Pi genes, such as those designated ZZ, SZ, and PS, have serum concentrations of AAT that are, 40% of normal. Such individuals are at risk for hepatic cirrhosis in childhood or pulmonary emphysema as young adults.

Interest in AAT as an immunohistochemical marker arose in the early 1980s because of the search for a marker of histiocytes (monocytes/macrophages). AAT,α-1-antichymotrypsin (AACT) and muramidase (lysozyme) were touted as specific markers of histiocytes (Isaacson et al, 1981), launching their use as a marker of such cells and malignant tumors derived from them. Monocytes have recently been shown to be another site of synthesis of AAT, short-term cultures of plasticadherent peripheral blood cells releasing isotopically labeled AAT into the supernatant. AAT was used to distinguish histiocytic from lymphoid neoplasms (Isaacson and James, 1983). In particular, this enzyme was used to characterize a large pleomorphic lymphoma of the intestine as "malignant histiocytosis" (Isaacson et al, 1982). Many studies employed AAT to support the histiocytic differentiation of malignant fibrous histiocytoma and other so-called "fibrohistiocytic" tumors (Meister & Nathrath, 1981; Du Boulay 1982; Kindblom et al, 1982; Roholl et al, 1985).As with many previous claims in immunohistochemistry for "specific markers", the initial enthusiasm was soon tempered by caution when it was shown that AAT, AACT and lysozyme immunoreactivity can be commonly found in a large variety of tumors of both epithelial and mesenchymal differentiation. These included carcinoid tumors, malignant melanomas, schwannomas (Permanetter & Meister, 1984; Soini & Miettinen, 1989), islet cell tumors of the pancreas (Ordonez et al, 1983), mixed mesodermal tumor of the ovary (Dictor 1981), uterine sarcomas (Marshall & Braye 1985) and ameloblastoma (Takahashi et al, 1995). The enthusiasm for AAT, AACT and lysozyme as immunohistochemical markers fell off rapidly as this information became more widely known (Dar et al, 1992). Interestingly, the entity of so-called "malignant histiocytosis" in the intestine was soon shown to be of T-cell lineage (Isaacson et al, 1985). Immunoreactivity for this group of proteolytic enzymes may well be a reflection of the intracytoplasmic accumulation of

phagolysosomes and to imply histiocytic differentiation.

Applications

AAT, AACT and lysozyme can still provide useful diagnostic information but they have to be used in the context of a panel of antibodies directed to the diagnostic entities considered in differential diagnosis. For example, in pleomorphic tumors of the skin, these markers are useful for the separation of atypical fibroxanthoma from its mimics (Leong & Milios, 1987), although other markers can provide more relevant information to separate such entities (Appendix 1.22), and in identifying tumors rich in phagolysosomes such as granular cell tumors. Immunolabeling for AAT remains an important way of demonstrating the presence of accumulated enzyme within hepatocytes in AAT deficiency (Palmer et al, 1978). Recently, it was suggested that this protease inhibitor is found in thyroid papillary carcinoma but not in normal thyroid tissue, a finding confirmed by Western blotting and immunoprinting (Poblete et al, 1996).

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α-Fetoprotein (AFP)

Source/Clones

Accurate, Biodesign (polyclonal), Biogenesis (219-2, BIOAFP003, polyclonal), Biogenex (A-013-01), Bioprobe (F2, C3), Biotec (XFD05, polyclonal), Camon, Cymbus Bioscience (946.11), Dako (polyclonal), Immunotech (IC5, C3), Pierce (ZGAFP1), Sigma (C3) and Zymed (ZSA06, ZMAF2, polyclonal).

Fixation/Preparation

The antibody is immunoreactive in routinely prepared sections. HIER enhances staining.

Background

α-Fetoprotein (AFP) is a glycoprotein composed of 590 amino acid residues. Cells of the embryonic yolk sac, fetal liver and intestinal tract synthesize this glycoprotein. By immunostaining, the antigen is detectable in hepatocellular carcinoma, and gonadal and extragonadal germ cell tumors, including yolk sac tumors. It is otherwise not present in adult tissues.

Applications

Staining for AFP is largely used for the identification of the glycoprotein in germ cell tumors and in the separation of hepatocellular carcinoma (HCC) from its mimics such as cholangiocarcinoma and metastatic carcinoma in the liver (Appendix 1.8). Unfortunately, although specific, AFP is of low sensitivity and estimated to be present in no more than 44% of hepatocellular carcinomas (Chedid et al, 1990). Other antibodies employed in a panel may be useful in this context. They include antialbumin (specific to HCC but not a sensitive marker), cytokeratin 19 (expressed by bile duct epithelium and cholangiocarcinoma), cytokeratin 20 (expressed by both cholangiocarcinoma and gastrointestinal tract tumors), polyclonal CEA (highlights bile canaliculi in HCC but stains the cytoplasm of cholangiocarcinoma and metastatic adenocarcinoma diffusely),α-1-antitrypsin (found in HCC but of low specificity, being expressed in various carcinomas) (Leong et al, 1998) and sialoglycoproteins such as B72.3 and Leu M1 (found in some metastatic adenocarcinomas) (Fucich et al, 1994; Guindi et al, 1994). Another mimic of HCC is the recently described hepatoid tumor that has immunophenotypic characteristics similar to that of HCC, including staining for AFP, canalicular staining for CEA and α-1-antitrypsin. Such tumors have been described in the urinary bladder, lung, gastrointestinal tract and focally in germ cells tumors (Ishikura et al, 1990; Sinard et al, 1994). They represent areas of true hepatocellular differentiation.

Comments

We employ clone A-013-01, routinely following HIER.

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Amyloid

Sources/Clones

Amyloid-A (AA)

Dako (mc1), polyclonal anti-AA, Calbiochem/Novocastra (polyclonal), Axcel/Accurate (mc1), American Research Products (REU86.2), Biogenesis (polyclonal), Biosource (5G6), and Sanbio/Monosan/Accurate (REU86.2).

Transthyretin (ATTR/Prealbumin)

Axcel/Accurate (polyclonal), Biodesign (polyclonal), Biogenesis (polyclonal), Dako (polyclonal), and Fitzgerald (polyclonal).

β2-Microglobulin (Aβ2M)

Accurate (FMC16, polyclonal), Accurate/Sigma Chemical (BM63), Advanced Immunochemical (1F10, 2G3, 6G12), American Research Products (1672-18), Biodesign (GJ14, polyclonal), Biogenesis (B2M01), Biosource (MIG-85), Cymbus Bioscience (GJ14, polyclonal), Pharmingen (TU99), Sanbio/Monsan (B2M01), and Zymed (Z022).

Amyloid β Precursor Protein (βAPP)

Boehringer Mannheim (polyclonal), Dako (6F/3D), and Zymed (LN27).

Fixation/Preparation

These antibodies are applicable to formalin-fixed paraffin-embedded tissue sections.

Background

The amyloidoses are characterized by local, organ-limited or generalized proteinaceous deposits of autologous origin (Glenner, 1980a, b). The pattern of distribution, progress of disease and complications are dependent on the fibril protein. Amyloid is characterized by the following:

a typical green birefringence with polarized light after Congo red staining;

non-branching linear fibrils with a diameter of 10 - 12 nm;

an X-ray diffraction pattern which is consistent with Pauling's model of a cross-β-fibril (Lansbury, 1992).

The diagnosis and classification of amyloidosis requires both histological proof and detection of the amyloid fibril - histochemical confirmation of amyloid deposits using Congo red evaluation in polarized light followed by identification of the fibril protein by immunostaining, thereby revealing the probable underlying disease. Apart from the rare familial syndromes, localized forms of amyloid affect certain organs or lesions (Aβ in brain; calcitonin in medullary carcinoma; islet amyloid polypeptide in insulinomas or islets of Langerhans). The five major different fibril proteins are usually associated with the most common generalized amyloid syndromes: amyloid A (AA), amyloid of λ- (Aλ) and κ- (Aκ) light chains, of transthyretin (ATTR) and β2-microglobulin origin. These fibril proteins may be deposited in a wide variety of tissues and organs (Glenner, 1980a, b). They therefore have to be considered in the investigation of any biopsy considered to be amyloidogenic.

Applications

In most instances good correlation is achieved between the immunohistochemical classification of amyloid and the underlying diseases (R鯏ken et al, 1996). AA-amyloidosis is commonly associated with chronic inflammatory disorders. AL-amyloidosis (either λ- or κ-light chain origin) is linked mainly to the plasma cell dyscrasias or interpreted as being idiopathic. ATTR-amyloidosis is found in cases with familial amyloidosis. Aβ2M-amyloidosis is associated with long-term hemodialysis.

However, a critical issue in the clinicopathological typing of amyloidosis is the interpretation of the immunostaining (R鯏ken et al, 1996). Occasionally, more than one antibody may show immunostaining of amyloid deposits. Immunohistochemistry detects any associated contaminating component in the amyloid deposit (amyloid P component, apolipoprotein E and glycosaminoglycans) and not merely the currently known obligate fibril proteins. Further, the five syndromic fibril proteins originate from plasma proteins (Glenner, 1980a, b), which may themselves `contaminate' amyloid deposits. The most critical of these are the immunoglobulin light chains (R鯏ken et al, 1996). Based on these aberrant staining patterns, R鯏ken et al have proposed that the identification of a fibril protein with a single antibody demonstrates an even and homogeneous immunostaining for the entire amyloid deposit, whilst staining of the contaminant protein remains uneven. Instances also arise where two immunoreactive antibodies demonstrate similar uneven staining patterns, interpreted as being due to the irregular presentation of the epitope of the fibril protein, resulting in a similar staining pattern as contaminating proteins. These workers strongly recommend testing an additional specimen or biopsy to determine the causative fibril protein. In addition, the correlation of immunohistopathological observations and the clinical diagnosis is also mandatory to arrive at the correct classification of the amyloid fibril.

Another problem area is the false-negative detection of amyloid. This can be avoided by increasing the sensitivity of detection by using both immuno- and Congo redstaining methods (R鯏ken et al, 1996). The latter method of detection is also influenced by the sample quality. It has long been recognized that the diagnostic yield of gastrointestinal biopsies (especially rectal) is extremely high, but should contain submucosa. Other recommended sites include subcutaneous fat, sural nerve, heart, kidney and bone marrow. Whilst AA-amyloidosis is commonly detected in rectal biopsies, any involved organ or tissue is suitable for identification/classification of AL-amyloidosis. Interestingly, a recent study has shown that long-term hemodialysis-associatedβ2-microglobulin amyloid may also involve the gastrointestinal system in addition to the usual osteoarticular involvement (Shimizu et al, 1997).

The distinction and classification of amyloidosis has major therapeutic implications, as studies have recommended that AL-amyloidosis be treated with cytotoxic drugs (melphalan and prednisolone), whilst AA-amyloidosis responds better to colchicine and dimethylsulfoxide (Kyle et al, 1985; Ravid et al, 1982).

The role of antibodies against amyloidβ precursor protein has assisted in the diagnosis of Alzheimer's disease (Iwamoto et al, 1997) and early detection of axonal injury (Sherriff et al, 1994) in the brain. Antibodies to transthyretin amyloid protein are useful in the diagnosis of cardiac amyloidosis (Jacobson et al, 1997).

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Androgen Receptor

Sources/Clones

Accurate (polyclonal), Biogenex (F39.4.1), Novocastra (2F12, polyclonal), Pharmingen (G122-25.3, G122-434, G122-77.14, AN1-15) and Sanbio/Monosan (F39.4.1).

Fixation/Preparation

The antibodies are immunoreactive in frozen sections, cell preparations and paraffin-embedded sections. HIER enhances the latter.

Background

The intracellular action of androgens is mediated by the androgen receptor, which is a key element of the androgen signal transduction cascade and a target of endocrine therapy for prostatic carcinoma. Qualitative and quantitative alterations of androgen receptor expression in prostatic carcinomas and their possible implications for tumor progression and treatment are therefore of diagnostic and research interest. Findings in prostatic tumor cell lines of rat and human origin suggest a reduction of androgen receptor protein expression accompanied by an increase in tumor aggressiveness. However, immunohistochemical analysis and binding assays have demonstrated the presence of androgen receptors in all histological types of prostatic carcinoma and in both therapy-responsive and therapy-unresponsive tumors.

Applications

Many of the immunohistochemical studies of androgen receptors have been related to prostatic carcinoma. The androgen receptor content of prostatic carcinoma has been inversely correlated to Gleason grade in stage D2 carcinomas, although it was unrelated to extent of disease and response to hormonal therapy at 3 months. Patients with 48% or more androgen receptor-positive cells had statistically significant better outcome in terms of both progression-free and cause-specific survival (Takeda et al, 1996). Another study suggested that pretreatment androgen receptor expression alone is not related to prognosis of hormonally treated prostate cancer; however, when combined with bcl-2 expression, it acts as an independent prognostic factor for clinical progression (Noordzij et al, 1997). One explanation for the discrepancy in findings may relate to the mutations that occur in the androgen receptor, which account for the variable response to hormonal therapy. These mutations produce broadened ligand specificity so that transcriptional factor activity of the receptor can be stimulated not just by dihydrotestosterone but also by estradiol and other androgen metabolites. Such activation of mutant androgen receptors by estrogen and weak androgens could confer on prostate cancer cells an ability to survive testicular androgen ablation through the activation of the androgen receptor by adrenal androgens or exogenous estrogen. Thus, mutated androgen receptors that occur prior to therapy may characterize a more aggressive disease (Hakimi et al, 1996).

The variability of androgen receptor protein content per unit nuclear area has been shown to increase with increasing histological grade, suggesting that this variability might account for the variable response to endocrine therapy in high-grade tumors (Magi-Galluzzi et al, 1997). The extent of heterogeneity of androgen receptor expression may be a useful indicator of response to hormonal therapy (Kloker et al, 1994).

Immunostaining for androgen receptor expression has been

studied in other cell types including endometrium (Mertens et al, 1996), genital melanocytes (Tadokoro et al, 1997), meningiomas (Carroll et al, 1995) and urinary bladder carcinomas (Zhuang et al, 1997).

Comments

The receptor is intranuclear in location. A cut-off of 10% androgen receptor-positive cells has been suggested to maximize assay prognostic efficiency, with 48% positivity showing significant correlation with response, time to progression and survival, but not with grade or stage of prostatic cancer (Pertschuk et al, 1994). Clone G122-25 is immunoreactive in fixed, paraffin-embedded tissue sections and does not appear to crossreact with estrogen or progesterone receptors.

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Zhuang YH, Blauer M, Tammela T, Tuohimaa P 1997. Immunodetection of androgen receptor in human urinary bladder cancer. Histopathology 30: 556-562.

Antiapoptosis

Sources/Clones

Dako (BM-1), Oncor (Apop Tag), Monosan (Annexin V - polyclonal) and Pharmingen (APO-BRDU, Annexin V-FITC).

Fixation/Preparation

Various methods of detection of apoptotic bodies are available. All methods can be used on formalin-fixed, paraffin-embedded tissue sections. Some require proteolytic digestion. Acetone-fixed cryostat sections and fixed-cell smears may also be used.

Background

Cell death may occur by necrosis or apoptosis. Necrosis results from direct physical or chemical damage to the plasma membrane or disturbances in the osmotic balance of a cell (Wyllie et al, 1980). With the entrance of extracellular fluid into the cell, resultant cell swelling and lysis precedes a subsequent inflammatory response. Furthermore, necrosis affects groups of cells, with consequent disruption of normal tissue architecture.

In contrast to necrosis, apoptotic cell death is a highly regulated physiologic process. The balance between apoptosis and cell proliferation results in the maintenance of cell homeostasis (Kerr et al, 1972). Apoptotic bodies are rapidly engulfed by neighboring cells or macrophages, without an inflammatory response being elicited. The nuclear structure alteration in apoptotic cells is induced by endonuclease DNA cleavage that results in the generation of large 50-300 kb fragments. This produces the characteristic DNA "ladders" of apoptosis as viewed on agarose gel electrophoresis (Oberhammer et al, 1993).

Recently, reliable methods have been developed that enable the rapid assessment of apoptosis on sections prepared from paraffin-embedded material, e.g. the TUNEL method for TdT-mediated dUTP-biotin nick end labeling (Sarkiss et al, 1996). The APO-BRDU kit utilizes the same principle. The enzyme TdT is used to catalyze a template-independent addition of bromolated deoxyribonucleotide triphosphates (Br-dUTP) to the 3' -hydroxyl ends of the numerous fragments of double- and single-stranded DNA present in apoptotic cells. This allows the labeling of the very high concentrations of 3'-OH ends that are localized in apoptotic bodies. Br-dUTP is claimed to be more readily incorporated into the genome of apoptotic cells than are deoxyribonucleotide triphosphates complexed to larger ligands like fluorescein, biotin or digoxigenin (Nagata & Golstein, 1995). Although rather specific for cells undergoing apoptosis, these techniques may also label cells undergoing necrosis. However, this is seldom a problem since the distinction between focal apoptotic events and necrosis is fairly clear. The histologic features of apoptosis include cell shrinkage and loss of junctional contact, resulting in a "halo" around the cell. The nucleus shows condensation and margination of the chromatin. This is followed by the fragmentation or "pinching off" of pieces of nuclear material, which are surrounded by cytoplasm with intact cytoplasmic organelles as shown at ultrastructural level, These apoptotic fragments of pyknotic nuclear material and cytoplasm are phagocytosed by adjacent cells or macrophages. Apoptotic cells have been called by various names in different tissues and include "Councilman bodies", "Civatte bodies", "necrobiotic cells" and "nuclear dust".

The BM-1 antibody is directed to the Lewiš antigen which has been identified phenotypically as a

Lamant L, Meggetto F, Saati TA et al 1996. High incidence of the t(2;5) (p23;q35) translocation in anaplastic large cell lymphoma and its lack of detection in Hodgkin's disease. Comparison of cytogenetic analysis, reverse transcriptase-polymerase chain reaction, and P-

marker of specific types of cells, and possibly specific stages of differentiation. Lewis^y is totally absent at the morula stage, but is highly expressed on the blastocyst surface and has been shown to play a role in the implantation process (Fenderson et al, 1991). Recently, Lewis^y has been identified as a characteristic of cells undergoing apoptosis (Hiraishi et al, 1993). In Lewis^y-positive areas of tissue sections, typical apoptotic morphological changes and DNA fragmentation were frequently observed in certain loci, although not all Lewis^y positive cells showed such signs of apoptosis. Although the BM-1 antibody against the Lewis^y antigen is reputed to detect apoptotic cells, further studies to test its efficacy, including a comparative analysis with the insitu end-labeling techniques, are awaited.

Another method of detection of apoptotic bodies is the use of Annexin V, which is a 35-36 kD Ca^{2+}-dependent phospholipid-binding protein that has a high affinity for the membrane phospholipid phosphatidylserine (PS). In apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment and allowing its binding to Annexin V. Binding to a signal system such as fluorescein isothiocyanate allows the easy identification of apoptotic cells (in frozen sections and cell preparations). Annexin V is thought to identify cells at an earlier stage of apoptosis than assays based on DNA fragmentation because externalization of PS occurs earlier than the nuclear changes associated with apoptosis (Raynal & Pollard, 1994).

Applications

BM-1 antibody may be applied to neoplasms in general to assess the apoptotic index, e.g., endometrial adenocarcinoma (Kuwashima et al, 1995). Recently, apoptosis has been considered to be a key event in oncogenesis, e.g. apoptosis has been reported to be promoted by tumorsuppressor gene p53 and inhibited by oncogene *bcl*-2 (Arends & Wyllie, 1991). Although apparent cell loss by apoptosis occurs in carcinomatous tissue (Hiraishi et al, 1993), the physiological significance is unclear (Umansky, 1982). BM-1 positivity has been found to be as high as 25-35% in T cells of lymph nodes of patients with AIDS-related complex (ARC), in contrast to healthy controls who had less than 5% (Adachi et al, 1988).

Comments

Strong BM-1 immunoreactivity is observed in the apical surface of tubular urothelium, basal cells (glandular foveoli) of gastric and esophageal mucosa and these tissues may be employed as controls.

The optimal method for the identification of apoptotic cells depends on the experimental system and the mode of induction of apoptosis. The degree of DNA degradation can vary according to the cell type, the nature of the inducing agent and the stage of apoptosis. The application of multiple methods, each based on a different feature of the apoptotic process, may provide more information about the cell population than any one method would give alone.

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Anti-p80 (ALK-NPM Fusion Protein)

Sources/Clones

Rabbit polyclonal antibody (Nichirei Corporation, Japan).

Fixation/Preparation

This antibody is suitable for formalin-fixed paraffin sections. Microwave antigen retrieval is essential for immunoreaction.

Background

It was recently recognized that a translocation involving chromosome 2 and 5 was associated with anaplastic large cell lymphoma (ALCL) (Mason et al, 1990). However, the exact frequency of this association is not clear. The translocation breakpoint has been cloned with the candidate genes being identified as the nucleophosmin (NPM) gene on chromosome 5 and a putative tyrosine kinase gene, the appropriately named anaplastic lymphoma kinase (ALK) gene, on chromosome 2 (Morris et al, 1994). It has been postulated that the ectopic expression of ALK may contribute to the pathogenesis of ALCL, but the ability of ALK to transform hematopoietic cells has not yet been demonstrated experimentally. The cloning of the translocation breakpoint allows the demonstration of the t(2:5) (p23; q35) by techniques other than cytogenetics. This has permitted the demonstration of t(2;5) in 30% (89/297) of ALCL cases (for review, see Chan, 1996) from several studies. In addition t(2;5) was also demonstrated in 6% (21/345) of Hodgkin's disease cases. The significance of the latter has yet to be determined.

Shiota et al (1994) produced a rabbit polyclonal antibody (anti-p80) against the tyrosine kinase domain of ALK in the ALK-NPM fusion protein. In tonsils, only follicular dendritic cells and endothelial cells showed weak positive signals when immunostained with anti-p80. Of 50 lymphomas, only three cases of ALCL with t(2;5) (p23;q35) and p80 mRNA reacted with the antibody. Several subsequent studies have confirmed the sensitivity and specificity of anti-p80 in identifying ALCL expressing ALK. The incidence of Hodgkin's disease that are t(2;5) positive also appears to be very low (3/186) by immunohistochemical study using anti-p80 (Lamant et al, 1996).

Applications

Currently, anti-p80 appears to be the most well-characterized antibody for the identification of ALCL with t(2;5). Given the morphological heterogeneity of ALCL, this antibody would prove to be useful in confirming the diagnosis, albeit in approximately a third of cases (Shiota et al, 1995).

Comments

The cloning of the t(2;5) (p23; q35) has stimulated interest in studying this translocation in ALCL and Hodgkin's disease. The availability of a specific antibody to ALK not only facilitates the diagnosis of ALCL in pathology laboratories using routine immunohistochemical procedures, but may contribute to a greater understanding of the significance of t(2;5) in the pathogenesis of the heterogeneous nature of ALCL (Pittaluga et al, 1997).

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BCL-2

Sources/Clones

Dako (124), Immunotech (124) and Zymed (BCL2-100).

Fixation/Preparation

Antibodies to bcl-2 are reasonably robust and work very well on paraffin-embedded tissue. Staining is not too dependent on fixation protocols and good results may be obtained with formalin-fixed, B5-fixed, methacarn-fixed and fresh-frozen tissues. Staining is enhanced by the use of antigen retrieval with either microwave or pressure-cooking pretreatment. The bcl-2 antibody may be used for labeling acetone-fixed cryostat sections or fixed-cell smears.

Background

The bcl-2 gene was identified more than a decade ago with the discovery and analysis of the t(14; 18) (q32; q21) translocation (Bakhshi et al, 1985). This translocation occurs in 70-80% of follicular lymphoma, comprising juxtaposition of the bcl-2 gene with the immunoglobulin heavy chain (IgH) gene on chromosome 14q32 (Chen-Levy et al, 1989). This results in an overexpression of the translocated bcl-2 allele induced by enhancers in the IgH region, although the translocation is not a prerequisite for bcl-2 protein expression, since this occurs in many cases without this rearrangement (Pezzella et al, 1990). The bcl-2 polypeptide is a 26 kD protein that is found on intracellular (mitochondrial and nuclear) membranes and in the cytosol (on the smooth endoplasmic reticulum), rather than on the cell surface. bcl-2 is not an oncogene and has no effect on cell replication. bcl-2 protein does, however, prevent cells from undergoing apoptosis, conferring a survival advantage on cells harboring the t(14; 18) translocation. In normal lymphoid tissue, bcl-2 antibody reacts with small B lymphocytes in the mantle zone and many cells within T-cell areas. In the thymus many cells in the medulla are stained, with weak/negative reaction in the cortex (Chetty et al, 1997).

Applications

The most relevant use of bcl-2 immunostaining lies in the distinction of reactive follicular lymphoid hyperplasia from follicular lymphoma (Pezzella & Gatter, 1995; Veloso et al, 1995 Cooper & Haffajee, 1996). Positive staining is cytoplasmic in location. Follicular lymphomas show striking bcl-2 expression in neoplastic follicles, whilst only isolated individual cells within the reactive follicle centers are positive (mostly T-cells). This difference in staining pattern is not due to down regulation or decreased bcl-2 mRNA, but largely to a posttranslational mechanism that results in decreased protein levels. Furthermore, bcl-2 protein expression is demonstrated in all grades of follicle center cell lymphomas in both small and large cells (Cooper & Haffajee, 1997). Strongly bcl-2-positive lymphoid aggregates in the bone marrow of patients previously diagnosed with nodal follicular lymphoma are indicative of lymphoma involvement (Chetty et al, 1995). However, there is no practical value in applying the bcl-2 antibody for classification of a malignant lymphoid infiltrate, since many different lymphoma types can be bcl-2 positive. Nevertheless, it has been demonstrated that non-Hodgkin's lymphoma with bcl-2 expression has a significantly higher relapse rate and a lower cause-specific survival than those without (Hill et al, 1996).

Expression of bcl-2-has been studied in many epithelial neoplasms (Pezzella et al, 1993; Lu et al, 1993). In general, better

prognoses accompany bcl-2 positive neoplasms than negative ones, with some prostatic cancers being the exception to the rule (Colombel et al, 1993). A reciprocal relationship has been demonstrated between bcl-2 reactivity and p53 overexpression in 65% of colorectal neoplasia, with a bcl-2+ve/p53-ve subgroup showing a strong correlation with negative lymph node status, implying a less aggressive pathway of neoplastic transformation (Kaklamanis et al, 1996). Recently, bcl-2 protein was detected in all grades of cervical intraepithelial neoplasia, with a striking increase in the number of positive cells with increasing severity of CIN, in combination with a mild increase in staining intensity (Harmsel et al, 1996).

Bcl-2 expression has been demonstrated in 79% (15 of 19) of synovial sarcoma cases (Hirakawa et al, 1996), but was negative in 20 leiomyosarcomas, four malignant peripheral nerve sheath tumors and four fibrosarcomas. However, in another study, bcl-2 protein was expressed in seven rhabdomyosarcomas and 5/7 leiomyosarcomas, four epithelioid leiomyomas and 6/14 leiomyomas (Soini & P滢kk 1996).

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Ber-EP4

Sources/Clones

Axcel/Accurate, Dako and Diagnostic Bioscience.

Fixation/Preparation

Ber-EP4 can be used on formalin-fixed, paraffin-embedded tissue sections. Prolonged formalin fixation can be deleterious to immunoreactivity, which is enhanced by HIER or by enzymatic predigestion with proteolytic enzymes such as trypsin and pronase. Ber-EP4 may also be used to label acetone-fixed cryostat sections and fixed-cell smears. A major advantage of this antibody is the high sensitivity which allows it to be used at high dilutions.

Background

Ber-EP4 was raised against MCF-7 cells and is directed against two glycoproteins of 34 and 49 kD present on the surface and in the cytoplasm of all epithelial cells with the exception of the superficial layers of squamous epithelia, hepatocytes and parietal cells (Latza et al, 1990). Although it is not yet clear what antigen is recognized by the antibody, an absence of reactivity to keratins was found in immunoblotting experiments. A positive reaction is seen in epithelial cells known to contain large amounts of the Ber-EP4 antigen, e.g. epithelial cells in the bile ducts and ducts of the epididymis.

Applications

Ber-EP4 shows a broad pattern of reactivity with human epithelial tissues from simple epithelia to basal layers of stratified non-keratinized squamous epithelium and epidermis (Appendix 1.20). In addition' most cases of carcinoma demonstrated immunoreaction with this antibody (Latza et al, 1990). However, two cases of malignant mesothelioma studied reacted negatively. In a separate study (Sheibani et al, 1992), 87% of 83 adenocarcinomas were found to express Ber-EP4. The only adenocarcinomas that failed to react were of breast origin (eight of 25 cases non-reactive) and kidney (all three cases non-reactive). In contrast, only one of 115 mesotheliomas studied showed positivity.

Focal expression of Ber-EP4 in the mesothelium of the peritoneum and the ovarian surface epithelium adjacent to endometriotic lesions suggests that the mesothelium possibly acquires characteristics of epithelial nature, supporting a metaplastic process of the peritoneal mesothelium in the pathogenesis of endometriosis (Nakayama et al, 1994).

Comments

Any attempt to use Ber-EP4 to help distinguish epithelial mesothelioma from adenocarcinoma should be accompanied by a panel of antibodies (Appendix 1.16) including CEA, Leu-M1, B72.3 (all three antibodies in combination were reported to distinguish over 90% pulmonary adenocarcinomas from pleural mesotheliomas; Sheibani et al, 1991) and calretinin. In addition, anti-EMA has been shown to produce a distinctive pattern of membrane staining corresponding to the circumferential long microvilli which are pathognomonic of malignant mesothelial cells (Leong et al, 1990).

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β-hCG (Human Chorionic Gonadotropin)

Sources/Clones

Accurate (1039), American Research Products (1C5), Biodesign ([427,681], [812,813], [827,829,830], [827,31], 2B1-3, ME.1, ME.106, ME.108, polyclonal), Biogenesis (2F4/3, BIO-BCG-001, BIO-BCG-005, BHCG-010, polyclonal), Biogenex (D7), Caltag Laboratories (2092), Dako, Fitzgerald (M15292, M15294, M94138, M94139, M94140, M94141, polyclonal), Immunotech (2B1.3), Sanbio/Monosan (2092) and Zymed (ZMCG13, ZSH17).

Fixation/Preparation

These antibodies are applicable to both formalin-fixed, paraffin-embedded sections and to frozen sections. Neither enzyme digestion nor HIER appears to enhance immunoreactivity.

Background

Human chorionic gonadotropin (hCG) is a glycoprotein (40 kD) comprising a protein core and a carbohydrate side chain (Bellisario et al, 1973). The molecule is composed of two dissimilar subunits α and β. The α subunit is indistinguishable immunologically from the α subunit of pituitary glycoprotein hormones: luteinizing hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH). The β subunits are different from each other and confer specificity. hCG, secreted in large quantities by the placenta, normally circulates at readily detectable levels only during gestation (Braunstein et al, 1976).

The monoclonal antibody (IgG[1]) to β-hCG was produced by immunization with pure chorionic gonadotropin β subunit. β-hCG is demonstrable in syncytiotrophoblasts of normal human placenta.

Applications

hCG is the most important marker of gestational trophoblastic cells, being present in syncytiotrophoblastic cells and cells of the intermediate trophoblast but absent in cytotrophoblast (Appendices 1.29, 1.30). In syncytiotrophoblast cells, hCG is demonstrable from the 12th day of gestation, reaches a peak at 6 weeks and decreases thereafter; at term hCG is present only focally in these cells. In choriocarcinoma strong diffuse immunostaining for hCG occurs in syncytiotrophoblastic cells (and focal immunostaining for human placental lactogen). In contrast, placental site trophoblastic tumor shows focal hCG immunopositivity (and diffuse human placental lactogen immunoreaction) (Appendix 1.29).

β-hCG expression in non-trophoblastic tumors may indicate aggressive behavior of the tumor. It is worth noting that hCG may be demonstrated in 14% of patients with hepatocellular carcinoma (Braunstein et al, 1973). hCG may be demonstrated in the trophoblast-like cells which develop in undifferentiated carcinoma of the endometrium (Pesce et al, 1991); however, the presence of recognizable glandular structures and the lack of the biphasic pattern of alternating rows of syncytial and cytotrophoblasts rule out the possibility of choriocarcinoma.

hCG has also been demonstrated in poorly differentiated areas with cells resembling syncytiotrophoblasts in three women with serous papillary or mucinous adenocarcinomas of the ovary (Civantos & Rywlin, 1972). In 6-8% of dysgerminomas, there are individual or collections of syncytiotrophoblastic giant cells that contain/produce hCG.

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CA 125

Sources/Clones

Dako (OC125) and Immunotech (Ov185).

Fixation/Preparation

Monoclonal anti-CA 125 (M11) can be used on formalin-fixed, paraffin-embedded tissue sections. The deparaffinized tissue sections must be treated with heat (in citrate buffer or Dako Target Retrieval Solution) prior to the immunohistochemical staining procedure.

Background

CA 125 was discovered with a monoclonal screen for tumor-specific antigens of hybridomas derived from mouse lymphocytes immunized to an ovarian cell culture line, OVCA433 (Bast et al, 1981). The antigen is located on the surface of ovarian tumor cells with essentially no expression in normal adult ovarian tissue (Kabawat et al, 1983). Significantly, CA 125 is also found in sera of patients with ovarian, pancreatic (about 50%), liver, colon and other (22%-32%) adenocarcinomas (Kuzuya et al, 1986). Although CA 125 is not specific for ovarian carcinoma, it nevertheless does correlate directly with disease status (Bast et al, 1983). Similar to other tumor markers, CA 125 is also expressed normally in fetal development: the antigen has been localized to the amnion celomic epithelium and derivatives of M黩lerian epithelium (Hardardottir et al, 1990). In adult tissue, the monoclonal antibody OC 125 reacted with the epithelium of the fallopian tube, endometrium, endocervix, apocrine sweat glands and mammary glands (Kabawat et al, 1990; Hardardottir et al, 1983; O'Brien et al, 1991).

Presently, little is known of the structure of this extracellular matrix molecule, nor is there any indication of its function. It appears to be part of a large molecular weight mucin-like glycoprotein complex that can be resolved to a 200-250 kD species on gel electrophoresis. Although the antigen is thought to contain a carbohydrate component, the antigenic epitope recognized by OC 125 is considered to be peptide in nature (Davis et al, 1986).

Applications

The most important property of CA 125 is that it is regularly expressed on the tumor cell surface of serous cystadenocarcinoma of the ovary (>95%), whilst no expression is detected in mucinous cystadenocarcinomas. Although only a small number of tumors were examined, the following were also found to stain positively with CA 125: colonic adenocarcinoma (1/2), breast carcinoma (3/8), uterine papillary serous carcinoma (1/1), thyroid follicular adenoma (1/1), transitional cell carcinoma of the bladder (2/3), uterine adenomatoid tumor (1/1), lung bronchoalveolar carcinoma (1/1), endometrioid carcinoma of the ovary (2/2) and squamous cell carcinoma of the penis (1/1) (Dako specifications). Employed in an appropriate panel, CA 125 is useful for the separation of colonic carcinoma from ovarian endometrioid carcinoma in the pelvis (Appendix 1.13).

Recently, a mesothelioma was reported which demonstrated both serum and immunohistochemical positivity with CA 125 (Almud関ar Bercero et al, 1997). This indicates that CA 125 cannot reliably distinguish between metastatic serous epithelial tumors of the peritoneum and mesothelioma.

Comments

The major role of CA 125 in immunohistology is in the

identification of metastatic serous carcinoma of the ovary. Primary serous cystadenocarcinoma of the ovary is the recommended positive control tissue for optimization of CA 125.

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N/97-Cadherin/E-Cadherin

Sources/Clones

Monoclonal antibody anti-N-cadherin (clone 13A9) and anti-E-cadherin (clone E9) (Soler et al, 1995). Accurate (6F9), American Research Products (6F9), Calbiochem (HybEcad#1), Coulter (67A4), Eurodiagnostica/Accurate (5H9), Immunotech (67A4), Lab Vision Corp (HybEcad#1), Sanbio/Monosan/Accurate (5H9), Urodiagnostica/Accurate (5H9) and Zymed (HECD1, ECCD1, ECCD2, SHE78-7).

Fixation/Preparation

Applied primarily to frozen sections, both antibodies are now applicable to formalin-fixed, paraffin-embedded tissue with heat pretreatment in citrate buffer.

Background

It has long been recognized that cancer cells have differences in their adhesive properties when compared with non-transformed cells (Hedrick et al, 1993). There is evidence that among the different cell adhesion molecules, the cadherin family of calcium-dependent cell-cell adhesion molecules and their associated proteins are indeed tumor suppressors. The cadherin family includes several distinctive members, two of which include E(epithelial)-cadherin (Gumbiner and Simons, 1986), a 120 kD protein expressed in epithelial cells and concentrated in cell-cell adherens junctions, and N (nerve)-cadherin (Redies et al, 1993), a 135 kD protein expressed in nerve cells, developing skeletal muscle (Knudsen et al, 1990), embryonic and mature cardiac muscle cells (Soler & Knudsen, 1994) and pleural mesothelial cells (Hatta et al, 1987). During embryonic development, expression of distinctive members of the cadherin family determines the aggregation of cells into specialized tissues as they interact with identical cadherins within the same tissue. Hence, the mesoderm-derived mesothelial cells that form the pleura express N-cadherin, whilst epithelial cells of the lung express E-cadherin (Hatta et al, 1987). Therefore, the development of well-characterized monoclonal antibodies that recognize N-cadherin without crossreactivity with E-cadherin provided an opportunity for its application to immunohistology.

Applications

In 1995, Soler et al found a high level of expression of N-cadherin in all mesotheliomas and E-cadherin in all pulmonary adenocarcinomas on fresh-frozen sections. The same group of investigators recently confirmed these findings using antibodies to N-cadherin and E-cadherin that reacted with fixation and paraffin-embedding-resistant epitopes in a series of malignant mesotheliomas and adenocarcinomas (Han et al, 1997). Although one case of mesothelioma was negative for N-cadherin and one adenocarcinoma was weakly positive for N-cadherin (but strongly positive for E-cadherin), these antibodies appeared to offer a sufficient degree of sensitivity and specificity for use in the differential diagnosis of mesothelioma and adenocarcinoma.

The application of antibodies to N- and E-cadherin to ovarian epithelial tumors has revealed interesting findings (Soler et al, 1997). Both E- and N-cadherins were expressed in serous and endometrioid tumors, whilst mucinous tumors strongly expressed E-cadherin only. The expression of N-cadherin in serous and endometrioid tumors traces their origin to the mesoderm-derived ovarian surface epithelium. Another recent study (Dara et al, 1997) demonstrated

both E- and N-cadherins in benign but not malignant ovarian tumors, whilst only N-cadherin was present in borderline tumors. Further, negative E-cadherin ovarian carcinomas presented a shorter survival. These workers suggest that the E- and N-cadherin differential expression may be involved in ovarian carcino-genesis and may have diagnostic and prognostic value.

Reduction in E-cadherin expression has been associated with lack of cohesiveness, high malignant potential and invasiveness in epithelial neoplasms of the colon (Kinsella et al, 1993), ovary (Hashimoto et al, 1989), stomach (Matsuura et al, 1992), pancreas (Moller et al, 1992), lung (Williams et al, 1993), breast (Gamallo et al, 1993; Sormunen et al, 1998) and head/neck (Schipper et al, 1991). In contrast, N-cadherin has also been demonstrated in astrocytomas/glioblastomas (Shinoura et al, 1995) and rhabdomyosarcomas (Soler et al, 1993).

Comments

Confirmation of data reported by Han et al (1997) and availability of anticadherin antibodies, represent a breakthrough in the study and diagnosis of malignant mesothelioma. Malignant mesothelioma and pulmonary adenocarcinoma tissue are recommended for use as positive controls for N- and E-cadherin respectively. We have found trypsin predigestion followed by HIER to produce the greatest immunoreactivity for these antigens, particularly E-cadherin.

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Calcitonin

Sources/Clones

Axcel/Accurate (polyclonal), Biodesign (polyclonal), Biogenesis (polyclonal, 1I5), Biogenex (polyclonal), Caltag Laboratories (polyclonal), Chemicon (polyclonal), Dako (polyclonal, CAL-3-F5), Fitzgerald (polyclonal), Immunotech (polyclonal), Sanbio/Monosan (polyclonal), Seralab (polyclonal) and Zymed (polyclonal).

Fixation/Preparation

These antibodies are applicable to formalin-fixed paraffin sections. HIER does not appear to enhance immunoreactivity but is not deleterious.

Background

CAL-3-F5 was raised against the synthetic peptide corresponding to the C-terminal portion of human calcitonin (aa 24-32). The polyclonal antibodies were raised in rabbits using synthetic human calcitonin (35 kD). Molecular biology studies have shown that most regulatory peptides are cleavage products of larger precursor molecules (Sikri et al, 1985). The structure of the calcitonin precursor was predicted from the nucleotide sequence of cloned cDNA prepared from the mRNA obtained from medullary thyroid carcinoma (Allison et al, 1981; Amara et al, 1982a). In the human calcitonin precursor, calcitonin is flanked by two molecules: PDN (peptide-aspartic acid-asparagine), a 21-amino acid C-terminal flanking peptide, and a larger N-terminal peptide. Calcitonin gene-related peptide (CGRP)α is also encoded by the calcitonin gene and is produced as a result of differential RNA processing (Amara et al, 1982b). The differential production of CGRP and calcitonin from the calcitonin gene is regulated in a tissue-specific manner, with CGRP being produced in nervous tissue and calcitonin in thyroid C-cells. However, both CGRP and calcitonin is found in normal, hyperplastic and neoplastic C-cells in man, although the immunohistochemical pattern of localization is different for individual antigens.

Applications

Antibodies to calcitonin are useful to identify normal, hyperplastic and neoplastic C-cells. Medullary thyroid carcinoma (MTC) occurs in both a sporadic and inherited form, with a biological behavior between anaplastic and differentiated thyroid carcinomas. Given the morphologic heterogeneity of MTC, both in histological structure (solid, trabecular or insular) and cellular patterns (spindle, polyhedral, angular or round), as well as the description of papillary, follicular, clear cell and anaplastic variants (Schr鯨der et al, 1988), the role of antibodies to calcitonin becomes crucial in making the correct diagnosis. All MTC in a series of 60 (Schr鯨der et al, 1988) and 25 (Sikri et al, 1985) cases demonstrated immunoreaction with antibodies to calcitonin. It has also been suggested that calcitonin-rich tumors appeared to have a better prognosis than calcitonin-poor neoplasms (Saad et al, 1984). However, subsequent studies were at variance with these observations (Schr鯨der et al, 1988). Studies have also shown sporadic MTC to be a more life-threatening neoplasm than MTC occurring in the setting of MFN II a syndrome, whilst MEN IIb syndrome was most aggressive.

Finally, antibodies to calcitonin are useful to identify the concept of C-cell hyperplasia in benign and malignant thyroid glands (Santeusanio et al, 1997).

Comments

Antibody to calcitonin is a compulsory addition to any immunohistochemical

histopathology laboratory for the diagnosis of MTC. Normal parafollicular C-cells are suitable as positive control tissue.

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Calretinin

Sources/Clones

Polyclonal antibody, 7696 antiserum raised against human recombinant calretinin (Swart, Bellinzona, Switzerland) and Chemicon (AB149).

Fixation/Preparation

These antibodies are applicable to formalin-fixed paraffin sections. Pretreatment of tissue sections with citrate buffer in a microwave oven or with 0.01% pronase in phosphate-buffered saline at room temperature increases calretinin immunoreactivity (Doglioni et al, 1996).

Background

7696 antiserum was raised against human recombinant calretinin (Schwaller et al, 1993) whilst AB149 antiserum was raised against guinea pig calretinin. Calretinin is a calcium-binding protein of 29 kD. It is a member of the large family of EF-hand proteins, to which the S-100 protein also belongs. EF-hand proteins are characterized by a peculiar amino acid sequence that folds up into a helix-loop-helix that acts as a calcium-binding site. Calretinin contains six such EF-hand stretches (Rogers et al, 1990). The calretinin gene was initially isolated from a cDNA clone from the chick retina and shows 60% homology with the calbindin gene (Parmentier, 1990). It is abundantly expressed in central and peripheral neural tissues, particularly in the retina and in neurons of the sensory pathways (Schwaller et al, 1993; Andressen et al, 1993). In addition, consistent calretinin immunoreactivity is found in the normal mesothelial cell lining of all serosal membranes, eccrine glands of skin, convoluted tubules of kidney, Leydig and Sertoli cells of the testis, epithelium of rete testis, endometrium and ovarian stromal cells and adrenal cortical cells.

Applications

The polyclonal antisera to calretinin consistently immunostained mesothelial cells and malignant mesotheliomas both in routinely fixed, embedded tissue sections and in cytological preparations of serous effusions (Doglioni et al, 1996). The diagnostic sensitivity of this immunocytochemical approach reached 100%, allowing immunostaining of all 44 mesotheliomas investigated, which included five biphasic and three sarcomatoid types. The specificity of calretinin immunoreactivity was checked against 294 adenocarcinomas of different origin (19 serosal metastases and 275 primary tumors potentially capable of metastases to the serosal membranes). Only 28 cases showed focal immunoreactivity for calretinin.

Comments

It is recommended that calretinin, as a mesothelioma-binding antibody, be used in conjunction with the standard panel of antibodies used in the evaluation of malignant mesothelioma (Appendix 1.16). The further uses and limitations of calretinin await further evaluation.

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Carcinoembryonic Antigen (CEA)

Sources/Clones

Accurate (C234, 12-140-10, MIC0101), Axcel/Accurate (A5B7, polyclonal), Biodesign (ME. 104, CEJ 065, MAM6, 9207, 9201, 9203, polyclonal), Biodesign/Immunotech Inc (CEJ065), Biogenesis (6.2, 1G9/9, 10, MAC601, polyclonal), Biogenex (SP-651, TF3H8-1), Biosource, Calbiochem, Caltag Laboratories (CEA6.2), Cymbus Bioscience (85A12), Dako (11-7, polyclonal), E-Y Labs, Fitzgerald (M94129, M94130, M94131, M94132, M 2103124, M2103125, polyclonal), Harlan Sera Lab/Accurate (601), Immunotech Inc (FJ95, CEJ 065), Immunotech SA (F023C5), Novocastra (85A12, 12-140-10), Oncogene (TF3H8), Shandon Lipshaw (CEJ065), Sigma Chemical (C6G9) and Zymed (ZCEA1, COL-1).

Fixation/Preparation

This antibody can be used on formalin-fixed, paraffin-embedded tissue sections. Prolonged fixation in buffered formalin may destroy the epitope. Antibody to CEA may also be used for frozen sections. Trypsinization is essential for antigen unmasking.

Background

CEA consists of a heterogeneous family of related oncofetal glycoproteins (approximately 200 kD molecular weight) which is secreted into the glycocalyx surface of gastrointestinal cells. CEA was first described in 1965 as a specific antigen for adenocarcinoma of the colon and the digestive tract of a 2-6-month-old fetus. The monoclonal antibody to CEA was raised using tumor cells derived from a hepatic metastasis of colonic carcinoma (Rogers et al, 1976). CEA is a complex glycoprotein so even after purification, some degree of molecular heterogeneity exists (Sheibani et al, 1992). Therefore antibodies to CEA, particularly polyclonal, commonly react against a non-specific crossreactive antigen (NCA) located in normal colon and granulocytes. Because of the crossreactivity of most heterologous anti-CEA antisera with NCA, the results obtained when polyclonal anti-CEA antibody was used, have recently been questioned (Whitaker et al, 1982). The anti-NCA reactivity of anti-CEA antibody is demonstrated with positive immunoreaction in polymorphonuclear leukocytes and macrophages since the cells lack CEA antigen, but contain NCA. Therefore it is recommended that positive results obtained with a polyclonal anti-CEA antibody without preabsorption with NCA be interpreted as non-specific (Sheibani et al, 1992). Even monoclonal antibodies to CEA may crossreact with other molecules of the CEA family, including NCA (Sheibani et al, 1992). Therefore, each antibody needs to be evaluated to avoid non-specific results.

Applications

CEA is found in several adenocarcinomas, such as colon, lung, breast, stomach and pancreas. Some studies have found over 70% of adenocarcinomas from a variety of organs to be positive, with no evidence of expression of CEA by neoplastic cells in several hundred cases of malignant mesothelioma (Shebani et al, 1986, 1988). Hence, in these studies, expression of CEA by adenocarcinomas and their absence in mesothelioma represent valuable markers in the discrimination of mesotheliomas from morphologically similar adenocarcinomas involving any organ (Sheibani et al, 1992). However, it should be stressed that such results are dependent on the antibody evaluation in

independent laboratories. Another study (Stirling et al, 1990) obtained cytoplasmic or membrane-related staining in five of 45 cases of mesothelioma studied. Occasional hyaluronaterich epithelial mesotheliomas may produce false positivity with CEA, although this staining can be abolished by hyaluronidase digestion prior to immunoprocessing (Robb 1989). A study examining multiple-marker immunohistochemical phenotypes to distinguish between malignant pleural mesothelioma from pulmonary adenocarcinoma, demonstrated CEA to be the best single marker (Brown et al, 1993): positive-97% specific and sensitive for adenocarcinoma; negative-97% specific and sensitive for mesothelioma (Appendix 1.16). Polyclonal CEA is also useful for the demonstration of bile canaliculi in hepatocytes and the cells of hepatocellular carcinoma in both cytologic preparations and tissue sections (Appendix 1.8). Although the presence of bile canaliculi is specific for hepatocytes, its sensitivity is low.

Comments

The fact that no single antibody is sufficiently specific and sensitive for the distinction of mesothelioma from adenocarcinoma necessitates the use of a panel of antibodies comprising a broad-spectrum cytokeratin, monoclonal CEA, Leu M1 and BER-EP4, which allows for confident differentiation of these tumors in approximately 90% of cases (Leong & Vermin-Roberts, 1994; Attanoos et al, 1996). Colonic carcinoma is the favored positive control tissue for antibodies against CEA.

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Catenins, α, β, γ

Sources/Clones

α-catenin: Becton Dickinson (1G5) and Transduction Laboratories.

β-catenin: Transduction Laboratories and Zymed (5H10).

γ-catenin: Becton Dickinson (10C4) and Transduction Laboratories.

Fixation/Preparation

HIER is necessary for the immunoreactivity of these antibodies in fixed paraffin-embedded sections. Immunoreactivity is preserved in frozen sections and cell preparations.

Background

There is currently a great deal of interest in the adhesion molecules and their expression and localization. Cell-to-cell adhesion plays a major role not only in embryogenesis but also in the intercellular adhesion of cancer cells and hence their motility and metastasis (Jiang 1996; Ilyas & Tomlinson, 1997). The transmembrane molecule E-cadherin is considered to be one of the key molecules in the formation of the intercellular junctional complex and establishment of polarity in epithelial cells. The cytoplasmic domain of E-cadherin in adherens junctions interacts via intracellular catenins with the actin-based cytoskeleton and includes fodrin, whereas the extracellular domain is involved in homotypic cell-to-cell adhesion through the formation of a molecular zipper complex. The integrity of the E-cadherin adhesion system has been shown to be disturbed or disrupted in experimental and human carcinomas and reduced expression of E-cadherin induces dedifferentiation and invasiveness in tumor cells (Tsukita et al, 1992; Birchmeier & Behrens, 1994; Ilyas & Tomlinson, 1997).

Catenins, the α-subunit (102 kD), β-subunit (88 kD) and γ-subunit (82 kD), are a group of proteins that interact with the intercellular domain of E-cadherin, resulting in complexes of E-cadherin/β-catenin/α-catenin or E-cadherin/γ-catenin/α-Catenin (Hinck et al, 1994). a-Catenin shows sequence homology to vinculin and interacts with the actin cytoskeleton, either directly or indirectly via α-actinin; β-catenin is the vertebrate homologue of the Drosophila segment polarity gene armadillo; and γ-catenin, which is identical to plakoglobin, is also found in desmosomes (Jiang, 1996). The regions of both α- and β-catenin, located on 5q21-22 and 3p21, have been shown to be involved in the development of certain tumors (see Jiang, 1996) and reduced expressions of both α- and β-catenin have been described in various tumors including breast carcinoma (Hashizume et al, 1996). Besides adhesion functions, β-catenin binds to APC (adenomatous polyposis coli) protein, a putative tumor suppressor. APC mutation disturbs the equilibrium and levels of free β-catenin level in the cell and may have a role in tumorigenesis. β-catenin has recently been shown to have a function in signal transduction when bound with members of the Tcf-LEF family of DNA-binding proteins (Behrens et al, 1996).

Applications

Although currently not of diagnostic importance, the expression of the catenin proteins and their localization are potentially important markers to predict motility and invasiveness of epithelial neoplasms (Sormunen et al, 1998). Our studies suggest that the detachment of β-catenin from the cell membrane heralds the breakdown of the cadherin-catenin-fodrin-cytoskeletal

complex both in vitro and in vivo. The loss of cell-to-cell adhesion is concomitant with a change in cell shape, from epithelioid to fibroblastoid (Sormunen et al, 1998).

Comments

The monoclonal antibodies from Transduction Laboratories are immunoreactive in routinely fixed, paraffin-embedded tissue section but only following HIER in citrate buffer.

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Tsukita S, Nagafuchi A, Yonemura S 1992. Molecular linkage between cadherins and actin filaments in cell-cell adherens junctions. Current Opinion in Cell Biology 4:834-839.

Cathepsin D

Sources/Clones

Accurate (polyclonal), Axcel/Accurate (polyclonal), Biodesign (polyclonal), Biogenesis (C5), Biogenesis/Novocastra (C5, polyclonal), Biogenex (C5, M1G8, polyclonal), Calbiochem (polyclonal), Caltag Laboratories (NCL-CDm), Chemicon (polyclonal), Dako (polyclonal), Fitzgerald (polyclonal), Immunotech (C5), Lab Vision Corp (C5), Novocastra (polyclonal), Oncogene (OS13A) and Zymed (polyclonal).

Fixation/Preparation

The antigen is immunoreactive in fixed, paraffin-embedded tissue sections only following HIER. Staining can also be performed on frozen sections and cell preparations.

Background

The cathepsins are ubiquitous lysosomal proteases and are classified both functionally and according to their active site. Cathepsin D, cathepsin B and, to a lesser extent, other cathepsins have been described as prognostic markers in cancer. Cathepsin D is the most widely studied of the cathepsins. It is an estrogen-regulated protease. A precursor form of 52 kD is processed in lysosomes into the mature 14 kD and 34 kD forms. This enzyme is thought to have proteolytic activity, which may facilitate the spread of neoplastic cells through different mechanisms and at different levels of the metastatic cascade. Cathepsin D is thought to promote tumor cell proliferation by acting as an autocrine mitogen through the activation of latent forms of growth factors or by interacting with growth factor receptors. The enzyme has also been shown, in vitro, to degrade extracellular matrix and to activate latent precursor forms of other proteinases involved in the invasive steps of the cancer metastasis. Although its active role in promoting these processes in vivo has yet to be proven, recent clinical observations which show a positive correlation between levels of cathepsin D activity and malignant progression of some human neoplasms further support this hypothesis (Duffy 1992; Leto et al, 1992). Cathepsin B, which catalyzes the degradation of laminin, may also play a role in the rupture of the basal membrane and may be of relevance in colorectal and pancreatic cancer (Schwartz, 1995).

Immunohistochemical studies in breast cancer have found that cathepsin D expression was significantly associated with poor overall survival in node-positive (Aaltonen et al, 1995) and node-negative patients (Isola et al, 1993). Recent observations that high levels of cathepsin D activity may be observed in macrophage-like stromal cells may account for some of the previous apparently conflicting reports concerning the prognostic relevance of biochemical and immunohistochemical estimations of cathepsin D in breast cancers. Cytosol assays measured total cathepsin D levels, whereas the immunohistochemical assessment was restricted to enzyme expression within tumor cells (Razumovic et al, 1997). When stromal cathepsin D levels were taken into account in immunohistochemical studies, significant associations were found with high tumor grade, increased tendency to local recurrence, regional recurrence, poorer disease-free survival and poorer overall patient survival (O'Donoghue et al, 1995; Joensuu et al, 1995; Charpin et al, 1997).

Applications

Cathepsin D expression has been studied in a variety of other tumors including carcinomas of

the lung (Sloman et al, 1996; Higashiyama et al, 1997), stomach (Allgayer et al, 1997), uterine cervix (Kristensen et al, 1996), endometrium (Losch et al, 1996) and urinary bladder (Dickinson et al, 1995), medullary carcinoma of the thyroid (Holm et al, 1995) and colorectal cancer (Theodoropoulos et al, 1997).

Comments

Cathepsin D has recently been advocated as a marker of immature ganglion cells in suspected cases of Hirschsprung's disease, the intense granular cytoplasmic reactivity for the enzyme forming a collarette around the nucleus (Abu-Alfa et al, 1997).

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SlomanA, D'Amico F, Yousem SA 1996. Immunohistochemical markers of prolonged survival in small cell carcinoma of the lung. An immunohistochemical study. Archives of Pathology and Laboratory Medicine 120: 465-472.

Theodoropoulos GE, Panoussopoulos D, Lazaris AC, Golematis BC 1997. Evaluation of cathepsin D immunostaining in colorectal adenocarcinoma. Journal of Surgical Oncology 5: 242-248.

CD 1

Sources/Clones

Accurate (WM35-1a), Becton Dickinson (Leu5), Biodesign (ALB1, ALB2), Biogenesis (DMC1), Biogenex (T6-1a), Bioprobe, Biosource (BB5), Boehringer Mannheim (YIT6), Caltag Lab (66-11-C7, VIT66-1a), Coulter (T6), Cymbus Bioscience (CBNT6-1a), Dako (NA1/34), Immunotech (010), Oncogene, Sanbio (66-11-C7), Serotec (4A76, NAI-34-1a), Seralab (CD 1) and Immunotech (CD 1a).

Fixation/Preparation

Fresh-frozen tissues. CD 1a (clone 010) is effective in paraffin-embedded tissues, with immunoreactivity enhanced by HIER.

Background

Human CD1 genes are a family of five non-polymorphic genes that, although homologous to both class I and II major histocompatibility complex genes, map to chromosome 1. Four isoforms of the CD 1 proteins have been clustered, namely CD 1a, -b, -c and -d and are expressed on the surface of cells in association with β 2-microglobulin and may function as non-classic antigen-presenting molecules. While CD 1 genes have been found in a wide variety of vertebrates, they have shown differences in size and complexity in different mammals. Most CD 1 molecules can be separated into two groups based mainly on homology of nucleotide and amino acid sequences. Group 1 includes the human CD 1a, -b and -c proteins, which are the classic CD 1 antigens first identified on human thymocytes and now recognized on a variety of specialized Ag-presenting cells, including dendritic cells in lymphoid and non-lymphoid tissues. These proteins can also be induced in vitro on virtually all circulating human monocytes by exposure to granulocyte-macrophage-CSF, suggesting that they might be upregulated on tissue macrophages in many inflammatory lesions. The Group 2 CD 1 proteins include the human CD 1d and mouse CD 1, which so far have been found to be most prominently expressed by gastrointestinal epithelia and B lymphocytes (Boumsell, 1989).

CD 1a, -b and -c are expressed in about 70% of all thymocytes, predominantly the cortical thymocytes. CD 1 is not expressed in early thymocytes or by mature resting or activated T lymphocytes. This distribution is reflected by neoplastic populations of T cells in that precursor T-ALL/LBLs expressing cortical or immature phenotypes are CD 1+, in contrast to those with prothymocyte or medullary thymocyte phenotypes. All postthymic or T dt-negative T-cell neoplasms such as T-CLL, T-PLL, Tγ-lymphoproliferative disorder, S閃ary syndrome, cutaneous T-cell lymphoma and node-based T-cell lymphoma are consistently negative for CD 1 (Porcelli & Modlin, 1995).

Applications

CD 1a, CD 1b and CD 1c antigens are membrane glycoproteins with MWs of 49 kD, 45 kD and 43 kD, respectively. Their expression on thymocytes and also on a variety of antigen-presenting cells including Langerhans cells and interdigitating dendritic cells makes detection, particularly of CD 1a, useful in the diagnosis of Langerhans cell histiocytosis and the classification of thymomas and malignancies of T-cell precursors.

CD 1a is a specific marker for Langerhans cells (Shinzato et al, 1995). Thymic lymphocytes that are CD 1+ represent cortical thymocytes.

Comments

Antibodies to CD 1a are useful in diagnosis of Langerhans cell histiocytosis, in the classification of thymomas and malignancies of T-cell precursors. While most of the antibodies available are only reactive in fresh-frozen tissues, a recently developed antibody to CD 1a, clone 010, is reactive in paraffin sections following heat-induced epitope retrieval and is available through Immunotech (Krenacs et al, 1993).

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Shinzato M, Shamoto M, Hosokawa S et al 1995. Differentiation of Langerhans cells from interdigitating cells using CD1a and S-100 protein antibodies. Biotechnology and Histochemistry 70: 114-118.

CD 2

Sources/Clones

Accurate (CLBT11, F923A11, MEM65, MT910), Ancell (1E7E8), Becton Dickinson (Leu 5, S5.2), Biodesign, Bioprobe/Tha, Biosources (BH1), Boehringer Mannheim (MT26), Caltag Labs (G11), Coulter (T11, 39C15), Cymbus Bioscience (GJ12), Dako (MT910), Exalpha (T6.3), Immunotech (39C1.5), Novocastra (X1X8), Ortho (OKT2), Pharmingen (RPA2.10), Sanbio (MEM65), Serotec and Zymed (RPA2.10).

Fixation/Preparation

Fresh-frozen tissue, fresh air-dried cell preparations.

Background

Human T lymphocytes were initially distinguished from B lymphocytes by their ability to produce spontaneous rosettes with sheep red blood cells, a phenomenon mediated by the CD 2 molecule, a glycosylated transmembrane receptor molecule also referred to as T11 antigen or LFA-3 antigen (leukocyte function associated antigen-3). Three functionally important epitope groups have been defined on the human CD 2 molecule, designated $T11_1$, $T11_2$ and $T11_3$ (CD 2R). $T11_1$ is the epitope responsible for E-rosetting and T-cell stimulation through this epitope is mediated by an IL-2-dependent pathway. Stimulation of the $T11_2$ and $T11_3$ epitopes occurs via an alternative pathway (Meuer et al, 1984; Knowles, 1985).

CD 2 is one of the earliest T-cell lineage-restricted antigens to appear during T-cell differentiation and only rare CD 2+ cells can be found in the bone marrow. It is found in all T lymphocytes and natural killer cells but not in B cells or any other cell population. CD 2 binds to its counter receptor CD-58 (LFA-3), a member of the Ig gene superfamily, which locates on the surface of target cells. CD 2 binding to LFA-3 activates T cells and may also have a role in prothymocyte homing as it is known to mediate thymocyte-thymic epithelium adhesion. Although it is known that CD 2 appears after CD 7 but before CD 1, its temporal relationship with CD 3 is less definite, with some recent evidence suggesting that CD 3 appears in the cytoplasm before CD 2 (Osborn et al, 1995).

Applications

CD 2 can be considered a pan-T cell antigen and is therefore useful for the identification of virtually all normal T lymphocytes. It is also very useful in the assessment of lymphoid malignancies as it is expressed in the majority of precursor and postthymic lymphomas and leukemias and is not expressed by B neoplasms (Foon & Todd, 1986). As with other pan-T-cell antigens, CD 2 may be aberrantly deleted in some neoplastic T cell populations, especially peripheral T-cell lymphomas. Rarely, sIg+ B cell neoplasms have been described to form spontaneous E-rosettes but these reactions are not mediated via the CD 2 receptor (Knowles, 1989).

Comments

CD 2 antibodies can be used for identification of lymphomas and leukemias of T-cell origin. Currently, the monoclonal antibodies available are reactive only in fresh-frozen tissue and not in fixed paraffin-embedded sections.

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Osborn L, Day ES, Miller GT et al 1995. Amino acid residues required for binding of lymphocyte function-associated antigen 3 (CD58) to its counter-receptor CD2. Journal of Experimental Medicine 181: 429-434.

CD 3

Sources/Clones

Accurate (CLBT3, T3, UCHT1), Ancell, Becton Dickinson (Leu 4), Biodesign, Biogenex (CD 3, 12F6), Bioprobe, Biosource (BB11), Boehringer Mannheim (4B5), Coulter (T3, CD 3), Cymbus Bioscience, Dako (T3-4B5, UCHT1), Exalpha (M2AB), Gen Trak, Immunotech (SPV-T3b), Novocastra (UCHT1) Ortho (OKT3), Pharmingen (HIT3A), Sanbio (MEM57), Serotec, Zymed (SPV-T36) and polyclonal CD 3 antisera from Dako, Serotec and Bioprobe/Tha.

Fixation/Preparation

The monoclonal antibodies are immunoreactive only in fresh-frozen section and cell preparations, whereas polyclonal antisera will react in fixed paraffin-embedded tissue, but only following HIER or prolonged enzyme digestion.

Background

The CD 3 antigen consists of at least four structurally distinct membrane glycoproteins of molecular weight 20-28 kD. This complex, comprising extracellular, transmembrane and intracellular domains, is non-covalently associated with the polymorphic TCRα/β or, alternatively, the TCRγ/δ heterodimer. Stimulation of the CD 3 complex results in T-cell proliferation, release of cytokines and display of non-specific cytotoxicity, properties requiring the participation of accessory cells. It is believed that the CD 3 complex is responsible for mediating signal transduction to the internal environment upon antigenic recognition by the TCR, although the actual mechanisms of T-cell activation following antigen binding to the TCR are not known (Campana et al, 1987).

CD 3 is present in the cytoplasm prior to its detection on the cell surface of thymocytes and more than 95% of thymocytes bear surface and/or cytoplasmic CD 3. The antigen is one of the earliest to be expressed in T-cell differentiation and begins during the prothymocyte stage prior to entrance into the thymus. It is a T cell-specific surface marker normally present in resting and activated T lymphocytes. Cytoplasmic CD 3 expression is lost as common thymocytes differentiate into medullary thymocytes and the antigen is found only on the cell surface in postcortical T-cells but not in B cells, monocytes/macrophages, myeloid cells or any other cell type except for weak expression in Purkinje cells of the cerebellum. The polyclonal anti-CD 3 may produce weak staining of squamous epithelium and Hassal's corpuscles in the thymus but this lacks the distinct membrane ring-like pattern seen in T-cells and may represent weak cross-reactivity (Campana et al, 1989).

Applications

CD 3 is therefore a useful marker to distinguish precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma from their B cell counterparts and acute myeloid leukemia (Chetty & Gatter, 1994). CD 3 is a pan-T-cell lineage-restricted antigen, which is useful for labeling both neoplastic and non-neoplastic T-cells and surface CD 3 is expressed by all categories of postthymic T-cell lymphomas as well as lymphoblastic lymphoma but not lymphomas of B cell lineage. CD 3 may be aberrantly deleted in some peripheral T-cell lymphomas (Van Dongen et al, 1988; Cabecadas & Isaacson, 1991).

Comments

The appropriate antibody should be used for cytocentrifuge

preparations as MoAb OKT3 (Ortho) detects surface CD 3 but not the cytoplasmic antigen in cytocentrifuge preparations. MoAbs Leu 4 (Becton Dickinson) and UCHT1 (Ancell, Immunotech, Sera Lab, Biodesign) detect cytoplasmic CD 3 in cytocentrifuge preparations and many other antibodies are not reactive at all in such preparations. The polyclonal CD 3 antibody is a useful reagent for paraffin-embedded sections as well as cytocentrifuge smears, especially following heat-induced epitope retrieval. It is reactive against both normal and neoplastic T-cells. CD 3 is absent in a subpopulation of T-cell neoplasms including cases of mycosis fungoides, pleomorphic small cell lymphoma, pleomorphic medium and large cell lymphoma and anaplastic large cell lymphoma. This may reflect aberrant gene expression by the malignant T-cells with loss of the antigen at the outset; alternatively, deletion may occur during the process of large cell transformation as seen in anaplastic large cell lymphoma (Picker et al, 1987).

A recently developed monoclonal anti-CD 3 clone (NCL-CD3-PS1) generated to a recombinant fusion protein representing theε subunit of the CD 3 molecule is reactive in paraffin-embedded sections and promises to be very useful but is currently not commercially available (Steward et al, 1997).

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Picker LJ, Weiss LM, Medeiros JL et al 1987. Immunophenotypic criteria for the diagnosis of non-Hodgkin's lymphoma. American Journal of Pathology 128: 181-201.

Steward M, Bishop R, Piggott NH et al 1997. Production and characterization of a new monoclonal antibody effective in recognizing the CD3 T-cell associated antigen in formalin-fixed embedded tissue. Histopathology 30: 16-22.

Van Dongen JJM, Krissansen GE, Wolvers-Tettero ILM et al 1988. Cytoplasmic expression of the CD3 antigen as a diagnostic marker for immature T-cell malignancies. Blood 71: 603-612.

CD 4

Sources/Clones

Ancell (QS4120), Becton Dickinson (Leu3), Biodesign (13B8.2, BL4), Biogenesis (T138A), Biotest (T4, TT1), Coulter (T4), Cymbus Bioscience (MEM115), Dako (MT310), GenTrak, Immunotech (BL4, 13B8.2), Oncogene (VIT4), Sanbio (BL-TH4), MEM115), Seralab, Serotec (B-A1, B-F5, B-B14, 13B8.2) and Pharmingen (RM-4-4, RM-4-5).

Fixation/Preparation

Fresh-frozen tissue and cell preparations.

Background

After the discovery that lymphocytes could be divided into B cells and T-cells, discrete subsets of T-cells which function as helper, suppressor and cytotoxic cells were recognized. The CD 4 molecule is a non-polymorphic glycoprotein belonging to the Ig gene superfamily that is expressed on the surface membrane of functionally distinct subpopulation of T cells, mutually exclusive of the CD 8 molecule (Maddon et al, 1985). The CD4 molecule is a 55 kD glycoprotein with five external domains, each homologous to an Ig light chain-variable region, a transmembrane domain and a highly conserved intracellular domain. The CD 4 gene has been mapped to the short arm of chromosome 12 (Isobe et al, 1986; Brady & Barclay, 1996).

The CD 4 molecule acts as a coreceptor with the TCR complex and appears to bind to the non-polymorphic region of the MHC class II molecule and may serve to increase the avidity of cell-to-cell interactions. It also serves as a receptor for the human immunodeficiency virus on T-cells, monocytes/macrophages and in some neural cells (Dalgleish et al, 1984; Doyle & Strominger, 1987).

The CD 4 antigen, like CD 8, appears at the common thymocyte stage of T-cell differentiation and is expressed in about 80-90% of normal thymocytes. CD 4 thus marks helper/inducer T-cells and is expressed in 55-65% of mature peripheral T-cells. It should be noted that the phenotype-functional association of CD 4 to helper and CD 8 to suppressor/cytotoxic function is not universal. Subpopulations of suppressor or cytotoxic T cells can be identified among CD 4+ T-cells. Although also expressed on monocytes/macrophages, Langerhans cells and other dendritic cells, CD 4 is not expressed by B cells.

Applications

The CD 4 antibody is useful for the identification of T helper/inducer cells and plays an important role in the immunophenotyping of reactive lymphocytes and in lymphoproliferative disorders. The majority of peripheral T-cell lymphomas are derived from the helper T-cell subset so that most postthymic T-cell neoplasms are CD 4+CD 8-. Tγ-lymphoproliferative disease is an exception where the proliferative cells are CD 4-CD 8+. As with other T-cell antigens, CD 4 may be aberrantly deleted in neoplastic T-cells so that the evaluation of such tumors requires the application of a panel of markers in order to identify tumors with such anomalous antigenic expression.

Comments

Current anti-CD 4 antibodies are immunoreactive only in fresh-frozen tissue sections and fresh cytologic preparations. In the latter preparations, fixation in 10% buffered formalin or in 0.1%

formal saline produces consistent immunostaining especially if heat-induced epitope retrieval is employed. There is satisfactory staining of reactive T-cells in paraffin-embedded sections following heat-induced epitope retrieval but the staining is weak in neoplastic T-cells. Many phagocytic histiocytes and dendritic cells are also CD 4+, making interpretation of frozen section staining difficult.

OPD4 (CD45RA) was initially claimed to be specific for CD 4+ T-cells, but this has not been proven to be so and OPD4 labels both CD 4+ and CD 8+ cells.

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Isobe M, Huebner K, Maddon PJ et al 1986. The gene encoding the T cell surface protein T4 is located on human chromosome 12. Proceedings of the National Academy of Science USA 83: 4399-4402.

Maddon PJ, Littman DR, Godfrey M et al 1985. The isolation and nucleotide sequence of a cDNA encoding the T cell surface protein T4: a new member of the immunoglobulin gene family. Cell 42: 93-104.

CD 5

Sources/Clones

Ancell (UCHT2), Becton Dickinson (Leu1), Biodesign (BL1a, UCHT2), Biogenex (T1), Bioprobe (T1), Coulter (T1), Cymbus Bioscience (UCHT2), Dako (DK23), GenTrak, Immunotech (BL1a), Oncogene (UCHT2), Sanbio (BL-TP), Seralab (UCHT2), Serotec, Sigma and Pharmingen (UCHT2).

Fixation/Preparation

Fresh or frozen tissue.

Background

The CD 5 molecule is a transmembrane glycoprotein of 67 kD, with the typical tripartite structure of a signal peptide. The human CD 5 has a sequence similar to that of the Ly-1 antigen in mouse and both are distantly related members of the immunoglobulin superfamily of genes. CD 5 is expressed on both T and some B lymphocytes. It is weakly positive in the most immature T-cell precursors which are CD 34+, with the intensity of expression increasing with maturation. CD 5 expression is first seen in intrathymic T-cell progenitors (CD 5+/CD 34+) which differentiate into CD 3+/CD 4+/CD 8+ T-cells. This antigen is expressed in the majority of T-cells with only about 11% of CD 4+ lymphocytes being CD 5-. Two-thirds of these CD5-negative cells are $\alpha\beta$ T-cell receptor-positive cells and one-third are $\gamma\delta$ T-cells. Anti-CD 5 antibodies have been shown to prolong the proliferative response of anti-CD 3 activated T lymphocytes by enhancing signal transduction by the T-cell receptor antigen, a process associated with increased IL-2 production and increased IL-2 receptor expression by the T-cells. The CD 5 antigen may also act as a signal-transducing molecule in a manner independent of CD 3. It has also been suggested that the B-cell surface protein CD 72 (Lyb-2) is the ligand or counterstructure for CD 5 and occupancy of CD 72 by anti-CD 72 antibodies, and possibly CD 5+ T-cells, enhances IL-4-dependent CD 23 expression on resting B- ymphocytes.

When CD 5 is expressed on B lymphocytes, it is usually weakly staining compared to the strong expression of mature T lymphocytes. This weak expression makes precise identification of the CD 5+ and CD 5- B-cell populations difficult. CD 5+ B cells (B-1 cells) are first seen in the peritoneal and pleural cavities of the fetus at gestation week 15. The cells become prominent in the fetal spleen with 60% or more of splenic B cells expressing the antigen. At birth, about 68% of cord blood B cells and approximately half of the peripheral blood B lymphocytes are CD 5+ and this level drops dramatically in the peripheral blood to near adult levels within the first year of life. Fifteen to twenty-five percent of peripheral blood B lymphocytes in adults are positive for CD 5.

There is some suggestion that CD 5+ B lymphocytes represent a distinct subpopulation. Although both CD 5+ and CD 5- B cells produce immunoglobulin, upon activation CD 5+ cells selectively produce primarily IgM antibodies, while CD 5- B cells make primarily IgG antibodies, an observation made in cord blood. CD 5+ B cells have also been reported to be associated with usually low affinity, polyreactive antibody production, often called autoantibodies. About 50% of autoantibody-associated crossreactive idiotype-bearing B lymphocytes are CD 5+. It is possible that some of these differences may be due to lineage differences or are simply secondary to some type of B-cell activation and require further investigation (Arber & Weiss, 1995).

Applications

CD 5 is a fairly specific and sensitive marker of T-cell lineage. Almost 85% of T-cell acute lymphoblastic leukemias are CD 5+ and lack of CD 5 expression in T-ALL in patients with a white cell count of less than 50 000/ml is reported to be associated with a worse prognosis than corresponding patients with CD 5+ T-cells. CD 5 expression has been reported in 3-10% of cases of acute myeloid leukemia. As CD 5 is a pan-T-cell marker, it is not surprising that the majority of T-cell malignancies (76%) are CD 5+ (Shuster et al, 1990). In peripheral T-cell lymphomas including cutaneous T-cell lymphomas, the loss of CD 5 expression can be employed to support a diagnosis of malignancy. In cutaneous T-cell lymphoma, CD 5 is not as frequently lost when compared to loss of CD 7.

With B-cell neoplasms, CD 5 expression has been considered an almost defining characteristic of many entities. Chronic lymphocytic leukemia (CLL) is the most common CD 5+ B cell malignancy. It is assumed that the small population of CD 5+ B cells found in normal healthy adults and prominent in cord blood is the non-neoplastic counterpart of this type of CLL. B-cell CLL is also associated with polyspecific antibodies or autoantibodies and frequently expresses crossreactive idiotypes. Over 90% of cases of typical CLL are CD 5+. CD 5 expression may be lost when the large cell lymphoma of Richter's syndrome supervenes in CD 5+ CLL (Matutes & Catovsky, 1991).

With rare exceptions, monocytoid B-cell lymphoma and low-grade B-cell lymphoma of mucosa-associated lymphoid tissue are usually CD 5-, a feature which can be employed to distinguish these disorders. CD 5+ B-cells have been reported to be increased in some patients with monoclonal gammopathy of undetermined significance and in cases of multiple myeloma.

CD 5 positivity has been found in cases of thymic carcinomas and some cases of atypical thymomas but not in typical thymomas (Hishima et al, 1994). Carcinomas of the lung, breast, esophagus, stomach, colon and uterine cervix have been reported to be all CD 5-.

Comments

We employ clone Leu1 in our laboratory and although most publications indicate that the CD 5 antigen is only demonstrable in fresh and frozen tissues, we have successfully demonstrated CD 5 using Leu1 antibody following MW epitope retrieval with TUR (Leong et al, 1996). A recently produced antibody to CD5 (clone NCL-CD5, Vector Laboratories) was claimed to be immunoreactive in paraffin-embedded tissues following steam heat-induced epitope retrieval but in a study of 12 CD 5+ malignancies, only one, a small cell lymphoma, was positive in fixed tissues (Ben-Ezra & Kornstein, 1996).

References

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CD 7

Sources/Clones

Becton Dickinson (Leu 9), Biodesign (WT1, WM31, 8H8.1), Biogenesis (WM31), Coulter (3A1), Cymbus Bioscience (WM31), Dako (DK24), GenTrak, Immunotech, Oncogene U3A1E), Sanbio (WT1), Seralab and Serotec (B-F12, B-5, HNE51).

Fixation/Preparation

Fresh frozen-tissue and fresh cytologic preparations.

Background

CD 7 antigen is a cell surface glycoprotein of 40 kD expressed on the surface of immature and mature T-cells and natural killer cells. It is a member of the immunoglobulin gene superfamily and is the first T-cell lineage-associated antigen to appear in T-cell ontogeny, being expressed in prethymic T-cell precursors (preceding CD 2 expression) and in myeloid precursors in fetal liver and bone marrow and persisting in circulating T-cells. While its precise function is not known, there is a recent suggestion that the molecule functions as an Fc receptor for IgM (Lazarovits et al, 1994).

Applications

CD 7 is the most consistently expressed T-cell antigen in lymphoblastic lymphomas and leukemias and is specific for T-cell lineage and is therefore a useful marker in the identification of such neoplastic proliferations. In mature postthymic T-cell neoplasms, it is the most common pan-T antigen to be aberrantly absent and its absence in a T-cell population is a useful pointer to a neoplastic conversion.

Comments

Current antibodies are not immunoreactive in fixed tissues.

References

Lazarovits AI, Osman N, Le Feuvre CE et al 1994. CD7 is associated with CD3 and CD 45 in human T cells. Journal of Immunology 153: 3956-3966.

CD 8

Sources/Clones

Accurate, Ancell (UCHT4), Becton Dickinson (Leu2), Biodesign (UCHT4, CD8.C12, B9.11, B9.2), Biogenex (T8), Biotest (Tu102), Biogenesis (T80C), Caltag, Coulter (T8), Cymbus Bioscience (UCHT4), Dako (DK25, C8/144B), Exalpha, GenTrak, Immunotech (B9.2, B9.11), Oncogene (UCHT4), Pharmingen (RPA-Y8), Research Diagnostics (CLB-T8/4, UCHT4), Sanbio (MEM31, BLT58/2), Seralab (UCHT4) and Serotec (BHT, MF8).

Fixation/Preparation

Fresh-frozen section and fresh cytological preparations. Clone C8/144B is also immunoreactive in fixed paraffin-embedded tissue sections following HIER.

Background

Like CD 4, the CD 8 molecule is composed of non-polymorphic glycoproteins, belonging to the Ig superfamily, that are expressed on the surface membrane of mutually exclusive, functionally distinct T-cell populations. The CD 8 molecule is a 34 kD glycoprotein that forms disulfide-linked homodimers and homomultimers on the cell surface of peripheral T-cells, the CD 8 gene being linked to the κ locus on chromosome 2. The CD 8 molecule comprises an external domain and highly conserved transmembrane and intracellular domains, the external domain showing striking homology with other members of the Ig gene superfamily (Eichmann et al, 1989). The CD 8 molecule functions as a TCR coreceptor on suppressor/cytotoxic T-cells and recognizes foreign antigens as peptides presented by MHC class I molecules. In the thymus, the CD 8 molecule forms complexes with the CD 1 glycoprotein, an MHC class I-like molecule. CD 8 appears to bind to the non-polymorphic regions of MHC class I molecules and may thus serve to enhance the avidity of cell-to-cell interactions (Christinck et al, 1991). Both CD 4 and CD 8 antigens appear during the common thymocyte stage of T-cell differentiation and CD 8 is expressed by about 80% of normal thymocytes. Thereafter, CD 4 and CD 8 are retained by those maturing thymocytes destined to become helper/inducer and suppressor/cytotoxic T-cells respectively, CD 8 being expressed by about 25-35% of peripheral T-cells, specifically of the suppressor/cytotoxic subset (Martz et al, 1982). In addition, about 30% of NK cells express low levels of CD 8. This phenotypic-functional association is not universal and subpopulations of suppressor/cytotoxic T cells can be identified among CD 4+ cells (Parnes, 1989).

Applications

As with the CD 4 marker, CD 8 has an important role in the immunophenotypic analysis of reactive and neoplastic populations of T-cells, being used to identify a mature T-cell subset with suppressor/cytotoxic function. Like the CD 4 marker, CD 8 may also be aberrantly deleted from neoplastic T-cells. It is expressed on T-cell lymphoblastic lymphomas (Picker et al, 1987).

Comments

With the exception of clone C8/144B, most anti-CD8 antibodies are only immunoreactive in fresh-frozen sections and cytologic preparations.

References

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Martz E, Davignon D, Kurzinger K, Springer TA 1982. The molecular basis for cytotoxic T lymphocyte function: analysis with blocking monoclonal antibodies. Advances in Experimental Medicine and Biology 146: 447-465.

Parnes JR 1989. Molecular biology and function of CD4 and CD8. Advances in Immunology 44: 265-311.

Picker LJ, Weiss LM, Medeiros JL et al 1987. Immunophenotypic criteria for the diagnosis of non-Hodgkin's lymphoma. American Journal of Pathology 128: 181-201.

CD 9

Sources/Clones

Biodesign (ALB6, MM2/57), Cymbus Bioscience (MM2/57), GenTrak, Immunotech (ALB6), Research Diagnostics (MM2/57), Sanbio (CLB/CD9), Seralab (FMC56, FMC8) and Serotec (MM 2/57).

Fixation/Preparation

Fresh-frozen tissue and cytologic preparations and formalin-fixed paraffin-embedded tissue.

Background

The CD 9 antigen is a cell surface glycoprotein (p24) of molecular weight 24 kD belonging to the tetra-membrane-spanning protein family, coded by chromosome 12. The antigen is present on pre-B cells, monocytes and platelets and has protein kinase activity. The majority of mature peripheral blood or lymphoid tissue B cells or other normal circulating hematopoietic cells other than platelets do not express it. It is present on activated T-cells, mast cells and some dendritic reticulum cells (Kersey et al, 1981; Ash et al, 1982; Carbone et al, 1987).

Applications

The expression of CD 9 in malignant cells is complex and not strictly lineage, activation or differentiation associated. It is found in >75% of precursor B cell ALL/LBL, about 50% of B cell CLL and some better differentiated B-cell neoplasms such as prolymphocytic leukemia and multiple myeloma as well as some T-cell lymphomas, and acute myeloid leukemias. Other B cell lymphomas including centrocytic lymphoma, follicle center cell lymphoma and Burkitt's lymphoma, may also express this antigen and there is also variable expression on neuroblastomas and some epithelial tumors (Komada et al, 1983; San Miguel et al, 1986; Lardelli et al, 1990).

Comments

Because of its expression by a wide spectrum of B- and T-cell neoplasms and non-lymphoid tumors, this marker has limited application in the phenotypic analysis of hematopoietic neoplasms.

References

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Pathology 14: 752-763.

San Miguel JF, Caballero MD, Gonzalez M et al 1986. Immunological phenotype of neoplasms involving the B cell in the last step of differentiation. British Journal of Haematology 62: 75-83.

CD 10 (CALLA)

Sources/Clones

Accurate, Ancell, Biodesign (ALB1, ALB2), Coulter (J5), Cymbus Bioscience (Mem 78), Dako (SS2/36), GenTrak, Immunotech (ALB1, ALB2), Research Diagnostics (MEM 78, J-149), Sanbio (MEM 78, BFA.11), Seralab (B-E3) and Serotec.

Fixation/Preparation

Current antibodies are immunoreactive only in fresh-frozen tissue.

Background

The common acute lymphoblastic leukemia antigen (CALLA) is a 100 kD single-chain glycoprotein whose sequence is virtually identical to that of neutral endopeptidase (NEP-24.11 enkephalinase). It is a metalloenzyme that requires zinc as a cofactor and is thought to inactivate regulatory peptides favoring cell differentiation. It was originally defined by hetero-antiserum raised in rabbits by immunization with cells of a "non-B, non-T" cell acute lymphoblastic leukemia. CD 10 is present on the cell surface of stem cells in the bone marrow and fetal liver that are also TdT and HLADR antigen positive (Anderson et al, 1984; Letarte et al, 1988).

Applications

CD 10 was originally used as a specific marker for non-B, non-T cell ALL. It is expressed in approximately 75% of precursor B-cell ALL and more than 90% of cases of myelogenous leukemia in lymphoid blast crisis, but it is not a leukemia-specific antigen nor is it B- or T-cell lineage restricted (Carrel et al, 1983). The antigen is found on variable proportions of cells making up T-cell ALL/LBL, Burkitt's lymphoma, follicular lymphoma and multiple myeloma (Durie & Grogan, 1985; Ruiz-Arguelles & San Miguel, 1994). In addition, CD 10 is expressed on the renal glomerular and tubular cells, fibroblasts, bile canaliculi, melanoma cell lines and various other epithelial cells.

Comments

While CD 10 is neither lineage specific nor tumor restricted, it remains a useful marker, especially in the analysis of childhood ALL/LBL and follicular lymphomas (Greeves et al, 1975, 1983).

References

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Ruiz-Arguelles GJ, San Miguel JF 1994. Cell surface markers in multiple myeloma. Mayo Clinic Proceedings 69: 684-690.

CD 103

Sources/Clones

Biogenex (2G5), Coulter (2G5), Dako (Ber-ACT8), Immunotech (2G5) and Serotec (295.1).

Fixation/Preparation

The antibodies are mainly immunoreactive in cryostat sections of fresh-frozen tissue. Immunoreactivity in fixed paraffin-embedded sections has not been reported.

Background

The antibody to CD 103, also known as antihuman mucosal lymphocyte 1 antigen (HML-1), recognizes a T-cell-associated trimeric protein of 150, 125 and 105 kD (Falini et al, 1991), which is expressed on 95% of intraepithelial lymphocytes and only on 1-2% of peripheral blood lymphocytes (Spencer et al, 1988; Kruschwitz et al, 1991). CD 103 (α E-integrin) antigen is part of the family of β 7 integrins on human mucosal lymphocytes which play a specific role in mucosal localization or adhesion (Parker et al, 1992). CD 103 is a receptor for the epithelial cell-specific ligand E-cadherin and is expressed by a major subset of CD 3+, CD 8+, CD 4- lymphocytes present in the intestinal mucosa. About 40% of isolated intestinal lamina propria lymphocytes (LPL) expressed HML-1, the majority being CD 8+.Virtually all LPL expressed CD 45RO whereas only about 50% were CD 29+, a percentage similar to that in peripheral blood lymphocytes. HML-1 + cells were almost exclusively CD 45RA- and the in vitro expression of HML-1 was inducible on T-cells by mitogen (Schieferdecker et al, 1990).

Applications

Antibodies to CD 103 are used for the diagnosis of intestinal T-cell lymphoma (Schmitt-Graff et al, 1996). CD 103 has been found to be a useful marker of B-cell hairy cell leukemia which shows strong reactivity for CD 22, CD 25, CD 103, DBA.44 as well as immunoglobulin light-chain restriction (Harris et al, 1994; Cordone et al, 1995). The antigen may be occasionally expressed by some B-cell lymphomas (Moller et al, 1990) and has also been demonstrated in T-lymphoblastic lymphoma (Falini et al, 1991).

Comments

Current diagnostic applications of antibodies to CD 103 are restricted by their immunoreactivity only in fresh cell preparations and cryostat sections.

References

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CD 11

Sources/Clones

CD 11a

Ancell (38), Biodesign (MEM 25, SPV-L7), Cymbus Bioscience (38), Dako (MHM24), Gen Trak, Immunotech (25.3), Sanbio (MEM 25), Serotec (B-B15) and Pharmingen (2D7).

CD 11b

Ancell (44), Biodesign (44, Bear-1), Biogenex, Boehringer Mannheim, Cymbus Bioscience, Dako (2LPM19c), GenTrak, Immunotech (Bear-1), Japan Tanner, Research Diagnostics (CD 44), Sanbio (Bear-1), Seralab (44), Serotec (ED7) and Pharmingen (M1/70).

CD 11c

Ancell (3.9), Becton Dickinson (Leu M5), Biodesign (FK24, BU15), Cymbus Bioscience, Dako (KB90), Gen Trak, Immunotech (BU15), Oncogene (3.9), Research Diagnostics (CD39), Sanbio (FK24), Seralab (Fk24) and Serotec (3.9).

Fixation/Preparation

Current antibodies are only immunoreactive in fresh-frozen tissue.

Background

Each of the CD11 subtypes represents a different β chain which forms one of the β2 family of integrin adhesion receptors when linked non-covalently to β2 (CD 18) to form a heterodimer. CD 11a, leukocyte function-associated protein (LFA-1) with a molecular weight of 180 kD, is present on B cells, T cells, NK cells, monocytes, granulocytes, megakaryocytes and activated platelets. CD 11b (Mac-1), the C3bi receptor, has a molecular weight of 165 kD and it is present on granulocytes, monocytes and some histiocytes. CD 11c, which has a molecular weight of 150 kD, is present on monocytes, tissue macrophages, granulocytes, some suppressor/cytotoxic T cells and a subset of B cells. It is usually positive on true histiocytic malignancies and some B-cell lymphomas including hairy cell leukemia and monocytoid B-cell lymphoma (Chadburn et al, 1990).

CD 11/CD 18 integrins have a function in intercellular communication between lymphocytes and between lymphocytes and endothelial cells. The interaction between leukocytes and endothelial cells involves CD 11/CD 18 integrins which bind to intercellular adhesion molecules ICAM-1 (CD 45) and ICAM-2 (Albelda et al, 1994).

Applications

Currently, the diagnostic applications for this marker are very limited and available antibodies are reactive only in frozen sections. Differential expression of CD 11a (LFA-1) has been described in small cell lymphocytic lymphoma and CLL and has been used to account for the difference in peripheral blood involvement in these entities but the findings require confirmation. In the immunophenotypic separation of monocytoid B-cell lymphoma from other small cell lymphomas such as plasmacytoid small cell lymphoma, CLL and mantle cell lymphoma, CD 11c has been suggested to be a useful discriminant, being more frequently expressed in monocytoid lymphoma.

References

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Chadburn A, Inghirami G, Knowles DM 1990. Hairy cell leukemia-associated antigen Leu M5 (CD 11c) is preferentially expressed by benign activated and neoplastic CD8 cells. American Journal of Pathology 136:29-37.

CD 15

Sources/Clones

Accurate (C3D-1), Becton Dickinson (Leu M1), Biodesign (B428, 80H5, G15), Biogenex (Tu9), Cymbus Bioscience (28), Dako (C3D-1), Immunotech (80H5), Novocastra, Research Diagnostics (28), Sanbio (BL-G15), Seralab (MC-1) and Serotec (NH6, B-H8).

Fixation/Preparation

Fresh-frozen tissue and formalin-fixed, paraffin-embedded tissue. Muramidase pretreatment increases reactivity, particularly in acute myeloid leukemia.

Background

A variety of antibodies to CD 15 have been generated in different ways but appear to have similar immunoreactivity patterns. Some antibodies were developed by immunization and screening against human hematopoietic cell lines and were originally felt to be specific for myeloid leukemias, while other antibodies were developed from specific human and mouse carcinoma cell lines and were later found to react with granulocytes and a variety of human carcinomas. The antibodies are mostly of IgM isotype and have the common property of being able to recognize a specific sugar sequence that occurs in the glycolipid lacto-N-fucopentaose III ceramide and is also found in several glycolipids such as glycoproteins. The sugar sequence is referred to as X hapten or Lĕ and its highly immunogenic nature in mice has led to the production of several IgM monoclonal antibodies to the CD 15 cluster. The lacto-N-fucopentaose III has been identified in human milk and is virtually absent in benign human epithelium. A related substance lacto-N-fucopentaose II, is present in many benign human epithelial cells. The glycolipid, lacto-N-fucopentaose III has a structure similar to the Lewis blood group antigens. The CD 15 antigen exists in sialylated or unsialylated form, the former requiring prior digestion with muramidase to enable detection. Mature granulocytes and monocytes express the unsialylated molecule (Arber & Weiss, 1993).

Applications

CD 15 antibodies react with mature neutrophils; generally the reactivity is less with the less mature forms of the granulocyte series. Normal bone marrow myeloblasts are negative and some promyelocytes may not stain. Paraffin-embedded cells show both membrane and cytoplasmic staining. Normal platelets, red blood cells and B lymphocytes are routinely negative as are the vast majority of T lymphocytes. Mitogen-activated lymphocytes show positivity with the Leu M1 antibody and these are mostly T lymphocytes of the T4 subset. While some T8+ cells also express the antigen, a longer period of stimulation was needed to induce this finding.

In leukemia, CD 15 antibodies react with all neoplastic myeloid and monocytic proliferations although there is a variable pattern with different antibodies. CD 15 positivity is reported to be lost in cases of relapsed acute myeloid leukemia, correlating with a poorer survival. Almost all cases of chronic myelogenous leukemia have demonstrated the presence of CD 15 while in chronic phase. Approximately 16% of cases of acute lymphoblastic leukemia demonstrate the coexpression of at least one myeloid antigen and up to 50% of such cases are reportedly CD 15+ although the range of positivity is between 2% and 6%. CD 15 expression is highest in common acute lymphoblastic leukemia antigen

(CALLA)-negative cases which generally have a worse prognosis than cases of CALLA-positive ALL (Bernstein et al, 1982).

CD 15 expression is very helpful in the diagnosis of Hodgkin's disease as almost all the CD 15 antibodies available react with Reed-Sternberg cells and the mononuclear variants. Characteristically, the staining is membranous with globular, juxtanuclear staining of the Golgi complex. The cytoplasmic membrane staining has been confirmed by ultrastructural studies and lysosomal granules contiguous with perinuclear vesicles representing the Golgi apparatus are also stained. Reed-Sternberg cells and atypical mononuclear variants in Hodgkin's disease of mixed cellularity type, nodular sclerosing and lymphocyte-depleted type show staining with CD15 antibodies. However, lymphocyte-predominant Hodgkin's disease is CD 15- particularly in the nodular and in some cases of the diffuse subtype (Stein et al, 1986). Digestion with neuraminidase has been reported to result in staining of the L&H cells in lymphocyte-predominant Hodgkin's disease although the staining has been described to be less intense and predominantly cytoplasmic in distribution. Similarly, enzyme pretreatment has been reported to produce positivity in T-cell lymphomas mostly of the mature phenotype, particularly in advanced stage mycosis fungoides. A smaller percentage of low-grade B-cell lymphomas have also been reported to be CD 15+.

CD 15 is a useful marker for granulocytic sarcoma, staining the majority of cases (Swerdlow & Wright, 1986).

Strong CD 15 positivity has been found in carcinomas from a wide variety of sites. It is employed in a panel for the discrimination of adenocarcinoma from malignant mesothelioma, the latter being generally CD 15-. Cytomegalovirus infected cells have also been found to react with CD 15 antibodies, predominantly with cytoplasmic staining.

Comments

CD 15 antibodies are particularly useful for the identification of Reed-Sternberg cells, especially when they are employed in a panel which includes CD 45 (LCA), Reed-Sternberg cells showing the characteristic membranous and Golgi staining for CD 15 and negative staining for CD 45. It is also a useful discriminant when used in an appropriate panel for the separation of adenocarcinoma from malignant mesothelioma; adenocarcinomas and the antibodies label the myeloid cells of granulocytic sarcoma (Sewell et al, 1987). Staining is enhanced with microwave epitope retrieval using citrate buffer and enzyme digestion should not be performed when employed for the identification of Reed-Sternberg cells and adenocarcinomas.

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Swerdlow SH, Wright SA 1986. A spectrum of Leu M1 staining in lymphoid and hemopoietic proliferations. American Journal of Clinical Pathology 85: 283-288.

CD 19

Sources/Clones

Accurate (B19, CLB/B4/1, FMC63, polyclonal), Becton Dickinson (SJ25C1), Biodesign (BC3), Biogenex (B4), Biosource (BC3, SJ25C1), Caltag Laboratories (SJ25C1), Coulter (B4), Cymbus Bioscience (RFB9, SJ25-C1), Dako (HD37), Immunotech (386.12, J4.119), Novocastra (4G7/2E, FMC63), Pharmingen (B43, HIB19), Sanbio/Monosan (SJ25C1), Seralab, Sigma Chemical (SJ25C1) and Zymed (SJ25-C1).

Fixation/Preparation

The majority of these antibodies are only applicable to cryostat sections, although they may be used in acetone-fixed cryostat sections and smears. They are not suitable for formalin-fixed, paraffin-embedded sections.

Background

The CD 19 gene (along with CD 20 and CD 22) encodes transmembrane proteins with at least two extracellular immunoglobulin-like domains that are of vital importance to B-cell function (McMichael, 1987). Similar to the immunoglobulin genes, they are expressed in a lineage-specific and developmentally regulated manner (Kehrl et al, 1994). In normal cells, CD 19 antigen (90 kD polypeptide) is the most ubiquitously expressed protein in the B-lymphocyte lineage (Scheuermann & Racila, 1995). CD 19 expression is induced at the point of B-lineage commitment during the differentiation of the hemopoietic stem cell. Its expression continues through pre-B and mature B-cell differentiation, being downregulated during terminal differentiation into plasma cells. Furthermore, CD 19 expression is maintained in neoplastic B cells, enhancing its diagnostic usefulness. Since CD 19 is not expressed in pluripotent stem cells, it has become the target for a variety of immunotherapeutic agents (Scheuermann & Racila, 1995).

Applications

B43 monoclonal antibody recognizes the same surface epitope as several other anti-CD 19 monoclonal antibodies. Using clone B43 to test for CD 19 expression on 340 leukemias and 151 malignant lymphomas, Uckun et al (1988) showed CD 19 to be the most reliable B-lineage surface marker. The advantage of immunodetection of CD 19 expression is that B lineage leukemias and lymphomas rarely lose the epitope (Scheuermann & Racila, 1995). Furthermore, CD 19 is not expressed on myeloid, erythroid, megakaryocytic or multilineage bone marrow progenitor cells (Uckun et al, 1988).

Comments

Although most B cells carry the CD 19 antigen, the use of anti-CD 19 is restricted to cryostat sections and is therefore not helpful in routine diagnostic histopathology practice.

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1988. Detailed studies on expression and function of CD 19 surface determinant by using B43 monoclonal antibody and the clinical potential of anti-CD19 immunotoxins. Blood 71: 13-29.

CD 20

Sources/Clones

Accurate (L26), Becton-Dickinson (Leu 16), Biodesign (BB6), Biogenesis (MEM97), Biogenex (L260), Biotek (126), Coulter (B1), Cymbus Bioscience (MEM97, BC1), Dako (L26), Immunotech (L26, HRC20-B9E9), Monosan (MEM97), Research Diagnostics (MEM97), Sanbio (MEM97), Seralab (BC1), Serotec (B>B6, BC1), Signet, Novocastra, Pharmingen (2H7) and Zymed (L26).

Fixation/Preparation

All the available antibodies to CD 20 react in paraffin and frozen sections and can be used to label cells in suspension. Immunoreactivity is enhanced by heat-induced antigen retrieval but not proteolytic digestion.

Background

The CD 20 molecule is one of the best markers of B-cell lineage. It is a membrane-embedded, non-glycosylated phosphoprotein which appears in early pre-B cells and throughout maturation into late pre-B cells. It is expressed on the surface of all mature B lymphocytes but not in secreting plasma cells. The CD 20 gene is a single copy gene located on chromosome 11q12-q13, near the site of the t(11; 14)(q13; q32) translocation which is commonly noted in mantle zone lymphoma. The complete gene is 16 kb long and comprises eight exons, with six exons encoding the protein (Dorken et al, 1989; Tedder et al, 1989).

The exact function of the CD 20 molecule is unknown but it is involved in the regulation of B-cell activation, proliferation and differentiation. Certain anti-CD 20 antibodies trigger resting B cells to enter the cell cycle and induce IgM production, while other antibodies to CD 20 can inhibit B-cell activation (Ishii et al, 1984).

The CD 20 antigen appears on the cell surface after light chain gene rearrangement and before the expression of intact surface Ig, remaining throughout the course of B-cell development, and is lost only prior to plasma cell differentiation. While it is expressed on both resting and activated B cells, its expression is about fourfold greater in the latter.

Virtually all lymphoid cells in the germinal center express CD 20 besides CD 19, CD 22 and other pan-B cell antigens and CD 20 and CD 19 are also expressed by cells of the mantle zone, but in lesser intensity. In the thymus, CD 20 stains medullary B cells and cells within the epithelial meshwork of the thymic parenchyma. Cortical cells are negative for this antigen (Norton & Isaacson, 1989).

Weak expression of CD 20 may be seen in a subpopulation of T cells but the antigen is not expressed in normal myeloid, erythroid, monocytic or mesenchymal cells. Antigen-presenting dendritic cells in the blood do not stain for CD 20 and the antigen is not expressed in cells of the normal skin or adnexal structures (Chang et al, 1996).

Applications

CD 20 is the most useful marker for neoplasms of B-cell derivation and is almost always expressed in B-cell lymphomas of small cell type, prolymphocytic leukemia, follicular center cell lymphomas, large or small cell types of both diffuse and follicular patterns, monocytoid lymphomas, mantle cell lymphomas, hairy cell leukemias/lymphomas, immunoblastic lymphomas but not plasmacytomas The staining of CD 20 in chronic lymphocytic leukemia/small cell lymphoma may be weak and often not in all cells. It has not been shown to

stain the neoplastic cells of T lymphomas. While CD 20 has great diagnostic utility, it is of no prognostic relevance. Homogenous staining for CD 20 in bone marrow lymphoid aggregates is more common in neoplastic aggregates than in benign ones and may be a useful discriminator in such settings.

About 10-20% of lymphoblastic lymphomas are non-T cell lineage and express B-cell antigens, about half the latter group expressing CD 20.

In Hodgkin's disease, 60-100% of cases of the nodular lymphocyte-predominant subtype show CD 20 staining of the L&H malignant cells. The positivity in Reed-Sternberg cells of other subtypes of Hodgkin's disease is variable but much lower, less than 20%, (Zukerberg et al, 1991).

Occasional cases of acute myeloid leukemia and extramedullary myeloid tumors may show aberrant expression of CD 20 but this is estimated to involve only 3% of cases, with no correlation between any lymphoid antigen expression and morphology. In the case of chronic myelogenous leukemia, about 25-30% of the cases that show blastic transformation display lymphoid differentiation by morphology, cytochemistry and immunophenotyping. The lymphoid cells usually display the immunophenotype of precursor B cells, including the expression of CD 20 as well as other B-cell antigens such as CD 10, CD 19, increased TdT and rearranged immunoglobulin genes.

Immunoreactivity for CD 20 has been observed in the epithelial cells of a subset of thymomas and seems to correlate with spindling of the neoplastic cells.

Comments

Antibodies to CD 20 are mostly reactive in formalin-fixed paraffin-embedded tissues and it is by far the most superior marker for B lymphocytes, with a sensitivity and specificity of 95% and 100% respectively (Bluth et al, 1993). The pattern of staining is membranous and continuous. It may be accompanied by nuclear, paranuclear and diffuse cytoplasmic staining but this should be generally weak. Heat-induced epitope retrieval has been reported to produce nucleolar staining. Very rare cases of low-grade B-cell lymphomas may not stain for CD 20 and may express CD 43 in paraffin sections, suggesting an erroneous interpretation of T-cell lineage (Norton & Isaacson, 1989). However, an awareness of this and the proper use of antibody panels will avoid such pitfalls. Clone L26 is the most commonly used of the CD 20 antibodies.

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CD 21

Sources/Clones

Dako (1F8), Coulter (B2) and Immunotech (BL13).

Fixation/Preparation

CD 21 is applicable to formalin-fixed, paraffin-embedded tissue sections. Enzymatic digestion with proteolytic enzyme trypsin is essential for positive immunoreaction but HIER produces significant enhancement of immunoreactivity. CD 21 may also be used for labeling acetone-fixed cryostat sections or fixed-cell smears.

Background

CD 21 antigen (CR2) (Isotype: IgG1 k) represents the purified receptor of the C3d fragment of the third complement component from human tonsils (Weiss et al, 1984). This membrane molecule is a glycoprotein of molecullar weight of 145 kD and is involved in the transmission of growth-promoting signals to the interior of the B cell. CD 21 also functions as a receptor for Epstein-Barr virus (Nemerow et al, 1985). IF8 reacts with an epitope localized on trypsin fragments of CR2 of molecular weights 95,72,50,32 and 28 kD (Mason et al, 1986). The 28kD and 72 kD molecular weight fragments of CR2 contains the binding site for the C3d receptor.

The CD 21 antigen is a restricted B-cell antigen expressed on mature B cells. The antigen is also present on follicular dendritic cells (FDCs), the accessory cells of the B zones. IF8 labels B cells moderately and demonstrates FDCs strongly on cryostat sections. However, on paraffin sections, B-cell immunoreaction is abolished whilst the FDCs remain highlighted, similar to the cryostat sections. Hence, in normal and reactive lymph nodes, tonsils and extranodal lymphoid tissue, the antibody demonstrates the FDC meshwork remarkably clearly defined in the germinal centers (Mason et al, 1986).

Applications

On paraffin sections, antibodies to the CD 21 antigen are useful to demonstrate FDC meshwork in lymphoid proliferations where the germinal centers may be illdefined and difficult to delineate morphologically, e.g. HIV lymphadenopathy. In the early stages of progressive generalized lymphadenopathy (PGL, stage I), the large geographic reactive germinal centers may occupy large areas of the lymph node, giving an appearance of effacement of the architecture. Similarly, in the late stage of PGL (stage III), the atrophic germinal centers are not easily definable.

The demonstration of the nodular dense FDC meshwork of follicular lymphomas is also a potential application of the CD 21 antibody. Similarly, the follicular/nodular architecture of nodular lymphocyte-predominant Hodgkin's disease may be highlighted. Residual germinal centers that have been colonized in low-grade B-cell MALT lymphomas may also be demonstrated with antibody to CD 21 which reveals the FDC meshwork. Nodal mantle cell lymphoma and multiple lymphomatous polyposis are characterized by the presence of monotonous small lymphoid B-cell population and interspersed cells with "naked" nuclei (FDCs), which is helpful in distinguishing this lymphoma from other low-grade B-cell lymphomas (Chan, 1996).

The demonstration of a FDC meshwork is also characteristic of peripheral T-cell lymphomas of angioimmunoblastic lymphadenopathy (AILD) type. The FDC meshwork in AILD is typically around hyperplastic venules.

The diagnosis of angiofollicular lymph node hyperplasia or Castleman's disease (hyaline-vascular type) may also benefit from highlighting the follicles with anti-CD 21. Dysplastic FDCs have been demonstrated in association with Castleman's disease and are thought to be the precursor to FDC tumors. Again, the characteristic dendritic processes in FDC tumors are well demonstrated with CD 21 antibodies (Chan et al, 1997).

Comments

Although sometimes patchy and focal, reactivity with the paraffin section-reactive CD 21 is essential for the diagnosis of FDC tumors which are probably underdiagnosed through underrecognition.

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CD 23

Sources/Clones

Accurate, Biodesign (BB-10, BU38, 9P.25), Biotest (TU1), Cymbus Bioscience, Dako (MHM6), Gen Trak, Immunotech (9P25), Pharmingen (B3B4), RDI (TU1), Sanbio (BL-C/B8) and Serotec (B-G6, BSL-23).

Fixation/Preparation

Current available antibodies are immunoreactive only in fresh-frozen sections and fresh cytologic preparations.

Background

The CD 23 antigen is an integral membrane glycoprotein of molecular weight 45-60 kD. It has been identified as a low-affinity receptor for IgE and may be involved in the regulation of IgE production as well as also being a receptor for lymphocyte growth factor. Following crosslinkage of antigen and Ig, CD 23 becomes expressed and serves as an autocrine stimulus driving B-cell proliferation. CD 23 appears on B cells within 24 h following a variety of stimuli. Surface CD 23 has a half-life of only 1-2 h and is shed in the form of soluble fragments of varying molecular weight which display the autocrine-promoting activity. Two species of CD 23 have been described FcεRIIa and FcεRIIb, differing in the N-terminal cytoplasmic region and sharing the same C-terminal extracellular region. FcεRIIa is strongly expressed on IL-4-activated B cells and weakly on mature B cells; it also stains some dendritic reticulum cells which probably acquire the antigen from neighboring B cells (Kikutani et al, 1986). FcεRIIa is not found on circulating B cells and its expression can only be induced on surface IgMD+ cells and not on those B cells that have lost IgD, undergone isotype switch and express IgG, IgA or IgE. FcεRIIb is expressed weakly on a range of cell types including monocytes, eosinophils, platelets, some T cells and NK cells. IL-4-treated monocytes show stronger staining (Zola, 1987; Armitage & Goff, 1988).

Applications

CD 23 is found in most low-grade B-cell lymphomas and in Reed-Sternberg cells in Hodgkin's disease (Rowlands et al, 1990). Activated B cells within germinal centers express CD 23 in high density but mantle zone (resting) B cells are negative or stain only weakly. The majority of B-cell CLLs and a variable proportion of B-cell non-Hodgkin's lymphoma are CD 23+, whereas mantle cell lymphomas are generally negative, so that this marker is useful when applied with other markers to separate the small cell lymphomas. Precursor B-cell and T-cell ALL/LBL, acute myeloid leukemia, chronic myeloid leukemia and post-thymic T cell neoplasms are CD 23- (Raghoebier et al, 1991). The marker is upregulated by EBV infection (Thorley Lawson et al, 1985).

Comments

Anti-CD 23 (clone BU38, Biodesign) which is reactive to a variable extent in paraffin-embedded sections following treatment with protease-1 (Kumar et al, 1996), is no longer available.

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CD 24

Sources/Clones

Biodesign (ALB9), Cymbus Bioscience (ALB9), Dako (SN389), Immunotech (ALB9), RDI (ALB9) and Serotec (ALB9).

Fixation/Preparation

Current antibodies are reactive in fresh-frozen sections and cell preparations only.

Background

Antibodies to CD 24 react with a 42 kD single-chain sialoglycoprotein which is expressed throughout B-cell differentiation but, like other pan-B-cell antigens, is lost following activation and before the secretory (plasma cell) stage. CD 24 is not entirely restricted to B cells and is expressed on granulocytes, interdigitating cells, and renal epithelial cells, as well as some benign and malignant epithelial tumors (Abramson et al, 1981; Kemshed et al, 1982; Melink & LeBien, 1983; Hsu & Jaffe, 1984).

Applications

CD 24 is expressed on the majority of precursor B-cell ALL/LBLs and by virtually all mature, TdT-, SIg+ and SIg- B-cell non-Hodgkin's lymphoma (Kersey et al, 1982). It is not found in multiple myeloma nor on benign and neoplastic T cells. Anti-CD 24 has been used for purging bone marrow of B-ALL cells in autologous bone marrow transplantation.

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CD 30

Sources/Clones

Accurate (Ki-1, Ber-H2), Biodesign (HRS4), Bioprobe (IC)-88), Cymbus Bioscience (Ki-1), Dako (Ber-H2, Ki-1), Diagnostic Biosystems (Ki-1, Ber-H2), Immunotech (HRS4, Ki-1) and Serotec.

Fixation/Preparation

The Ki-1 antibody produces membrane staining only in frozen sections and does not stain paraffin-embedded tissues. BER-H2 labels an epitope that survives routine fixation and processing.

Background

The first CD 30 antibody to be generated was called Ki-1 and was thought to be specific for Reed-Sternberg cells. The Ki-1 antibody recognizes an intracellular protein and a membrane-bound glycoprotein which are apparently not related. The membrane-bound glycoprotein is often referred to as the true CD 30 antigen. It has a molecular mass of 105-120 kD and is phosphorylated at serine residues and contains an N- and O-glycosidyl bound carbohydrate portion. The extracellular domain of CD 30 shows significant homology with members of the tumor necrosis factor/nerve growth factor receptor superfamily (Stein et al, 1985). The human CD 30 gene has been localized to the short arm of chromosome 1 at 1p36, a band frequently involved in neoplastic disorders (Fonatsch et al, 1992). Deletions, duplications, translocations and inversions of this band have been observed in non-Hodgkin's lymphomas and abnormalities of the short arm of chromosome 1 have been described in Hodgkin's disease. 1p36 is also the location for the TNF receptor-2 gene and appears to be a preferential site for integration of viruses such as the Epstein-Barr virus.

CD 30 appears to be a lymphoid activation antigen and its expression can be induced on B and T lymphocytes in vitro by a number of stimuli which include viruses and lectins. CD 30 may act as a receptor whose ligand is a cytokine. Recombinant CD 30L exhibits pleiotropic cytokine activities, with CD 30L inducing proliferation of activated T cells in the presence of an anti-CD 3 costimulus and enhancing the proliferation of a Hodgkin's cell line HDLM2. CD 30L mRNA expression can be induced on Tcells and macrophages, suggesting that a variety of autocrine and paracrine mechanisms may be operative. Immunoelectron microscopic studies have localized the antigen in the cytoplasm and in association with the nuclear envelope, chromatin structures and nucleoli.

Applications

CD 30 antibodies do not react against any resting peripheral blood cells. Staphylococcus-stimulated B lymphocytes and phytohemagglutinin-stimulated T lymphocytes become CD 30+, and expression of the antigen can be induced by activating T-helper lymphocytes with autologous and allogeneic stimulator cells. The antigen is also expressed in Epstein-Barr virus-transformed B cells and human T lymphotrophic virus-transfected T-cell lines. Activated T-cells express CD 38, CD 71, CD 25, epithelial membrane antigen, HLA-DR and CD 15 together with α-1-antitrypsin and CD 11C prior to the expression of CD 30. Scattered large B and T cells localized around lymphoid follicles and at the margin of germinal centers show CD 30 positivity in normal and reactive lymph nodes. These cells may also coexpress Ki-67 nuclear antigen, indicating their proliferating state. Similarly,

macrophages which are generally negative for CD 30 may become CD30+ in conditions such as miliary tuberculosis, sarcoidosis and other granulomatous reactions such as cat scratch disease and toxoplasmosis. BER-H2 may also label a subpopulation of plasma cells. Among non-hemopoietic tissues, exocrine pancreatic cells, some cerebral cortical neurons and Purkinje cells may be positive for CD 30.

In initial studies, Hodgkin's disease was the only neoplasm that was CD30+. About 89% of cases of non-lymphocyte predominant Hodgkin's disease are positive for CD 30 and the staining pattern is membranous, often with a strong paranuclear globule in the region of the Golgi and weaker cytoplasmic staining. In frozen sections, BER-H2 produces stronger staining than Ki-1 and staining is also stronger in frozen sections than in paraffin sections. A variable degree of positivity is seen in the L&H cells of lymphocyte-predominant Hodgkin's disease. About 25% of cases show positivity in paraffin sections and the staining is generally weaker and is limited usually to the cell membrane (Swerdlow & Wright, 1986). It is reported that a much higher incidence of positivity is seen in frozen sections.

CD 30 expression is a characteristic of anaplastic large cell lymphoma (ALCL) which is defined in part by its nearly constant CD 30 positivity. The pattern of staining is similar to that seen in Reed-Sternberg cells and may be expressed by ALCLs of both T- and B-cell lineage as well as "null" cell types. CD 30 expression, however, is not limited to ALCL and may be found in other types of non-Hodgkin's lymphoma. In one study of about 500 cases of non-Hodgkin's lymphomas, 36 cases of lymphomas other than ALCL were CD 30+. The expression of CD 30 is highest in immunoblastic lymphomas, and among the T-cell lymphomas both mycosis fungoides as well as other types of peripheral T-cell lymphomas including AILD-like T-cell lymphoma, Lennert's lymphoma and HTLV-I+ T-cell leukemia/lymphoma, may show a relatively high incidence of CD 30 positivity. It has been suggested that primary CD30+ lymphomas, particularly primary cutaneous lymphomas, have a better prognosis than their CD 30-counterparts (Stein et al, 1985; Piris et al, 1990). However, the expression of CD 30 in cutaneous lymphomas which arise in patients with a preceding history of another lymphoma may have a particularly poor prognosis. The expression of CD 30 in lymphomatoid papulosis and regressing atypical histiocytosis has suggested a close relationship between these disorders and cutaneous CD 30+ ALCL. These three lesions may represent a spectrum with their histologic and clinical characteristics determined by the degree of biological aggressiveness of the neoplasm and the host immune defenses.

Occasional cases of plasmacytomas and myelomas may show CD 30 positivity. Hairy cell leukemia is consistently negative for CD 30 and Langerhans' cell histiocytosis is also CD 30-. Staining has also been reported to be negative in three cases of dendritic reticulum cell sarcoma and the expression in true histiocytic tumors is not known. CD 30 positivity has not been reported in cases of leukemia (Piris et al, 1990).

CD 30 positivity has been reported in embryonal carcinomas and in the embryonal elements of mixed germ cell tumors and, less commonly, has been observed in pancreatic and salivary gland carcinomas (Pallesen & Hamilton-Dutoit, 1988). Occasionally, other paraffin-embedded carcinomas and malignant lymphomas may show weak, diffuse cytoplasmic staining and CD 30 positivity has more uncommonly been observed in mesenchymal tumors including leiomyoma, leiomyosarcoma, rhabdomyosarcoma, synovial sarcoma, giant cell tumor of tendon sheath, malignant fibrous histiocytoma, osteogenic sarcoma, Ewing's sarcoma, malignant schwannoma, ganglioneuromas and aggressive fibromatosis. Occasional lipoblasts in liposarcoma may show positivity (Mechterscheimer & Moller, 1990; Chang et al, 1993).

Comments

We have found that BER-H2 staining is enhanced by MW epitope retrieval in citrate buffer with or without enzyme pretreatment. Because it is expressed in stimulated B-and T-lymphoid cells, BER-H2 should not be employed as a primary marker of Reed-Sternberg cells. However, it should be used in a panel for the identification of ALCL, bearing in mind that such tumors may be CD 45- and EMA+, an

immunophenotype which may be mistaken for carcinoma. From a practical standpoint, ALCLs do not express cytokeratin.

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CD 31

Sources/Clones

Accurate (JC70A, CLB-HEC75), Becton Dickinson (L133.1), Biogenex (9G11), Coulter (56E), Dako (JC/70A), Monosan (CLB-58, VM64), Novocastra (HC1.6), Pharmingen (2ET, M290, WM59), Research Diagnostics and Sanbio (VM64).

Fixation/Preparation

Antibodies to CD 31 are generally immunoreactive in fixed, paraffin-embedded tissue sections as well as fresh cell preparations and cryostat sections. HIER enhances immunoreactivity and, if employed, enzyme predigestion is not necessary.

Background

CD 31 is a 130 kD glycoprotein, also designated platelet endothelial cell adhesion molecule-1 (PECAM-1), that is normally expressed on endothelial cells and circulating and tissue-phase hematopoietic cells, including platelets, monocytes/macrophages, granulocytes and B cells. This antigen is also expressed by sinusoidal endothelial cells in the liver, lymph node and spleen (Parums et al, 1990). The same endothelial cells display variable staining with *Ulex europeaus* agglutinin-I (UEA-1) and for von Willebrand factor (factor VIII - related protein), indicating that the sinusoidal endothelium differs from other vascular endothelium. CD 31 does not label connective tissue, basement membrane, squamous epithelium or adnexal structures of the skin (Suthipintawong et al, 1995). The exact function of CD 31 has not been fully elucidated but it appears to mediate platelet adhesion to endothelial cells and may promote vascular adhesion of leukocytes (Stokinger et al, 1990; Albelda et al, 1991).

Applications

The main application of CD 31 is as a marker of both benign and malignant endothelial cells (Leong et al, 1997). It is an apparently more sensitive marker than CD 34, von Willebrand factor or UEA-1 as a marker of malignant vascular endothelium (DeYoung et al, 1993) (Appendices 1.22 and 1.23). Despite the earlier suggestion that CD 31 is specific for vascular endothelium (Parums et al, 1990) with no expression by lymphangiomas, we clearly showed that there was distinct staining for CD 31 in all 19 cases of lymphangioma studied, albeit of lesser intensity than that observed in vascular endothelium (Suthipintawong et al, 1995). Indeed, the endothelium of blood and lymphatic vessels share many common antigens such as CD 34, von Willebrand factor and UEA-1 and none provides absolute distinction between the two types of vessels. In the light of these findings, claims that Kaposi's sarcoma shows vascular endothelial differentiation or derivation (Scully et al, 1988; Hoerl & Goldblum, 1997) will need to be reassessed.

Comments

Some form of HIER should be used with anti-CD 31 to produce optimal immunoreactivity in fixed tissue sections (we employ microwave-stimulated HIER). The antigen is localized to the cell membrane with some weaker staining of the cytoplasm.

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CD 34

Sources/Clones

Becton Dickinson (MY10), Biodesign (QBEND/10), Biogenex (QBEND/10), Cymbus Bioscience, Dako (BIRMA-K3), Gen Trak, Immunotech (QBEND/10, IMMU133.3), Oncogene, PerSeptive, RDI (9BI-3c5, ICH3), Selinus (B1-3C5), Seralab (BI-3C5) and Serotec (QBEND/10).

Fixation/Preparation

Antibodies are immunoreactive in fixed tissue and staining is significantly enhanced by HIER.

Background

The CD 34 antigen is a 110 kD, heavily glycosylated transmembrane protein of generally unknown function. Some evidence suggests that CD 34 might play a role in cell adhesion with the highly glycosylated molecule allowing it to act as a ligand for lectins. In this way, CD 34+ hematopoietic precursors might bind to lectin-expressing cells of the bone marrow stroma. The CD 34 antigen was originally defined by monoclonal antibody MY10 raised against the human myeloid leukemia cell line KG1a. The gene for CD 34 has been localized to chromosome 1 in the region of 1q32 and the DNA sequence demonstrates no homology with any previously known human genes (Baum et al, 1992; Greaves et al, 1992).

The CD 34 antigen is present on ~ 1% of normal bone marrow mononuclear cells including hematopoietic precursors/stem cells. Thus, antibodies to CD 34 can be used to purify the CD 34+ stem cell population from CD 34- malignant cells. The CD 34+ bone marrow population contains not only hematopoietic stem cells but also more mature lineage-committed precursor cells for the erythroid, myeloid and lymphoid lineages. Included among these CD 34+ cells are stromal cells necessary for the appropriate bone marrow environment for hematopoiesis (Baum et al, 1992).

The demonstration of CD 34 on immature leukemias and vascular neoplasms has been the main contribution to its diagnostic utility. Besides bone marrow stem cells and normal endothelial cells, the antigen is found on cells in the splenic marginal zone and dendritic interstitial cells around vessels, nerves, hair follicles, muscle bundles and sweat glands in a variety of tissues and organs. CD 34+ cells appear in the peripheral blood after treatment with chemotherapy or cytokines. In blood vessel endothelium the antigen may be absent from large veins and arteries and from sinuses in the placenta and spleen. It is expressed on the luminal surface and membrane processes that interdigitate between endothelial cells. In new vessels such as in tumors, the location of the antigen is altered and it is found on the abluminal microprocesses of such vessels (Van De Rijn & Rouse, 1994).

Among the hematopoietic neoplasms, CD 34 is seen in the immature leukemias such as acute lymphoblastic leukemia of both T-and B-cell lineage and acute myeloblastic leukemia. In myelodysplastic syndromes the expression of CD 34 was predictive of transformation and poor survival outcome. There is some confusion over the value of CD 34 as a prognostic parameter in the leukemias. Some studies have suggested that its expression is a poor prognosticator in AML whereas it is a marker of good prognosis in childhood ALL, probably those restricted to B-cell lineage all these studies being performed with flow cytometry analysis (Van De Rijn & Rouse, 1994).

Applications

The expression of CD 34 is retained in malignant endothelial cells so that it is a good marker for vascular tumors (Appendices 1.22 and 1.23). The endothelial cells of both vascular and lymphatic vessels express the antigen (Ramani et al, 1990; Suthipintawong et al, 1995). There is variable staining for CD 34 in smooth muscle cells and their tumors. Antibodies to CD 34 label gastrointestinal stromal tumors (GIST) very strongly (Appendix 1.25). Epithelioid smooth muscle tumors stain less frequently but the marker may serve as a useful discriminator from epithelial tumors which are generally negative for CD 34 (Sirgi et al, 1993). The antigen is displayed by nerve sheath tumors although in some series both neurofibromas and schwannomas failed to stain. In the latter, staining may be mainly in the Antoni B areas. While the staining in malignant nerve sheath tumors is largely negative, some series report a high frequency of reactivity, suggesting that CD 34 may be a useful inclusion in the diagnostic panel for such tumors as S100 and CD57 are negative in such tumors (Weiss & Nickoloff, 1993). Epithelioid sarcoma and hemangiopericytoma show staining for CD 34 and the marker is invariably found in solitary fibrous tumors and dermatofibrosarcoma protuberans, two tumors which are generally recognized from their histologic mimics by the absence of specific markers (Kutzner, 1993; Westra et al, 1994). Recently, CD 34 was also demonstrated in four of 12 cases of angiomyofibroblastomas (Neilsen et al, 1996). Interestingly, reactivity for CD 34 was found in giant cell fibroblastomas and one Bednar tumor, supporting the relationship of such tumors to dermatofibrosarcoma protuberans. CD 34 is also a useful marker for early myeloid cells and hence stains granulocytic sarcoma.

Comments

Much of the earlier controversy concerning the staining of CD 34 in spindle cell tumors was due to the sensitivity of the staining technique. CD 34 staining is greatly enhanced by heat-induced epitope retrieval, especially microwave-induced techniques.

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Westra WH, Gerald WL, Rosai J 1994. Solitary fibrous tumor. Consistent CD 34 immunoreactivity and occurrence in the orbit. American Journal of Surgical Pathology 18: 992-998.

CD 35

Sources/Clones

Dako (Ber-MAC-DRC, To5) and Immunotech (J3D3).

Fixation/Preparation

This antibody can be used on formalin-fixed, paraffin-embedded tissue section. Enzymatic digestion with proteolytic enzymes (e.g. pronase) for antigen retrieval must be performed for optimum immunoreaction. HIER enhances immunoreactivity, especially when Target Retrieval Solution is employed. The CD 35 antibody may also be applied to acetone-fixed cryostat sections or fixed-cell smears.

Background

DAKO-CD 35 (isotype: IgG 1, k) reacts with a formalin-resistant epitope of the receptor for the C3b fragment of the third component of human complement (Gerdes et al, 1982). This receptor, which is often referred to as CR1, consists of a single glycoprotein chain with a molecular weight of approximately 220 kD. The antigen has been designated CD 35 in the system for classifying human leukocyte antigens and is therefore equivalent to To5 (Bettelheim, 1989).

In frozen sections of normal tissues, DAKO-CD 35 shows immunostaining of B-cell follicles of lymphoid tissue. The most strongly labeled cells within B-cell follicles are follicular dendritic cells (FDCs), but mantle zone lymphoid cells also immunoreact to a lesser degree. The C3b receptor on epithelial cells of renal glomeruli may also be clearly demonstrated with this antibody. Further, enzyme-treated, routinely processed paraffin sections show strong immunoreaction of FDCs in lymphoid tissue (both nodal and extranodal). The well-defined dense meshworks of FDCs in germinal centers are well demonstrated with this antibody (Fearon, 1980).

Applications

Immunohistological analyses of FDCs in paraffin sections are confined to the demonstration of FDC meshworks in reactive and neoplastic lymphoid tissue. In this regard identical immunoreactions of the dendritic cell processes of FDC are demonstrated with antibodies to both CD 21 and CD 35. Hence, the application of antibody to CD 35 in surgical pathology (being similar to CD 21) remains largely for the demonstration of FDC meshworks in follicles of HIV lymphadenopathy, Castleman disease, follicular lymphoma, follicular colonization by low-grade B-cell MALT lymphoma and nodular lymphocyte-predominant Hodgkin's disease. Demonstration of FDCs with CD 35 antibody is also useful in mantle cell lymphoma and peripheral T-cell lymphoma-AILD type (Chan, 1996). In contrast to follicular lymphomas in which the lymphomas cells are encased within a network of proliferating FDCs, the network of FDCs in mantle cell lymphoma is loosely arranged. In angioimmunoblastic T-cell lymphoma, there is a pronounced proliferation of FDCs around postcapillary venules. Finally, the application of antibody to CD 35 in the diagnosis of FDC tumors is discussed in the section on CD 21 (p 71)

Recently, another antibody to FDC has been generated. The CNA.42 antibody is reactive in fixed, paraffin-embedded sections and stains FDCs but apparently identifying an antigen different from other known anti-FDC antibodies (Raymond et al, 1997). The antibody also labels some T-cell lymphomas as well as a variety of soft tissue tumors and a proportion of carcinomas of the gastrointestinal tract and lung. The

antigen is conserved in a wide spectrum of animal tissues other than human.

Comments

In postchemotherapy excision specimens, immunostaining with a CD 21/CD 35 antibody cocktail is useful to highlight dispersed small islands of residual tumor among the negative foamy histiocytes (Chan et al, 1997). Reactive germinal centers highlighted by antibodies to FDCs are ideal for use as positive control tissue.

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CD 38

Sources/Clones

Accurate (BCAP38), Advanced Immunochemical (24G3), Biodesign (MIG-P12, T16), Biosource (BA6), Caltag Laboratories (BL-AC38, HIT2), Coulter (CD 38, T16), Cymbus Bioscience (BA6), Dako (AT13/5), Immunotech (T16), Pharmingen (HIT2), Sanbio/Monosan (BL-D2, MIG-P12), Sanbio/Monosan/Accurate (BLD2), Seralab and Serotec (B-A6, AT13/5, T16).

Fixation/Preparation

Most antibodies are reactive in fixed paraffin-embedded sections and HIER in Target Retrieval Solution enhances staining (Leong et al, 1997).

Background

The CD 38 molecule, initially described as T10, consists of a single chain of 46 kD, spanning the membrane with its carboxyl terminus located in the extracellular compartment. CD 38 has been one of the most elusive molecules within the family of leukocyte multilineage markers (Reinherz et al, 1980) that has emerged as a multifunctional protein (Mehta et al, 1996). It is expressed on different precursor cells, monocytes, activated T cells and terminally differentiated B cells, including plasma cells (Malavasi et al, 1994). This transmembrane glycoprotein appears to mediate several diverse functions such as signal transduction, cell adhesion (including binding to endothelium), with an important role in lymphocyte homing (Dianzani et al, 1994), and cyclic adenosine diphosphate-ribose synthesis, but its activities remain elusive (Malavasi et al, 1994). Immunoreactivity for CD 38 has also been described in a subset of pyramidal neurons and astrocytes and was predominantly distributed in the perikarya and dendrites in association with rough endoplasmic reticulum, ribosomes, small vesicles, mitochondria and cell membranes (Yamada et al, 1997). CD 38 has also been demonstrated in normal prostate epithelium within both basal and secretory epithelial cells and appeared to be lost in some cases of prostatic carcinoma, hyperplasia and in non-malignant glands surrounding tumor. It was speculated that the role of CD 38 in intracellular calcium mobilization may contribute to smooth muscle contraction and/or sperm motility (Kramer et al, 1995).

The source of the antigen for raising anti-CD 38 specific monoclonal antibody had mainly been preparations obtained from MLC cells, normal thymocytes and the plasmacytoma cell line LP-1 (Alessio et al, 1990). This was used in the context of endometrial biopsy specimens to allow the definitive diagnosis of chronic inflammation to be made (Leong et al, 1997).

Applications

The expression of CD 38 is not restricted to a specific lineage nor to a discrete activation step. It is found on precursor cells in the bone marrow, activated cells (T and B blasts), terminally differentiated cells (such as plasma cells), monocytes and most peripheral blood NK cells (Allessio et al, 1990; Malavasi et al, 1994). CD 4+CD 45RA+ cells also preferentially express CD 38, but the antigen is not expressed by CD 4+CD4 5RO+ cells. From a practical standpoint, CD 38 has been useful in the immunophenotyping of acute leukemias and in research into the role of activated T cells in immunodeficiency diseases and in autoimmune diseases. It is a useful marker for plasma cells as poorly differentiated plasma cells may

mimic other blastic lymphoid cells and suboptimal cytomorphologic preservation may impede the accurate recognition of plasma cells (Appendix 1.6). We have found CD 38 to be a better antibody than VS 38 when employed to identify plasma cells such as in the diagnosis of chronic endometritis (Leong et al, 1997), as the latter also stains stromal and endometrial cells, reducing its usefulness in this setting. CD 38 shows strong labeling of plasma cells, enhancing their distinctive cytologic characteristics.

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CD 40

Sources/Clones

Ancell (BE1), Biodesign (BL-C4), Caltag Laboratories (BLB40), Coulter/Immunotech (MAB89), Cymbus Bioscience (B-B20), Immunotech (MAB89), Pharmingen (5C3), Sanbio/Monosan (BL-C4), Sanbio/Monosan/Accurate (BLC4) and Serotec (B-B20).

Fixation/Preparation

The antigen is resistant to formalin fixation and immunostaining can be performed on cryostat sections and cytological preparations.

Background

CD 40 is a 48 kD integral membrane protein expressed by B lymphocytes, dendritic cells, monocytes, epithelial cells, endothelial cells and tumor cells including carcinomas, B-cell lymphomas/leukemias and Reed-Sternberg cells of Hodgkin's disease. It has been clustered as a member of the nerve growth factor (NGF)/tumor necrosis factor (TNF) receptor superfamily. Its corresponding counterstructure, the CD 40 ligand (CD 40L) being mainly expressed by activated CD 4+ T cells and also some activated CD 8+ T cells, basophils, eosinophils, mast cells and stromal cells. CD 40L shares significant amino acid homology with TNF particularly in its extracellular domain, and is therefore viewed as a member of the TNF, ligand superfamily. The recent flurry of publications relating to CD 40 suggest that this receptor may have a pivotal role in the function of B lymphocytes and their survival (Klaus et al, 1997; Van Kooten & Banchereau, 1997; Liu & Arpin, 1997; Lipsky et al, 1997; Gulbranson-Judge et al, 1997). Binding of CD 40L+ T cells to CD 40+ B-cells is thought to play a major role in the T cell-dependent B-cell activation, B-cell proliferation, Ig isotype switching, memory B-cell formation and rescue of B-cells from apoptotic death in germinal centers (Gruss et al, 1997). Mutations of the CD 40L gene have been associated with the X-linked hyper-IgM immunodeficiency syndrome, indicating the critical role of the CD 40/CD 40L interaction in the T-cell B cell-interplay. Accordingly, expression of CD 40 has been found in most of the B-cell neoplasms, Reed-Sternberg cells of Hodgkin's disease and some carcinomas. In contrast, functional CD 40/CD 40L interactions appear to be critical for cellular activation signals during immune responses and neoplastic tumor cell growth. Lack of this important interaction results in greatly reduced activation of CD 4+ T cells (Gray et al 1997), while successful interaction of these molecules results in full activation of T-cell effector functions such as help for B-cell differentiation and class switch, activation of monocytes and macrophages to produce lymphokines and to kill intracellular pathogens and activation of autoreactive T-cells to mount an autoimmune response (Klaus et al, 1997; Grewal & Flavell, 1997a, b).

Applications

The intense research interest in CD 40 and its ligand has yet to be translated into diagnostic applications. Current uses of CD 40 have mostly been for the immunodetection and identification of tumor cells in all subtypes of Hodgkin's disease. As many as 100% of Hodgkin's disease cases have displayed positivity for CD 40, irrespective of their antigenic phenotype (Carbone et al, 1995). In contrast, CD 40 was immunodetected in only one-third of anaplastic large cell lymphomas, whereas almost 83% of B-cell non-Hodgkin's

lymphomas were positive (Carbone et al, 1995). In vitro engagement of CD 40 by its soluble ligand CD 40L enhanced both clonogenic capacity and colony cell survival of Hodgkin's disease cell lines. Recombinant CD 40L induced interleukin-8 secretion and enhanced IL-6, TNF and lymphotoxin-α release from cultured Reed-Sternberg cells. These cytokines play a significant role in the clinical presentation and pathology of Hodgkin's disease, a tumor of cytokine-producing cells. CD 40L has pleiotropic biologic activities on Reed-Sternberg cells and the CD 40-CD 40L interaction might be a critical element in the deregulated cytokine network and cell contact-dependent activation cascade typical of Hodgkin's disease (Gruss et al, 1994; Carbone et al, 1995).

Comments

CD 40 shows distinctive immunolocalization to the cell membrane and as a paranuclear dot similar to that of CD 30 and CD 15.

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CD 43

Sources/Clones

Advanced Immunochemical, Becton Dickinson (Leu22), Biodesign (BL-E/G3), Biogenesis (MEM59), Biogenesis/Biosource (WR14), Biogenex (MT1), Caltag Laboratories (BL-TP43), Coulter (DFT1), Cymbus Bioscience (DFT1), Dako (DF-T1), Labvision Corp (BRA7G), Novocastra (polyclonal), Pharmingen (HIS17, S7, 1G10), Sanbio/Accurate (BLEG3), Sanbio/Monosan (MEM-59), Serotec (DFT-1, DR-14) and Shandon Lipshaw (DFT1).

Fixation/Preparation

MT1 is applicable to formalin-fixed, paraffin-embedded sections, but requires enzyme (trypsin) pretreatment before immunostaining. HIER enhances immunoreactivity.

Background

MT1 (Poppema et al, 1987) and the identical antibody DFT-1 (Flavell et al, 1988) recognize a sialoantigen present on normal T cells, myeloid cells and macrophages. Megakaryocytes are variably positive. Both antibodies belong to the CD 43 cluster. There is evidence that the antibody MT1, originally thought to belong to CD 45 (Poppema et al, 1987), binds to an entirely unrelated molecule (Flavell et al, 1988). Both MT1 and DFT-1 recognize surface antigens (190, 110 and 100 kD).

Applications

In a review of several published series, MT1 was shown to immunoreact with 30% low-grade B-cell lymphomas, approximately 90% T-cell lymphomas, 69% B-cell and 97% T-cell lymphoblastic lymphomas and 44% anaplastic large cell lymphomas. However, it should be noted that MT1 highlights myeloid cells and macrophages (Norton & Isaacson, 1989a). Although normal small B lymphocytes are MT1- most low-grade B-cell lymphomas are MT1+. However, hairy cell leukemia, MALT lymphoma and follicle center cell lymphomas are notable exceptions. Therefore MT1 is not useful to distinguish between T- and B-cell lymphocytic lymphoma. Furthermore, although MT1 is a reliable marker of mantle cell lymphoma (MCL), it cannot immunophenotypically distinguish MCL from T- or B-cell lymphoblastic lymphomas (Norton & Isaacson, 1989b). Rarely, MT1 marks plasmacytoma/myeloma. It is more often positive than negative in peripheral T-cell lymphomas.

An investigation of 28 extramedullary myeloid cell tumors using paraffin section immunohistochemistry with a panel of myeloid markers revealed CD 43 to be the only antibody that was positive in 100% of cases irrespective of the differentiation of the myeloid cells (Traweek et al, 1993). Further, staining was always intense and widespread.

Comments

CD 43 remains less useful than CD 3 and UCHL1 as a marker of T-cell lymphomas. Nevertheless, in appropriate immunohistochemical panels CD43 does play a role in the identification of low-grade B-cell lymphomas and myeloid disorders. Normal tonsil is useful as a control since paracortical cells are MT1+, whilst follicle center cells are negative. The expression of CD 43 in a large B-cell lymphoma may be an indicator of dedifferentiation from a small cell lymphoma.

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Traweek ST, Arber DA, Rappaport H, Brynes RK 1993. Extramedullary myeloid cell tumors. An immunohistochemical and morphologic study of 28 cases. American Journal of Surgical Pathology 17:1011-1019.

CD 44

Sources/Clones

Available from Biodesign (T2.F4, BU52), Cymbus Bioscience (F10-44-2), Dako (DF1485, 2B11), Immunotech (J.173), Oncogene (A3D8, AIG3), Pharmingen (OX-49), RDI (F10-44-2), Sanbio (MEM-85), Seralab (A3D8, AIG3), Serotec (F10-44-2) and Sigma (A3D8).

CD 44v6

Available from R & D Systems (2F10) and various isoforms including v4, v5, v6, v7 and v7-v8 are available from Bender MedS.

Fixation/Preparation

The antibodies, particularly CA1G3, are effective in formalin-fixed, paraffin-embedded tissues but staining is optimal only after microwave-induced epitope retrieval in 10 mM citrate buffer at pH 6.0. Enzyme digestion should not be performed as this has been shown to alter the integrity of the antigen.

Background

The CD 44 receptor is also known as phagocytic glycoprotein (Pgp-1), extracellular matrix receptor III (ECM-III), B cell p80 antigen, lymphocyte homing receptor (Hermes antigen) and hyaluronate cellular adhesion molecule (H-CAM). CD 44 shows considerable homology with the cartilage link proteins involved in adhesion between hyaluronate and other proteoglycans in the extracellular matrix including collagen, fibronectin and ankyrin. Besides this function, CD 44 has since been found to have a role in recognition between lymphocytes and endothelial cells and in lymphocyte homing to the reticuloendothelial tissues. This latter function has led to interest in its possible role in the regulation of tumor cell dissemination.

The CD 44 family of glycoproteins exists in a number of variant isoforms, the most common being the standard 85-95kD or hematopoietic variant (CD 44s) found in mesodermal cells such as hematopoietic, fibroblastic and glial cells and in some carcinoma cell lines. The receptor is coded in five distinct domains located on the short arm of chromosome 11. The heterogeneity in the CD 44 molecule results from posttranslational modification of the protein and alternative splicing of up to ten exons results in variant isoforms of higher molecular mass (140-160 kD) which may be expressed individually or in various combinations, with potentially diverse functions. Higher molecular weight isoforms have been described in epithelial cells (CD 44v) and are thought to function in intercellular adhesion and stromal binding. While the other functions and distributions of the CD 44 family have not yet been completely elucidated, they are also known to participate in embryonic development and angiogenesis as well as other molecular processes associated with specific adhesions, signal transduction and cell migration. The recent demonstration of a concordance of the cell proliferation nuclear antigen Ki-67 and CD 44 expression in adenomatous polyps, colonic carcinomas and adjacent mucosa raises the possibility of CD 44 involvement in stimulating cell growth (Abassi et al, 1993).

Following the discovery that the splice variants, especially exon v4-7, initiated the lymphatic spread of rat pancreatic carcinoma cells, the role of the highly interspecies-conserved CD 44 in human tumor progression and metastasis has been examined. It appears that the CD 44-hyaluronate interaction is central

to tumor invasiveness, the receptor allowing the uptake and subsequent degradation of matrical hyaluronate. While many human tumors express CD 44, a positive correlation between increased CD 44v expression and tumor progression and/or dedifferentiation has been demonstrated in only some (East & Hart, 1993). Such tumors include non-Hodgkin's lymphoma (Stauder et al, 1995), hepatocellular carcinoma (Mathew et al, 1996), breast carcinoma, renal cell carcinoma (Terpe et al, 1993), colonic carcinoma (Abassi et al, 1993; Wielenga et al, 1993; Herrlich et al, 1995) and some soft tissue tumors (Wang et al, 1996). Conversely, CD 44v expression is downgraded in other tumors including neuroblastoma (Shtivelman & Bishop, 1991), squamous cell and basal cell carcinomas of the skin (Herold-Mende et al, 1996).

Applications

The suggestion that there is a positive association between CD 44 isoform expression and progression in human tumors has important implications for diagnosis and prognosis. Unfortunately, the situation is not yet clearcut. Confusion over the complicated exon boundaries and the different nomenclature employed by researchers have added to problems of identifying the true metastasis-associated isoform. Furthermore, stromal cells may contribute to the isoform pattern detected. For example, activated lymphocytes may express the so-called metastasis-associated variant of CD 44, emphasizing the importance of immunohistological assessment as a method that allows morphologic discrimination.

Comments

Currently, applications of CD 44 still lie in the research domain. While antibodies to specific isoforms are available, some reactive in fixed paraffin-embedded tissues, the antibody to pan-CD 44 molecule has been the most widely used in paraffin sections. Microwave epitope retrieval is essential for the demonstration of the antigen. While CD 44 is a plasmalemmal determinant, both cytoplasmic and cell membrane-staining patterns have been demonstrated in non-neoplastic and neoplastic cells. It has been suggested that exclusive cytoplasmic staining may reflect the overproduction of the protein so that not all of it can be incorporated into the cell membrane. Alternatively, the production of aberrant forms or massive shedding of the CD 44 molecule from the cell membrane could account for this pattern of staining.

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CD 45 (Leukocyte Common Antigen)

Sources/Clones

CD 45

Available from a large number of sources including Biodesign (ALB12, J.33, MEM 28, T29/33), Biogenex, Bioprobe (bra 55, ICO-46, LT46), Cymbus Bioscience (MEM 28, RVS-1, F10-89-4), Dako (T29/33, 2B11, PD7/26), Gen Trak, Immunotech (J.33, ALB12), Oncogene (MEM 28, T29/33, J.33), Pharmingen (H130, CT-1, 30F11.1), RDI (F-10-89-4, CLB-T200/1), Sanbio (BL-leuk-45), Seralab (F10-89-4), Serotec (YTH54.12, YTH24.5) and Sigma.

CD 45R

Available from Accurate/Ancell (351C5), Biodesign (DFB1, F8-11-13, MEM56), Biogenex, Bioprobe (LT45R), Cymbus Bioscience (DFB1), Gen Trak, RDI (DFB1), Seralab, Serotec and Pharmingen (HIS24, DNL-1.9, 16A, 23G2, RA3-6B2).

CD 45RO

Available from Accurate/Ancell (UCHL1), Biodesign (UCHL1), Biotest (UCHL1), Cymbus Bioscience (UCHL1), Dako (UCHL1, OPD4), Gen Trak, Immunotech (UCHL1), Seralab (UCHL1) and Serotec (UCHL1).

CD 45RA

Available from Accurate (YTH80.103), Biodesign (ALB11, F8-11-13), Cymbus Bioscience (F8-1-3, MEM 56), Dako (4KB5), Gen Trak, Immunotech (ALB11), RDI (F8-11-13), Sanbio (MEM-56), Seralab, Serotec (B-C15, F8-11-13) and Pharmingen (14.8).

CD 45RB

Available from Axcel/Accurate, Cymbus Bioscience and Dako (PD7/26).

CD 45RC

Available from Pharmingen (HIS25) and Serotec (YTH80.103).

Fixation/Preparation

The CD 45 antibodies that are commercially available are mostly effective in paraffin-embedded tissues as well as in frozen sections.

Background

The CD 45 cluster of antibodies recognizes a family of proteins known as the leukocyte common antigen (LCA) exclusively expressed on the surface of almost all hematolymphoid cells and their progenitors. The CD 45 antibody is one of the most specific currently available for diagnostic use. Virtually all hematolymphoid cells, including T and B lymphocytes, granulocytes, monocytes and macrophages, with the exception of maturing erythrocytes and megakaryocytes, express CD 45. This family of proteins has been called the leukocyte common antigen and, to date, has not been conclusively shown on any non-hematolymphoid cells.

The CD 45 proteins are coded for by a single gene located on chromosome 1q31-32. The gene is composed of 33 exons that code for the cDNA sequence as well as both 5' and 3' non-translated regions.

Differential usage of three exons termed A, B and C is known to generate eight different mRNAs and at least five proteins in the CD 45 protein family. The complete CD 45 protein consists of a large cytoplasmic domain of 707 amino acids, a transmembrane region of 22 amino acids and an external domain of 391-552 amino acids depending on the pattern of exon splicing. By electron microscopy, the CD 45 proteins consist of a globular structure of 12 nm, representing the cytoplasmic domain, and a rod-like structure of 18 nm, representing the external domain.

There is high conservation of the cytoplasmic domain among mammals and it shows homology with placental tyrosine phosphatases. Consistent with this homology, the CD 45 protein has intrinsic tyrosine phosphatase activity and belongs to a family of protein tyrosine phosphatases that includes 16 other members, at least seven of which are transmembrane proteins (Trowbridge et al, 1991).

The precise function of the CD 45 proteins is not known but they appear to play an important role in early lymphocyte activation. Protein tyrosine phosphatase can counter the actions of protein tyrosine kinases, enzymes known to be induced in early T-cell activation that may represent the primary signaling event initiated by the T-cell receptor. CD 45 expression is inversely related to spontaneous tyrosine phosphorylation of multiple proteins, which has a fundamental role in regulating T-cell calcium levels. CD 45 is required for both T-cell antigen receptor and CD 2-mediated activation of T-lymphocyte protein tyrosine kinase and is physically linked to both CD 2 and the T-cell receptor on the surface of memory T lymphocytes. The difference in structure among the external domains of the different CD 45 proteins probably determines the specific target stimuli for the different cell types expressing CD 45. Similarly, CD 45 may also be important for B-cell function. Antibodies to CD 45 inhibit an early phase in the activation of resting B cells and are able to inhibit c-myc induction in B-cells.

As a result of posttranslocational change of the mRNA of the A, B and C exons, several isoforms are produced. By strict definition, CD 45 antibodies are monoclonal antibodies, which react with all isoforms of CD 45 proteins, and there are several subclusters of antibodies that detect different species of CD 45 proteins. These have molecular weights of 220 kD representing the ABC isoform, 205 kD probably representing distinct AB and BC isoforms, 190 kD representing the B isoform and 180 kD representing the O isoform. The restricted CD 45 antibody refers to those that recognize subsets of CD 45 proteins but not the entire class and these CD 45R antibodies can be further subdivided into CD 45RA, CD 45RB and CD 45RO, depending on the isoform recognized by the antibody. To date, there are no monoclonal antibodies that specifically recognize the C isoform. CD 45RA antibodies generally precipitate the 200 and 205 kD (ABC and AB isoforms), CD 45RB the 220, 205 and 190 kD (ABC, AB, BC and B isoforms) and CD 45RO the 180 kD protein (O isoform).

Many of the CD 45 antibodies are sensitive to neuraminidase, consistent with the suggestion that these antibodies recognize epitopes that are associated with carbohydrates, and possibly, terminal sialic acids. PD 7 is a CD 45RB antibody and labels all known CD 45 proteins with the exception of the ones lacking exons A, B and C, whereas 2B11 reacts against AB protein but not others. The combination of PD 7 with 2B11 as a CD 45-CD 45RB cocktail (Dako) allows a reliable method of detecting LCA in hematolymphoid cells. CD 45 proteins are major components of the membranes of lymphocytes and form about 10% of the lymphocyte surface, accounting for much of the carbohydrate present on the membrane. The staining with CD 45 antibodies is membranous although there may be some staining of the Golgi. Histiocytes exhibit minimal cell membrane staining and phagocytic cells show immunolocalization of the antigen to secondary lysosomes (Weiss et al, 1993).

Applications

The CD 45 proteins are the most specific diagnostic antibodies currently available. A cocktail of PD7-2B11 (CD 45-CD 45RB) antibodies is a reliable marker of cells fixed in formalin as well as in cryostat sections and fresh cell preparations. It is, therefore, an essential component of the panel used to distinguish anaplastic large cell tumors, which include malignant lymphoma, melanoma and carcinoma. It is also an essential component of panels used to separate small cell tumors of lymph nodes, skin, bone and other sites, both in adults as well as in children. The reactivity of anti-LCA antibodies is between 93% and 99% for a cross-spectrum of different subtypes of B- and T-cell lymphomas.

In classic Hodgkin's disease, excluding the nodular L & H lymphocyte predominant subtype, membrane staining for leukocyte common antigen is rare, although cytoplasmic staining may be seen. Cytoplasmic staining may be spurious as similar cytoplasmic staining can be found in non-hematolymphoid neoplasms. By contrast, the majority of nodular

L & H lymphocyte-predominant Hodgkin's disease shows positivity for PD 7 and/or 2B11 and this subtype is now thought to be distinctly different from classic Hodgkin's disease.

Anaplastic large cell lymphoma may show positivity for LCA in only 50-87% of cases, although this figure may be higher in frozen section material. Furthermore, anaplastic large cell lymphoma may also show staining for epithelial membrane antigen, making its immunohistochemical differentiation from anaplastic carcinoma difficult. These tumors express CD 30 and, in 60% of cases, are of activated T-cell phenotype, showing staining for CD 45RO and/or CD 43 in paraffin sections (Chott et al, 1990).

Among other hematolymphoid neoplasms, plasmacytomas show a variable degree of positivity for LCA, ranging from 0% to 20% of cases. Hairy cell leukemia has been found to be uniformly positive for PD 7-2B11 and CD 45 expression has been found in all cases of acute leukemias of T-cell lineage and in over 80% of cases of B-cell lineage. Failure of CD 45 expression in acute childhood lymphoblastic leukemia appears to be associated with other favorable prognostic features such as lower leukocyte counts and serum lactic dehydrogenase levels and is also associated with chromosomal hyperdiploidy. Mast cell disease appears to be positive for PD 7-2B11 and polycythemia vera and extramedullary hematopoiesis were reported to be negative although only a few cases were studied. In keeping with the low expression in histiocytes, true histiocytic tumors were f鴅nd to be negative for PD 7-2B11, whereas cases of Langerhans' histiocytosis were reported to be positive. The rare cases of interdigitating reticulum cell sarcoma which have been studied have been reported to be positive for PD 7, similar to non-neoplastic interdigitating reticulum cells.

While larger series have reported a total absence of staining for LCA in non-hematolymphoid neoplasms, there have been rare case reports of staining examples of primitive sarcoma, probably rhabdomyosarcoma.

CD 45RA (4KB5, MB1, KiB3 and MT2)

The CD 45RA group of antibodies recognize the 220 kD and 205 kD variants of CD 45 encoded by exon A. These isoforms are expressed on the surface of most B cells, as well as postthymic, naive T cells and some medullary thymocytes. MT2 is thought to recognize a carbohydrate moiety and is negative in normal germinal centers, unlike antibodies MB1 and KiB3 which appear to bind to the peptide backbone of CD 45RA, staining mantle zone and follicular center cells. In the paracortical areas of lymph nodes, there are approximately equal numbers of CD 45RO+ and CD 45 RA+cells. In paraffin-embedded sections, MB1 and 4KB5 stain over 80% of cases of B-cell lymphomas, while MT2 stains only 57% of such cases. Small lymphocytic lymphoma has the highest rate of positivity while small non-cleaved cell lymphoma has the lowest. Fifty-seven percent of cases of follicular center lymphoma are positive for MT2 and this pattern of staining has been exploited for diagnostic purposes as only weak or absent scattered positivity for MT2 is seen in reactive germinal center cells (Browne et al, 1991).

Neoplastic follicles are labeled by MT2 whereas reactive follicles are not. This difference in staining patterns with MT2 has been postulated to be due to differences in the sialation of the CD 45 protein present on these B cells. T-cell lymphoma has a much lower incidence of positivity with CD 45RA antibodies and is seen in about 10% of cases.

CD 45RO (UCHL1, A6, OPD4)

CD 45RO antibodies recognize the 180 kD (O isoform) variant of CD 45. UCHL1 antibody reacts with approximately 90% of cortical thymocytes, 50% of medullary thymocytes and approximately 50-70% of CD 2-and CD 3+ peripheral blood and lymph node T cells. It rarely, if ever, reacts with benign B cells. While most mature T cells are CD 45RO+, some normal T-cell subsets are constitutively CD 45RO- and CD 45RA+ and the CD 45RO+ cells slowly increase in number to reach the adult level of about 50% by the age of 10-20 years. CD 45RO- cells include naive CD 4+ T cells, which predominate in neonates, and some CD 8+ or CD 4- CD 8-subsets found in intestinal intraepithelial T cells and enteropathy-associated T-cell lymphoma.

In the differentiation of low-grade B-cell from T-cell lymphomas, the approximated test analysis figures for UCHL1 are as follows: sensitivity 95%, specificity 95%, accuracy 95%. In contrast, in high-grade lymphomas, the same parameters are 80%, 85% and 83%

respectively. Stem cells giving rise to both erythroid and myeloid cells as well as primitive erythroid colony-forming cells express the 180 kD isoform of the CD 45 protein recognized by CD 45RO but more mature erythroid forms lack CD 45 expression. Most granulopoietic colony-forming cells are CD 45RO- while mature monocytes or macrophages and myeloid cells are generally CD 45RO+. These latter cells do not stain with the antibody OPD4, the difference in reactivity possibly being due to a difference in the carbohydrate structure of the epitope presented on these cells (Chittal et al, 1988; Norton & Isaacson, 1989).

The OPD4 antibody is not, as originally claimed, specific for CD 4+ T cells. It reacts very similarly to clone UCHL1 and differs only in having a low sensitivity for T-cell lymphoma and is not reactive with monocytic cells (Poppema et al, 1991).

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CD 54 (ICAM-1)

Sources/Clones

Accurate (1304.100.40), Biodesign (84H10, 15.2, MEM-111, MEM-112), Biogenesis (MEM-12), Biogenex (BBIG-1), Biosource (BC14, RR1-1), Caltag Laboratories (MEM111), Coulter (84H10), Dako (6.5B5), Exalpha Co. (D3.6), Immunotech (84H10), Novocastra (15.2), Pharmingen (3E2, HA58), Sanbio/Monosan (MEM-111), Serotec (84H10) and Zymed (MY13).

Fixation/Preparation

Apart from clone My13, which is applicable to both frozen and paraffin-embedded tissue sections, all the other antibodies are applicable to frozen sections only. In certain instances acetone fixation is recommended.

Background

Cell-cell adhesion is critical in the generation of effective immune responses and is dependent upon the generation of a variety of cell surface receptors (Ohh & Takei, 1996). Intercellular adhesion molecule-1 (ICAM-1; CD 54) is an inducible cell surface glycoprotein expressed at a low level on a subpopulation of hematopoietic cells, vascular endothelium, fibroblasts and certain epithelial cells. However, its expression is dramatically increased at sites of inflammation, providing important means of regulating cell-cell interactions and hence inflammatory responses. ICAM-1 is induced by proinflammatory cytokines such as interleukin-1, tumor necrosis factorα or interferon-γ (Stratowa & Audette, 1995).

The CD 54 antigen (ICAM-1) is a 90 kD integral membrane glycoprotein with seven potential N-linked glycosylation sites.

Applications

The CD 54 antigen is expressed on monocytes and endothelial cells. It is also a lymphokine-inducible molecule and has been shown to be a ligand for LFA-1-mediated adhesion. Expression of the antigen can be induced or upregulated on many cell types including B and T lymphocytes, thymocytes, fibroblasts, keratinocytes and epithelial cells. In its function of mediating immune and inflammatory responses, CD 54 antigen mediates adhesion of T-cells with antigen presenting cells and is involved in T-cell to T-cell and T-cell to B cell interactions (for review, see Fleming, 1990, 1991).

Increased expression of ICAM-1 has been associated with many types of atherosclerotic lesions (Poston et al, 1992). In rejecting kidneys the antibody highlights all infiltrating cells strongly as well as glomerulus epithelium, endothelium on capillaries, vessels and mesangium (Knapp et al, 1989).

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CD 56 (Neural Cell Adhesion Molecule)

Sources/Clones

Dako (MOC-1, T199), Monosan (123C3), Research Diagnostics (ERIC-1) and Zymed (123C3).

Fixation/Preparation

Applicable to formalin-fixed paraffin sections. Requires pretreatment with microwave or pressure cooker antigen/epitope retrieval in citrate buffer. Enzymatic pretreatment has been shown to markedly decrease reactivity. The antibody to CD 56 may also be applied to frozen sections or cell smears.

Background

CD 56, the neural cell adhesion molecule (NCAM), was discovered in a search for cell surface molecules that contribute to cell-cell interactions during neural development (Rutishauer et al, 1988). Human peripheral cells capable of non-MHC restricted cytotoxicity express the CD 56 antigen. NCAM has at least three isoforms, generated by differential splicing of the RNA transcript from a single gene located on chromosome 11 (Cunningham et al, 1987). The core polypeptide of the CD 56 appears to be the 140 kD isoform of NCAM, which is variably glycosylated and sialylated to produce mature species with molecular weights ranging from 175 to 220 kD. The CD 56 antigen itself appears not to participate directly in the cytolytic activity of NK cells (Ritz et al, 1988). Subsequent immunohistochemical studies have shown that NCAM is widely expressed in neural and neuroendocrine tissues (Bourne et al, 1991). Antibody clone 123C3 recognizes a heterodimeric glycoprotein with the 145 and 185 kD isoforms of NCAM (Schol et al, 1988), whilst clone ERIC-1 has been reported with two human isoforms, 145 and 180 kD, of NCAM. T199 is a 135-220 kD single-chain glycosylated and sialylated protein expressed on CD 2+, CD 3-, CD 16+ natural killer cells (NK) and neuroectodermal cells (Feidkert et al, 1989). Autopsy tissue has been used to demonstrate strong CD 56 immunoreaction in peripheral nerve, adrenal zona glomerulosa and medulla and synapses in cerebral cortex. CD 56 also marks thyroid follicular epithelium, proximal renal tubules, hepatocytes, gastric parietal cells and pancreatic islet cells (Shipley et al, 1997).

Applications

Merkel cell carcinoma, neuroblastoma, ganglioglioma, oligodendroglioma, glioblastoma multiforme, pheochromocytoma, retinoblastoma, laryngeal and pulmonary squamous cell carcinoma, pulmonary and intestinal carcinoid, pulmonary small cell undifferentiated carcinoma, pancreatic islet cell tumor, hepatocellular carcinoma, renal cell carcinoma and follicular and papillary thyroid carcinoma mark positively with CD 56 antibodies. CD 56 has been found to be negative in Ewing sarcoma, nasopharyngeal carcinoma, colonic adenocarcinoma, melanoma, meningioma, follicular center cell lymphoma, hairy cell leukemia (one case each respectively) and multiple myeloma (five cases). However, the current major application of CD 56 on paraffin sections is in the diagnosis of NK and NK-like T-cell lymphoma, i.e. CD 56 being a marker for natural killer cells (Chan, 1997). CD 56+ lymphomas are heterogeneous, encompassing several entities: nasal/nasopharyngeal NK/T-cell lymphoma, nasal-type (extranasal) NK/T-cell lymphoma, aggressive NK-cell leukemia/lymphoma and the newly described blastoid NK-cell lymphoma. The nasal form

represents the prototype of this group and is referred to as angiocentric lymphoma in the REAL classification. Since CD 56+ lymphomas do not always show angiocentricity, and angiocentricity may occur in other lymphoma types, the term NK/T-cell lymphoma or T/NK-cell lymphoma appears to be more appropriate. Two other types of T-cell lymphoma show a particularly high frequency of CD 56 expression: hepatosplenicδγT-cell lymphoma (63% CD 56+) and S-100 protein-positive T-cell lymphoma (Chan et al 1987; Wong et al, 1995).

Comments

Clearly CD 56 antibodies are essential for the diagnosis of NK/T-cell lymphomas which show a predilection for the upper aerodigestive tract, skin, testes, skeletal muscle, gastrointestinal tract and other extranodal sites and pursue an aggressive clinical course. Furthermore, this antibody may be used to detect residual disease in CD 56+ NK/T-cell lymphoma in which the neoplastic lymphoid cells are small and show minimal atypia, especially in small biopsies.

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CD 57

Sources/Clones

Becton Dickinson (Leu7), Biodesign (NC1), GenTrak, Immunotech (NC1), Sanbio (6-13-19-1) and Serotec (NC-1).

Fixation/Preparation

Most antibodies are reactive in fixed paraffin-embedded tissues and immunoreactivity is enhanced by heat-induced epitope retrieval.

Background

CD 57 antibodies detect a 110 kd protein encoded by a gene on chromosome 11. The protein is present on some peripheral lymphocytes but not in monocytes, granulocytes, platelets or erythrocytes. CD 57+ lymphocytes increase with age and represent 10-20% of lymphocytes in most adults. They mostly include a subset of CD 8+ T lymphocytes as well as natural killer (NK) cells (Abo & Balch, 1982). A subpopulation of peripheral lymphocytes that reacts with this marker includes large granular lymphocytes. This antibody also reacts with both CD 3+ and CD 3-, non-B lymphocytes. The CD 3- lymphocytes demonstrate NK cell activity and have large cytoplasmic granules that are not seen in the CD 3+ cells. CD 3+/CD 57+ T cells are primarily suppressor lymphocytes with CD 8 expression though CD 4+/CD 8/CD 57+ T cells have been described and CD 8+/CD 57+/HLA-DR+ T cells have also been identified. CD 3+/CD 8+/CD 57+ lymphocytes are positive for CD 45RA but not CD 45RO. While this phenotype is characteristic of na□e T lymphocytes, the CD 57+ cells differ from other na□e T cells by failing to lose the CD 45RA antigen when stimulated with alloantigens. These cells also differ from other T lymphocytes by their increased ability to acquire the HLA-DR antigen in the absence of antigen-specific cytotoxic activity against allogeneic target cells.

The frequency of CD 57+ lymphocytes in solid tissues varies according to site. They are increased in term placental tissue, but not in decidua of early pregnancy. They are decreased in bronchoalveolar lavage specimens compared with peripheral blood in the same patient and represent less than 2% of all nasal mucosal lymphocytes. CD 57+ lymphocytes are rare in both the endometrium and uterine cervix. They are also rare in the thymus and in the bone marrow; they constitute no more than 1% of all nucleated cells.

CD 57+ lymphocytes have a different distribution from that of CD 8+ cells in the tonsils and lymph nodes, with the CD 57 + cells located primarily within the germinal centers. These germinal center cells are CD 3+ T cells, which also express the CD 4 antigen. Similar to the CD 57+/CD 4+ T cells in cytomegalovirus (CMV) carriers, the CD 4+ germinal center cells do not display the usual helper activity of classic CD 4+ lymphocytes (Swerdlow & Murray, 1984).

CD 57+ cells in the spleen are seen mostly in the germinal centers of the white pulp or as a rim of cells around the central white pulp (Griffiths et al, 1989).

The HNK-1/Leu7 antibody also reacts with cells other than lymphocytes. CD 57 antibodies react with an antigen present in the central and peripheral nervous system myelin and oligodendroglia and Schwann cells. Some neural adhesion molecules also contain a carbohydrate epitope that is recognized by CD 57 antibodies. The reactivity is due to part of the myelin-associated glycoprotein having a similar molecular mass (110 kD) to the CD 57 lymphocyte antigen.

Besides neural-associated cells, CD 57 antibodies immunoreact with prostatic epithelium, pancreatic islets, adrenal medulla, renal loops of Henle and proximal tubules, chromaffin cells of the gut, gastric chief cells, epithelial cells of the outer thymic cortex and some cells in the fetal bronchus. They are also detected in the prostatic seminal fluid (Arber & Weiss, 1995).

Applications

CD 57+ lymphocytes are increased in patients following bone marrow transplantation. This increase often persists for years after the procedure. The majority of these cells are CD 57-/CD 8+ T lymphocytes which form up to two-thirds of the peripheral blood T lymphocytes, with a small expansion in CD 57+/CD 4+ cells.

The relationship of this increase in CD 57+ cells and graft-versus-host disease is controversial, some workers finding a correlation between the increase in CD 57+ cells and the onset of disease while others have not. Some investigators have noted the expansion of the CD 57+ population with reactivation of CMV after transplantation, similar to the increase in CD 57+ cells seen in healthy carriers of CMV.

CD 57+ cells are also elevated in the peripheral blood in some solid organ transplant patients. Up to 20% of renal allograft, 66% of cardiac allograft and 44% of liver allograft recipients had greater than 20% peripheral blood CD 57+/CD 3+ lymphocytes, the majority of these cells also being CD 8+. As with bone marrow transplantation, the elevation of CD 57+ correlated with a rise in CMV titers and may show poorer graft survival (Legendre et al, 1989).

CD 57+ cells are also elevated in human immunodeficiency virus infections. CD 57+/CD 8+ lymphocytes are increased through the clinical progression of the infection while CD 57+/NK and CD 57- NK cells remain normal.

Peripheral blood CD 57+ cells may be increased in patients with adult-onset cyclic neutropenia whereas no elevation was seen in childhood-onset cases. The adultonset variant of cyclic neutropenia was found to be steroid responsive.

Circulating CD 57- lymphocytes are elevated in patients with Crohn's disease with many of these cells being CD 8+, corresponding to the increase in suppressor cell function found in such patients, Elevations in peripheral blood CD 57+ cells may also be seen in rheumatoid arthritis.

Large granular lymphocytosis (LGL) is by far the most common CD 57+ lymphoproliferative disorder. LGL are usually CD 2+ and may be divided into T cell and NK cell types based on CD 3 expression. CD 3+ cases are generally associated with clonal T-cell gene rearrangement. The T-cell cases are usually CD 57- and CD 8+ and may be further typed according to the presence (type 1) or absence (type 2) of the NK-associated antigen CD 16. Immunostaining of the spleen may be useful in the evaluation of resected spleens in LGL patients. CD 57+ lymphocytes are found in the splenic red pulp, while the expanded while pulp nodules are usually not involved.

Elevations of peripheral blood CD 57+ lymphocytes may be associated with non-neoplastic states such as in CMV carriers, possibly in chronic hepatitis, in ankylosing spondylitis and more frequently in rheumatoid arthritis and Felty's syndrome. Synovial fluid CD 57+ cells may also be elevated in rheumatoid arthritis. Clonal T-cell receptor gene rearrangement has been demonstrated in some cases of rheumatoid arthritis, especially those with Felty's syndrome.

NK/T-cell lymphomas frequently affect the nasal and extranodal sites and show similarities to LGL. The lymphoma cells display large cytoplasmic granules with either a T cell or NK cell phenotype. They are also mostly positive for the Epstein-Barr virus and display an angiocentric pattern of infiltration with necrosis and an aggressive clinical course. Unlike LGL, NK/T-cell lymphomas are CD 57+ in less than 10% of cases, with most cases being CD 56+ so that CD 57 antibodies alone are unreliable NK cell markers of such lymphomas. (Ng et al, 1987; Nakumara et al, 1995)

CD 57 expression is seen in just over 20% of T-lymphoblastic lymphomas, but the expression of CD 57 does not correlate with NK activity in these cases and the significance expression of this antigen is unknown. Less than 2% of other types of T-cell lymphoma are CD 57+ and the antigen does not appear to be expressed in B-cell lymphomas, monocytic leukemia or Langerhans histiocytosis. Increases in presumably non-neoplastic CD 57+ cells may be seen in the neoplastic follicles of follicular lymphomas, especially of the small cleaved cell type, and in cases of nodular L&H Hodgkin's disease where the positive cells often

rosette around CD 20+ L&H cells, providing a useful pointer to the diagnosis. The CD 57+ cells in the latter condition are also CD 4+ and can be seen in about 25-30% of cases of nodular lymphocyte-predominant Hodgkin's disease. Similar distribution and increases in CD 57+ cells were not found in nodular sclerosing Hodgkin's disease, T-cell rich B-cell lymphoma or follicular lymphoma (Sun et al, 1992; Kamel et al, 1993).

CD 57 expression may be observed in a variety of solid tumors, the most common of which are lung tumors. Almost half of small cell lung carcinomas and about 85% of carcinoid tumors are CD 57+. In non-small cell lung carcinoma, the identification of neuroendocrine-associated antigens such as CD 57 has been shown to be predictive of response to chemotherapy. The expression of CD 57 antigen in small cell carcinoma and carcinoid is generally widespread in the tumor but only focal in non-small cell lung carcinomas. Sampling errors should be taken into consideration in the assessment and because of the low sensitivity and specificity of CD 57 antibodies, other neuroendocrine-associated markers such as chromogranin, synaptophysin and neuron-specific enolase should be employed.

Other non-hematopoietic neoplasms that express CD 57 include the majority of the thyroid carcinomas, especially papillary carcinoma, while it is present in only 30% of benign thyroid proliferations. CD 57 may be used to separate medullary carcinomas from other thyroid carcinomas, although there have been some reported examples of positivity in medullary carcinomas. Strong CD 57 staining of the majority of the tumor cells is indicative of papillary or follicular carcinoma and uncommon in benign thyroid proliferations and medullary carcinoma (Ghali et al, 1992).

The CD 57 antigen is expressed in prostatic epithelium but the marker does not discriminate between benign and neoplastic cells. Epithelial cells of thymomas are usually CD 57+ while only some thymic carcinomas express the antigen. Over half of malignant mesotheliomas are reported to express CD 57 although they generally do not react with other neuroendocrine markers. Among the soft tissue tumors, the majority of neural tumors, especially neuromas, schwannomas and neurofibromas, react with CD 57 antibodies. Most malignant peripheral nerve sheath tumors are CD 57+ but the antigen may also be expressed by other sarcomas such as synovial sarcoma and leiomyosarcoma. Therefore the marker on its own is not a useful diagnostic discriminant and should be used in an appropriate panel of antibodies in order to separate the various spindled and pleomorphic soft tissue tumors. Similarly, because CD 57 may be expressed by a variety of small round cell tumors including neuroblastomas, it is not a useful diagnostic discriminant for this group of poorly differentiated tumors (Bunn et al, 1985; Michels et al, 1987; Linnoila et al, 1994).

In the central nervous system, CD 57 expression may be seen in normal oligodendroglia and other nervous system cells as well as in their corresponding tumors (Motoi et al, 1985). Oligodendrogliomas perhaps show the most extensive degree of CD 57 positivity compared to astrocytomas and glioblastomas, which, demonstrate fewer positive cells. Among skin tumors, the expression of CD 57 closely paralleled that of S100 protein although the two were not identical. Neither was useful in the distinction of eccrine from apocrine tumors (Kanitakis et al, 1987). Melanocytic proliferations and melanomas may show variable positivity for CD 57, whereas reports of the expression of this antigen in Merkel cell carcinoma are conflicting, the antigen being absent in some series and positive in half the tumors in another study. CD 57 positivity is also seen in other tumors including a large proportion of granular cell tumors, paragangliomas and pheochromocytomas. Embryonal carcinomas and dysgerminomas are also reported to be positive for CD 57 in most cases.

Comments

The CD 57 antigen is most useful in the identification of large granular lymphocyte disorders and assists in the identification of L&H cells of lymphocyte-predominant Hodgkin's disease. The CD 57+ cells that are CD 4+ T cells form rosettes around CD 20+ L&H cells. Elevation of peripheral blood CD 57+ lymphocytes may be seen following some viral infections, such as CMV, in patients following bone

marrow or solid organ transplantation. CD 57 should not be used alone as a marker of NK cells neuroendocrine cells or neural cells and must be employed in

combination with other antibodies in a panel, particularly if used for diagnostic purposes. The antibody is immunoreactive in fixed, paraffin-embedded tissues, especially following heat-induced epitope retrieval.

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CD 68

Sources/Clones

Accurate (EVM11), Biodesign (BL-M68), Caltag Laboratories (BLM68), Dako (KP1, PG-M1, EBM 11), Sanbio/Monosan/Accurate (BLAD8) and Serotec (KiM6).

Fixation/Preparation

Apart from EBM 11 which is only applicable to frozen sections, KP1 and PG-M1 monoclonal antibodies are applicable to formalin-fixed paraffin sections, acetone-fixed cryostat sections and fixed-cell smears. Antimacrophage reagents recognizing formalin-resistant epitopes require microwave or enzyme pretreatment with trypsin or pronase before immunostaining to reduce background staining.

Background

The best macrophage reagents produced to date are those recognizing the CD 68 antigen (Knapp, 1989). This 110 kD antigen belongs to a family of acidic, highly glycosylated lysosomal glycoproteins that include the lamp-1 and lamp-2 molecules (Fukuda, 1991). CD 68 is the human homologue of the murine macrosialin antigen (Holness et al, 1993) and is present in the cytoplasmic granules of monocytes, macrophages, neutrophils, basophils and large lymphocytes (Pulford et al, 1990). This antigen is also expressed to some degree in the cytoplasm of some non-hemopoietic tissue. However, the function of the molecule is currently unknown.

The monoclonal antibody KP1 (IgG1, k) was raised against lysosomal granules prepared from lung macrophages (Pulford et al, 1989) and recognizes the 110 kD CD 68 antigen. This antibody labels monocytes and macrophages in a wide range of tissues, e.g. lung macrophages, germinal center macrophages and Kupffer cells. Osteoclasts and myeloid precursors in bone marrow are also strongly labelled. In frozen sections, KP1 stains endothelium and hepatocytes weakly. Strong labeling of blood monocytes (granular/cytoplasmic), neutrophils and basophils is also demonstrated with KP1. KP1 antigen is expressed as an intracytoplasmic molecule, associated with lysosomal granules.

The murine PG-M1 monoclonal antibody (IgG3, K) was raised against spleen cells of Gauchers disease (Falini et al, 1993). Reactivity with cells transfected with a human cDNA encoding for the CD 68 antigen confirms PG-M1 as a member of the CD 68 cluster. In normal tissue, PG-M1 is comparable to KP1; however, in bone marrow paraffin sections, PG-M1 strongly stains macrophages but *not* granulocytes and myeloid precursors. PG-M1 also shows immunopositivity with mast cells and synovial cells.

Applications

Malignant histiocytosis and true histiocytic lymphoma express the CD 68 macrophage marker (Ralfkiaer et al, 1990). These tumors should be CD 68+, but be unlabelled with antibodies to CD 30, T- and B-cell antigens and cytokeratins. Acute myeloid leukemias (AML) are identified by the presence of CD 68 antigen (Warnke et al, 1989; Thiele et al, 1992). Whilst KP1 recognizes M1-M5 types, PG-M1 immunoreaction is confined to M4 (myelomonocytic) and M5 (monocytic) types of AML. The CD 68 antibodies are also able to distinguish between monocyte/macrophage and lymphoid leukemias. Whilst this is useful in identifying granulocytic sarcoma, some B-cell neoplasms (notably small lymphocytic lymphoma and hairy cell

leukemia) show weak cytoplasmic staining in the form of a few scattered granules. Mast cell proliferations and "plasmacytoid monocytes" are usually stained by both the KP1 and PG-M1 antibodies. The CD 68 antigen is also expressed to varying degrees in Langerhans and interdigitating reticulum cell sarcomas, as well as Langerhans cell histiocytosis (Ruco et al, 1989).

Macrophages may be present either as rare scattered cells or large cellular infiltrates in some T- and B-cell lymphomas, leading to erroneous diagnoses of histiocytic malignancies. Dual immunocytochemical labeling with CD 68 antigen and T/B-cell antigen is useful in delineating the two populations. The identification of macrophages is also crucial in the diagnosis of granulomatous diseases, storage diseases and certain types of lymphadenitis, e.g. Kikuchi's lymphadenitis. In the latter condition, macrophages phagocytosing apoptotic bodies and cells known as "plasmacytoid monocytes" and "crescentic histiocytes" are easily recognized with antibodies against CD 68, avoiding the misdiagnosis of a high-grade lymphoma.

Comments

Caution is advised in the immunophenotypic interpretation of histiocytes, since the distinction between "uptake" and "synthetic" patterns should be borne in mind. KP1 would appear to be superior to PG-M1, particularly with respect to the wider recognition of AML. The latter antibody also carries the distinct disadvantage of being demonstrated in about 10% of melanomas. Tissue rich in macrophages is suitable as a positive control.

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CD 74 (LN2)

Sources/Clones

Ancell/Pharmingen (MB741), American Research Products (MB3), Biodesign (BU43, BU45), Biogenex (LN-2), Cymbus Bioscience (BU45), Harlan Seralab/Accurate (2G5), ICN Biomedicals (LN-2), Dako (LN2), Immunotech (LN2), Novocastra (LN-2), Pharmingen (LN2), RDI (BU45, LN2), Serotec (BU 45), Sigma (LN2) and Zymed (LN2).

Fixation/Preparation

This antibody is applicable to formalin-fixed, paraffin-embedded tissue sections, frozen sections and cytological preparations. Immunoreaction may be improved with microwave antigen retrieval in citrate buffer.

Background

The CD 74 antigen represents a membrane-bound subunit of the MHC class II-associated invariant chain (Wilson et al, 1993) that is encoded by the gene located on chromosome 5 region q31-q33 (Moller, 1995). The monoclonal antibody LN2 recognizes nuclear and cytoplasmic antigens of molecular weights 35 kD and 31 kD respectively in routinely processed tissues. MB-3, another mononuclear antibody, is thought to be identical to LN2. LN2 reacts with about 50% and 75% of activated and resting L20+ B cells in the peripheral blood and tonsils respectively. LN2 is positive in less than 3% of CD 3+ T cells. Very weak staining may be seen on circulating monocytes and granulocytes are negative.

In lymph nodes, LN2 positivity is seen primarily in germinal center and mantle cells. Staining is strongest in small germinal center cells and in mantle cells. Plasma cells are not labelled. The vast majority of cells in the interfollicular areas are negative except for interdigitating dendritic reticular cells, which are often strongly positive. Besides distinct staining of the nuclear membrane, there may be diffuse or paranuclear cytoplasmic staining, the pattern of staining being similar in both fixed and frozen tissue sections.

Thymocytes are negative for LN2 but thymic dendritic cells may often be positive. Other cells that may be positive for LN2 include sinusoidal histiocytes, epithelioid histiocytes, splenic red pulp histiocytes and Langhanstype giant cells. In addition, some epithelial cells and corresponding carcinomas may be positive but the staining of LN2 in these cells is often diffuse in the cytoplasm and the distinctive nuclear membrane staining is not observed (Epstein et al, 1984; Okon et al, 1985).

Applications

The LN2 antibody stains about 90% and 20% of low-grade B- and T-cell lymphomas respectively. In high-grade lymphomas, the corresponding figures are 85% and 75% respectively so that its value as a discriminator is less in large cell lymphomas. The pattern of labeling is also different. In small lymphocytic lymphoma, LN2 shows either nuclear membrane or dot-like cytoplasmic positivity whereas, in small cleaved cells nuclear membrane staining is the predominant pattern. In the mixed cell lymphomas and large cell lymphomas, LN2 stains the nuclear membranes of the small cleaved cells but only some of the larger cells, exhibit cytoplasmic staining, the minority displaying bright cytoplasmic globules.

Reed-Sternberg cells also stain with LN2, exhibiting cytoplasmic, cytoplasmic membrane and nuclear membrane staining in about two-thirds of cases. The antigen is expressed in about 60% of precursor B-cell ALLs/LBLs,

about 50% of AMLs (excluding FAB M6 AML), most cases of CML, granulocytic sarcomas and true histiocytic sarcomas (Norton & Isaacson, 1989a, b).

A recent study showed that LN2 antigen is strongly expressed by cells of malignant fibrous histiocytoma (MFH) but not atypical fibroxanthoma (AFX) (Lazova et al, 1997). LN2 immunoreactivity appears to distinguish between these two histologically similar yet biologically distinct tumors with a high degree of statistical significance. LN2 has also been observed to label some epithelial tumors, including adenocarcinoma of the uterus, squamous cell carcinoma of the lung and transitional cell carcinoma of the bladder (Epstein et al, 1984).

Comments

LN2 is immunoreactive in formalin-fixed, paraffin-embedded tissue sections. It is only poorly reactive in ethanol-fixed tissues and B5 fixation produces reduced positivity and a high background staining. The staining in B5-fixed tissues tends to be of the nuclear membranes and the perinuclear cytoplasm of B cells whereas, in formalin-fixed tissues, paranuclear dot-like globules are more common. Trypsinization destroys LN2 reactivity and neuraminidase treatment does not affect it (Yoshino et al, 1990). Given its contrasting immunoreactivity, it is possible that other applications of LN-2 are yet to be discovered. Benign or neoplastic B-cell tissue is recommended for optimization of this antibody.

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CD w75 (LN1)

Sources/Clones

Biogenex (LN1), Dako (LN1), Immunotech (LN1), Novocastra, Sigma Chemical (LN1), Zymed (LN1).

Fixation/Preparation

LN1 is applicable to formalin-fixed, paraffin-embedded tissue sections. Enzyme or heat pretreatment before immunostaining improves immunodetection.

Background

The CD w75 epitope is a sialylated carbohydrate determinant generated by the β-galactosyl α 2,6 sialyltransferase and has a molecular weight of 53 kD. Sialyltransferase catalyzes the incorporation of sialic acid to the carbohydrate group of glycoconjugates. Alterations on the cell surface of the oligosaccharide portion of glycoproteins and glycolipids are thought to play a role in tumorigenesis (Reed et al, 1993). Sialyltransferase has been found to be elevated in different tumor tissues and in serum of cancer patients. Further, the amount of sialic acid correlates with the invasiveness and metastasizing potential of several human tumors. Therefore the CD w75 epitope can be viewed as a target for identifying biologically aggressive tumors (David et al, 1993).

LN1 belongs to the CD w75 group of antibodies and recognizes a sialo antigen (45-85 kD). LN1 stains B lymphocytes in the germinal center with no reaction with T cells. It also reacts with a variety of epithelial cells including distal renal tubules, mammary glands, bronchus and prostate.

Applications

CDw 75 antigen expression has been examined in breast lesions (Reed et al, 1993). Duct carcinoma showed diffuse cytoplasmic staining in 21% of in situ and 35% of invasive carcinomas respectively. No correlation was demonstrated between immunoreactivity for CD w75 in breast carcinomas and their metastatic potential. However, CD w75 was more frequently expressed in high-grade carcinomas. A positive immunoreaction was demonstrated in benign proliferating lesions: intraductal papillomas (2/3) and epitheliosis in fibrocystic disease (10/14). This high frequency of immunoreactivity among the benign breast lesions was ascribed to activation of epithelial cells.

CD w75 epitope expression has also been examined in gastric carcinomas and their metastases (David et al, 1993). Forty-one cases (47%) were immunopositive for CD w75 antigen in the primary tumors or metastases. In contrast to breast carcinomas, a close relationship was found between antigen in primary tumors and their respective metastases. In addition, antigen expression correlated with an infiltrative growth pattern, lymphatic invasiveness and aneuploidy whilst no correlation was found with gastric carcinoma morphology, lymphoid infiltrate, vascular invasion and gastric wall penetration. Hence, CD w75 expression appears to be a good indicator of the biologic aggressiveness of gastric carcinoma.

LN1 is an excellent marker for B-cell lymphomas, especially follicular-derived lymphomas. In B cells, the LN1 antibody produces a typical membrane and cytoplasmic (paranuclear "dotlike" or Golgi) staining pattern. No immunoreaction is present with small lymphocytic lymphomas and T-cell lymphomas. LN1 also reacts with L&H cells in nodular lymphocyte-predominant Hodgkin's disease.

Comments

In view of the contrasting results between breast and gastric carcinoma, further studies examining CD w75 expression in these and other cancers are awaited. Follicular lymphomas or epitheliosis of the breast or gastric carcinoma are most suitable for use as positive control tissue.

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CD 79a

Sources/Clones

Dako (JCB117, HM57) and Immunotech (HM47).

Fixation/Preparation

This antibody is applicable to paraffin-embedded tissue sections. Heat pretreatment in citrate buffer is necessary for antigen retrieval to improve staining pattern. CD 79a may also be applied to acetone-fixed cryostat sections and fixed-cell smears.

Background

Membrane-bound immunoglobulin (mIg) on human B lymphocytes is non-covalently associated with a disulfide-linked heterodimer, which consists of two phosphoproteins of 47 kD and 37 kD, encoded by the *mb*-1 and *B*29 genes respectively (Mason et al, 1991; Van Noesel et al, 1991). Association of IgM with the *mb*-1 protein is necessary for membrane expression of the B-cell antigen receptor complex. When antigen is bound to this B-cell complex, a signal transduction is transmitted to the interior of the cell, accompanied by phosphorylation of several components following induction of tyrosine kinase activity. The *mb*-1/*B*29 dimer seems to be analogous to the association of the T-cell receptor with the CD 3 components (Homback et al, 1990).

Studies have shown that *mb*-1 is present throughout B-cell differentiation and is B-cell specific (Mason et al, 1991). Its high degree of specificity is probably a reflection of its crucial role in signal transduction after antigen binding to the B-cell antigen receptor complex. The *mb*-1 and *B*29 proteins were designated CD 79a and CD 79b at the Fifth International Workshop on Human Leukocyte Differentiation Antigens (Boston 1993). JCB117 was raised against the recombinant protein containing part of the extracellular portion of the CD 79a (*mb*-1) polypeptide (Mason et al, 1995). Clone HM57 was raised against a synthetic peptide sequence comprising amino acids 202-216 of *mb*-1 protein (Sakaguchi et al, 1988). This oligopeptide represents the intracytoplasmic C-terminal part of the *mb*-1 protein.

Applications

The *mb*-1 (CD 79a) chain appears before the pre-B cell stage and is still present at the plasma cell stage. JCB117 reacts with human B cells in paraffin-embedded tissue sections, including decalcified bone marrow trephines. When applied to 454 paraffin-embedded tissue biopsies, it reacted with the majority (97%) of B-cell neoplasms. This covered the full range of B-cell maturation including 10/20 cases of myeloma/plasmacytoma. This antibody also labeled precursor B-cell acute lymphoblastic leukemia, making it the most reliable B-cell marker detectable on paraffin-embedded specimens (Mason et al, 1995). T-cell and non-lymphoid neoplasms were negative, indicating that JCB117 may be of value in the identification of B-cell neoplasms.

The *mb*-1 protein has also been detected in nodular lymphocyte-predominant Hodgkin's disease using monoclonal antibody JCB117; however, only 20% of non-lymphocyte-predominant cases expressed *mb*-1.A rare phenotypic characterization has been demonstrated in mediastinal large B-cell lymphomas, with the majority being mb-1+/ Ig-(Kanavaros et al, 1995).

Comments

We have found JCB117 to be superior to HM57, the latter demonstrating cross-reactivity

with smooth muscle in paraffin sections.

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CD 99 (p30/32^{MIC2})

Sources/Clones

Dako (12E7), Pharmingen (MIC2) and Signet (013).

Fixation/Preparation

All three clones show enhanced immunoreactivity following some method of heat-induced epitope retrieval.

Background

The p30/32^{MIC2} antigen, also referred to as CD 99 or the MIC2 gene product, is a cell-surface glycoprotein of relative molecular mass of 30 000-32 000 that appears to be involved in cell adhesion processes. It is recognized by a number of monoclonal antibodies including RFB-1, 12E7, HBA71 and 013, although there is some demonstrable difference in sensitivity and perhaps specificity.

CD 99 was first described as a polypeptide expressed in T-cell acute lymphoblastic leukemia and T-ALL derived cell lines, as well as in a subset of cortical thymocytes. It was also found on a group of hematopoietic precursor cells in the human bone marrow including terminal deoxynucleotidyl transferase-positive cells and myelomonocyte progenitors, the expression decreasing with maturation of cells in the latter series. The MIC2 gene has been mapped to the terminal region of the short arm of the X chromosome (Xp22.32-pter) and the euchromatin region of the Y chromosome (Yq11-pter). The gene is expressed in both sexes and escapes X inactivation, making it the first described pseudoautosomal gene in humans (Dracopoli et al, 1985; Fellinger et al, 1991).

The main application of this antigen has been for the differentiation of the group of small round cell tumors in childhood as the marker is strongly expressed in Ewing's sarcoma and the closely related peripheral/primitive neuroectodermal tumors (PNETs). Both show strong membrane and cytoplasmic staining with clones 12E7, HBA71 and 013 (Stevenson et al, 1994; Weidner & Tjoe, 1994; Vartanian et al, 1996). Subsequent studies have also demonstrated positive staining in acute lymphoblastic lymphoma and related leukemias and rhabdomyosarcoma, although to a much lesser degree (Ramani et al, 1993). More recently, immunoreactivity for this marker has been shown in a much wider spectrum of normal tissues and ependymal cells, pancreatic islet cells, urothelium, some squamous cells, columnar epithelial cells, fibroblasts, endothelial cells and granulosa/Sertoli cells. Among the spindle cell neoplastic tissues which show variable positivity for CD 99 are synovial sarcomas, hemangiopericytomas, meningiomas, solitary fibrous tumors and, only very rarely, mesotheliomas. Epithelial tumors expressing CD 99 include neuroendocrine tumors such as islet cell tumors, carcinoid tumors and pulmonary oat cell carcinomas but apparently not Merkel cell carcinomas of the skin (Soslow et al, 1966). Granulocytic sarcomas have been shown to stain for CD 99 (Cooper & Haffajee, 1995).

Positive staining for CD 99 occurs as strong membrane immunolocalization whereas variable heterogeneous staining may be seen in some cases of non-Hodgkin's lymphoma and in occasional Reed-Sternberg cells and their variants.

Applications

CD 99 antibodies have proven usefulness for the separation of Ewing's sarcoma and PNETs from the other small round cell tumors in childhood (Pappo et al, 1993;

Lumadue et al, 1994) (Appendix 1.3). In addition, this marker can be employed as a diagnostic discriminator for the identification of thymic cortical T cells associated with thymic neoplasms (Chan et al, 1995; Dorfman & Pinkus, 1996) (Appendix 1.11) and in the differential diagnosis of spindle cell tumors. The latter include synovial sarcoma, hemangiopericytoma, meningioma and solitary fibrous tumors, all of which show variable extents of positivity (Renshaw, 1995). The recent demonstration of CD 99 in mesenchymal chondrosarcoma (Granter et al, 1996) emphasizes the need for caution if this marker is to be employed as a diagnostic discriminator for small round cell tumors.

Comments

Immunoreactivity is enhanced following heat-induced epitope retrieval. Both 013 and 12E7 have been very effective in our hands but it should be noted that they show different sensitivities, perhaps reflecting different specificities.

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c-erbB-2 (Her-2, neu)

Sources/Clones

Accurate (CB11, CBE1, polyclonal), Becton Dickinson (3B5), Biogenesis (2G2-91, LY369), Biogenex (EGFR), Coulter (3B5), Dako (polyclonal), Lab Vision (9G6.10, L87, N12, N24, N28.6), Novocastra (CB11, CBE1), Oncogene (CNeu), Pharmingen (9G6) and Dako (polyclonal antibodies).

Fixation/Preparation

Most antibodies are immunoreactive in fresh-frozen tissue sections as well as in fixed paraffin-embedded sections. HIER enhances immunoreactivity. Enzyme treatment is not necessary.

Background

The c-*erb*B-2 oncogene was discovered in the 1980s by three different avenues of investigation. The *neu* oncogene was detected as a mutated transforming gene in neuroblastomas experimentally induced in fetal rats. The c-*erb*B-2 was a human gene discovered by its homology to the retroviral gene v*erb*B, and HER-2 was isolated by screening a human genomic DNA library for homology with v*erb*B. When the DNA sequences were determined subsequently, c-*erb*B-2, HER-2 and *neu* were found to represent the same gene.

The c-*erb*B-2 gene is located on human chromosome 17q21 and codes for the c-erbB-2 mRNA (4.6 kb), which translates to the c-*erb*B-2 protein (p185). The c-*erb*B-2 oncogene is homologous with, but not identical to, c-*erb*B-1, which is located on chromosome 7 and encodes for the epidermal growth factor receptor. The c-*erb*B-2 protein is a normal cell membrane component of all epithelial cells with extracellular, transmembrane and intracellular tyrosine kinase activity (Lupu et al, 1992). Apart from this growth stimulatory function, the molecule plays an important role in the motility of tumor cells by the activity of a motility factor, which acts as a specific ligand for the c-*erb*B-2 protein. The motility factor induces chemotaxis of c-*erb*B-2 overexpressing breast cancer cells and may lead to an increased metastatic potential (De Potter, 1994).

c-*erb*B-2 gene alterations have been reported in diverse human neoplasms and almost exclusively involve amplification of the gene. Amplification involves the repeated duplication of a particular gene sequence, resulting in multiple gene copies within each cell. This results in overexpression of the gene product, as reflected in the levels of mRNA and gene oncoprotein. There is generally good correlation of the c-*erb*B-2 gene amplification with overexpression (Smith et al, 1994).

Applications

c-*erb*B-2 has been shown to be amplified in about 20-30% of invasive breast carcinomas and various studies have correlated the gene amplification or overexpression with other prognostic variables in breast cancer patients. Although these studies have not provided sufficient information on survival outcome to evaluate the prognostic implications of c-*erb*B-2 overexpression, almost all have shown a strong correlation with various established adverse factors including large tumor size, unfavorable histologic subtype, high histologic grade, high mitotic index and proliferative activity, positive nodal status, presence of hematogenous spread and aneuploidy (De Potter et al, 1990; Borg et al, 1991; Horiguchi et al, 1994).

c-*erb*B-2 overexpression is more common in invasive ductal and medullary carcinomas than in

lobular, colloid and papillary carcinomas. In intraductal carcinomas, it is almost exclusively seen in large cell, high nuclear grade, estrogen receptor-negative, comedo-type intraductal carcinoma. In contrast, in situ lobular carcinoma seldom shows overexpression of the oncoprotein. Overexpression is more common in invasive tumors associated with an intraductal component than in those without and there is usually concordance between the invasive and intraductal components of an individual tumor.

Despite the universal observation of a strong correlation with various adverse prognostic factors, the conflicting data regarding the prognostic value of c-*erb*B-2 suggest that overexpression of the oncoprotein may not be a powerful predictor by itself. In any individual patient, it should be employed as part of a multivariate approach to guiding treatment and determining prognosis. Overexpression of c-*erb*B-2 may also serve as a predictor of response to adjuvant treatment, predicting a poor response to chemotherapy and a lack of response to endocrine therapy on relapse and identifying those patients who are most likely to benefit from high-dose regimens (Muss et al, 1994).

Furthermore, as c-*erb*B-2 protein has an extracellular domain and tends to be expressed in more aggressive tumors, it is a potential target for immunotherapy.

Comments

Occasional reports have noted discrepancies between the demonstration of amplification of the *erb*B-2 gene and detection of protein overexpression by immunostaining, but despite this drawback, immunohistochemistry now appears to be the method of choice in most institutions for assessing c-*erb*B-2 overexpression (Bobrow et al, 1996). Only membrane staining should be accepted as positive staining and we have found the polyclonal antibody from Dako and monoclonal C*neu* to be the most sensitive. HIER enhanced staining and although it also produced some increase in cytoplasmic staining, this was not a hindrance to interpretation.

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Chlamydia

Sources/Clones

American Research Products (C512F), American Research Products/EY Labs (polyclonal), Biogenex (LM-9, 16-UB), Dako (RR402) and Fitzgerald (polyclonal).

C. Trachomatis

Accurate (115), Biogenesis (polyclonal), Biodesign (168, JDC1), Pharmingen (CHL888). Biokit, Barcelona, Spain and Syva Micro Trak, Palo Alto, CA.

C. Trachomatis 60 kD

Biodesign (168) and Biogenesis (polyclonal).

C. Psittaci

Biogenesis (73-0200, 77-05) and Kallestadt Diagnostics, Chaska, MN.

Fixation/Preparation

Most antibodies are applicable to routine formalin-fixed, paraffin-embedded tissue.

Background

Genital chlamydial infection is recognized as the world's most common sexually transmitted disease (WHO, 1990). In the majority of cases the condition is asymptomatic*C. trachomatis* is associated with various complications of pregnancy (Lan et al, 1995), premature birth and neonatal difficulties (Donders et al, 1991; Gencay et al, 1995). A monoclonal antibody specific for the outer membrane proteins of *C.trachomatis* is available.

C. psittaci is the causative agent of psittacosis. It infects a diverse group of animals, including birds, humans and other mammals. It is a cause of abortion in sheep, cattle and goats (Schlossberg, 1995). Transmission to humans is incidental, with a history of direct contact with contaminated products of conception. The disease is characterized as a mild-to-moderate flu-like illness (Gherman et al, 1985). However, in pregnancy the human host is especially vulnerable. Gestational psittacosis typically presents as a progressive febrile illness with headaches, complicated by abnormal liver enzymes, low-grade disseminated intravascular coagulopathy, atypical pneumonia and abnormal renal function (Hyde & Benirschke, 1997). Management includes termination of pregnancy with aggressive antibiotic therapy (Khatib et al, 1995).

Applications

Diagnosis of gestational psittacosis is dependent on histopathological findings which consist of an intense acute intervillositis, perivillous fibrin deposition with villous necrosis and large irregular basophilic intracytoplasmic inclusions within the syncytiotrophoblast (Wong et al, 1985).The application of genusspecific monoclonal antichlamydial antibody is useful for the rapid confirmation of the diagnosis (Mahoney et al, 1987).

C.trachomatis is a major cause of genital infection. The acquired infection tends to persist and is usually symptom free (Beatty et al, 1994). Consequently fetal exposure to chlamydial infection is high, with *C.trachomatis* being demonstrated in the placenta (Gencay et al, 1997). More often, basophilic intracytoplasmic inclusions are detected in cervical smears, where genus-specific antibody may be

applied for diagnostic confirmation. In lymphogranuloma venereum, a small ulcerating primary lesion develops in the genitalia, followed by involvement of draining lymph nodes with a suppurative granulomatous inflammation, necrosis and scarring.

Inclusion bodies may also be demonstrated in lung tissue and

secretions in atypical pneumonias caused by*C.trachomatis*. Trachoma/trachoma inclusion conjunctivitis or TRIC infection is common in the tropical zones, being responsible for blindness. The organism initially infects the conjunctival epithelium and it can be demonstrated in smears of these cells by the presence of characteristic intracytoplasmic inclusion bodies.

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Chromogranin

Sources/Clones

Antibodies to chromogranin A are available from Accurate (A3), Biogenesis (A11, LK2H10), Biogenex (A11, LK2H10), Camon (LK2H10), Cymbus Bioscience (LK2H10), Dako (DAK-A3, polyclonal), Diagnostic Biosystems (DAK-A3), Enzo (PHE5), Immunotech (LK2H10, C3420), Milab (CH), RDI (LK2H10), Novocastra (LK2H10), Medac, Sanbio (LK2H10), Saxon, Serotec (LK2H10, C3420) and Zymed.

Fixation/Preparation

The antibodies are immunoreactive in fixed, paraffin-embedded sections and frozen sections. HIER does not result in significant enhancement. Fixation in Bouin's or B5 fixative may improve immunogenicity. Proteolytic digestion does not improve immunostaining.

Background

The chromogranins are a family of soluble acidic proteins of about 68 kD. They are the major proteins in the peptide-containing dense core (neurosecretory) granules of neuroendocrine cells and sympathetic nerves. Ultrastructural examination has confirmed the localization of chromogranins to the matrix of neurosecretory granules of neuroendocrine cells. While having different molecular weights, the chromogranin subunits are neither identical nor entirely dissimilar and may differ in only two or three amino acid residues, with a minimum homology between any pair of polypeptides of about 33%. The chromogranins in neuroendocrine tissues display both quantitative and qualitative variability. They occur in the highest concentration in the following rank order: the adrenal medulla; anterior, intermediate and posterior pituitary; pancreatic islets; small intestine; thyroid C cells; and hypothalamus.

The antibody clone LK2H10 to chromogranin of 68 kD labels most normal neuroendocrine cells and their corresponding neoplasms. The LK2H10 clone was derived from human pheochromocytoma and exhibits crossreactivity with monkey and pig chromogranins (Wilson & Lloyd, 1984).

Chromogranins are thought to stabilize the soluble portion of neurosecretory granules by interaction with adenosine triphosphate and catecholamines and are released into the serum after splanchnic stimulation. They have multiple roles in the secretory process of hormones. Intracellularly, they are involved in targeting peptide hormones and neurotransmitters to granules of the regulated pathway by virtue of their ability to aggregate in the low-pH, high-calcium environment of the trans-Golgi network. Extracellular peptides formed as a result of proteolytic processing of chromogranins regulate hormone secretion. The synthesis of chromogranins is regulated by many different factors, including steroid hormones and agents that act through a variety of signaling pathways (Hendy et al, 1995).

Applications

The major applications of antibodies to the chromogranins are for the identification of neuroepithelial/neuroendocrine differentiation in normal and neoplastic tissues (Hirose et al, 1995; Blumenfeld et al, 1996), as well as the neural elements of the brain (Schiffer et al, 1995) and gut (Shen et al, 1994). Initial experience with clone LK2H10 to chromogranin A revealed less than 100% sensitivity for neuroendocrine cells, especially among those cells and tumors with low concentrations of

neurosecretory granules and among tumors such as insulinomas, somatostatinomas, prolactinomas and corticotropin-and growth hormone-producing adenomas. However, the rate of positivity has improved with the use of more sensitive immunolabeling procedures.

Chromogranin is the most specific marker for neuroendocrine differentiation and corresponds to the neurosecretory granule, the hallmark of the neuroendocrine cell (Appendices 1.3, 1.19, 1.26). While it may be used with other neuroendocrine markers such as NSE and PGP9.5 to improve the diagnostic yield, chromogranin and synaptophysin are the most specific of all neuroendocrine markers.

Comments

As neurosecretory granules tend to be localized beneath the plasma membranes of neuroendocrine cells, their highest density is within the cytoplasmic processes characteristic of such cells. As such, staining for chromogranin often highlights the cytoplasmic processes often not visible in H&E stains. Aberrant immunoreactivity for chromogranin has been described in normal and neoplastic urothelium, particularly in the umbrella cells, attributed to reactivity with chromogranin-like proteins in the transitional cells (Mai et al, 1994).

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c-Myc

Sources/Clones

Biogenesis (9E11), Caltag Laboratories (polyclonal), Chemicon, Fitzgerald (polyclonal), Novocastra (polyclonal), Oncogene (9E10, 8, 33), Pharmingen (9E10) and Serotec (CT14, polyclonal).

Fixation/Preparation

Several clones, including 9E10, are immunoreactive in acetone- or formalin-fixed, paraffin-embedded tissue sections.

Background

Myc is the product of the early-response gene *myc*. The *myc* family of oncogenes, *c-myc* and N-*myc*, on chromosome 8, encodes three highly related regulatory cycle-specific nuclear phosphoproteins. Myc protein contains a transcriptional activation domain and a basic helix-loop-helix-leucine zipper DNA-binding and dimerization domain. As a heterodimer with a structurally related protein, Max, Myc can bind DNA in a sequence-specific manner, suggesting that the Myc/Max heterodimer functions as a transcriptional activator of genes critical for the regulation of cell growth (Prins et al, 1993; Vastrik et al, 1994). When overexpressed or hyperactivated as a result of mutation in certain types of cells, *myc* can cause uncontrolled proliferation. There is evidence that *myc* may have a critical role in the normal control of cell proliferation and cells in which *myc* expression is specifically prevented in vitro will not divide even in the presence of growth factors. Conversely, cells in which *myc* expression is specifically switched on independently of growth factors cannot enter G_0. If the cells are in G_0 when Myc protein is provided, they will leave G_0 and begin to divide even in the absence of growth factors, a behavior that ultimately causes them to undergo programed cell death or apoptosis.

The presence of a single oncogene is not usually sufficient to turn a normal cell into a cancer cell. In transgenic mice that are endowed with *myc* oncogene, some of the tissues that express the oncogene grow to an exaggerated size and with the passage of time, some cells undergo further changes and give rise to cancers. However, the vast majority of cells in the transgenic mouse that express the *myc* oncogene do not give rise to cancers, showing that the presence of a single oncogene is not enough to cause neoplastic transformation. Nonetheless, *myc* expression produces an increased risk as the presence of another oncogene such as *ras* results in a synergistic effect known as oncogene collaboration. The synergism increases the incidence of cancers in the transgenic mouse to a much higher rate, although the cancers originate as scattered isolated tumors among noncancerous cells. Even with the presence of two expressed oncogenes, the cells must undergo further, randomly generated changes to become cancerous.

In follicular B-cell lymphomas, collaboration between myc and the *bcl*-2 gene occurs. If *myc* alone is overexpressed, cells are driven round the cell cycle inappropriately but this does not result in lymphoma because the progeny of such forced divisions die by apoptosis. If *bcl-2* is overexpressed at the same time, the excess progeny survive and proliferate as *bcl-2* acts as an oncogene by inhibiting apoptosis.

Applications

The ability to stain for c-Myc in tissue sections has understandably been received with great interest and several attempts have been made to use the oncoprotein as a prognostic marker. For example, in

squamous cell carcinoma of the head and neck significant negative correlation has been shown between c-Myc levels and the number of metastatic nodes and clinical stage of disease but no correlation was found with tumor size or degree of differentiation (Gapany et al, 1994). Other recent applications have included c-Myc protein expression, in prostatic carcinoma (Fox et al, 1993), pituitary adenomas (Lloyd & Osamura, 1997, ovary (King et al, 1996), lung (Prins et al, 1993) and colon (Agnantis et al, 1991), among other tumors.

The examination of c-Myc as a marker for persons at risk of various types of cancer including breast carcinoma is another potential useful application (Hehir et al, 1993).

Comments

Clone 9E10 is immunoreactive in formalin-fixed, paraffin-embedded tissue sections.

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Collagen Type IV

Sources/Clones

Accurate (COL-4), Axcel (CIV22), Biodesign (1o42, MC4HA), Biogenesis (2D8/29), Biogenex (CIV22), Biotec (XCD02), Dako (CIV22), ICN (polyclonal, 1042), Immunotech (CIV22), Milab, Sanbio (SB11), SeraLab (1042) and Serotec (CIV22, PHM-12)

Fixation/Preparation

Most commercial clones of antibodies are immunoreactive in fixed paraffin-embedded sections but only following HIER and enzymatic predigestion with trypsin before the application of the primary antibody.

Background

Basal lamina is mostly formed by a dense 40-60 nm-thick layer called the lamina densa and an electron-lucent layer adjacent to the cell membrane known as the lamina lucida. A loose layer of connective tissue, known as the lamina reticularis, may be present under the lamina densa. Type IV collagen localizes exclusively to the lamina densa and by immunoelectron microscopy is found in both lamina densa and lamina lucida. Laminin has the same distribution but appears to be more intensely localized to the lamina lucida. Other components of basal lamina include heparin sulfate proteoglycan, entactin, fibronectin and type V collagen, the latter probably a stromal rather than basal lamina component.

Applications

Diagnostic applications of collagen type IV immunostaining have mostly centered around the demonstration of basal lamina in invasive tumors, particularly epithelial tumors, and their changes with tumor invasion and metastasis (Birembaut et al, 1985). In particular, the demonstration of an intact basal lamina has been used to distinguish benign glandular proliferations such as microglandular adenosis and sclerosing adenosis from well-differentiated carcinoma like tubular carcinoma of the breast (Raymond & Leong, 1991; Tavassoli & Bratthauer, 1993). Immunostaining for collagen type IV has also been applied to discriminate between C-cell hyperplasia and microscopic medullary carcinoma of the thyroid. The former showed complete investment of the C-cells by a continuous rim of basal lamina, whereas the latter was typified by deficiencies of the basal lamina so that the constituent C-cells were extrafollicular in location (McDermott et al, 1995). There was also focal reduplication of basal lamina, apparently tumor derived. Studies of collagen type IV in the matrix proteins and basal lamina of glomeruli and tubules have been reported (Schleucher & Olgemoller, 1992; Ziyadeh, 1993). Immunostaining for basal lamina has been shown to be a rapid and useful way to distinguish major variants of congenital epidermolysis bullosa, especially when electron microscopy is not available (Bolte & Gonzalez, 1995).

Distinctive patterns of basal distribution were recently demonstrated in various types of soft tissue tumors, adding to the diagnostic armamentarium for this group of neoplasms which are often difficult to separate (Leong et al, 1997) (Appendix 1.15). While the presence of basal lamina cannot be used as an absolute discriminant for blood vessels and lymphatic spaces, the latter lack the reduplication of the basal lamina characteristic of blood vessels and generally show thin and discontinuous staining for collagen type IV and laminin (Suthipintawong et al, 1995). The distinctive staining observed around blood vessels has been

employed as a marker when performing capillary density measurements (Madsen & Holmskov, 1995).

Comments

Earlier work on basal lamina immunostaining was restricted to the use of immunofluorescence techniques in frozen sections because of the lack of sensitivity of the available antibodies and techniques. The application of HIER combined with proteolytic digestion makes it possible to produce consistent immunostaining of paraffin-embedded, routinely prepared tissue sections.

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Cyclin D1 (bcl-1)

Sources/Clones

Dako (DCS-6), Immunotech (5D4) and Novocastra (P2D11F11, DCS-6).

Fixation/Preparation

Clone DCS-6 is effective on paraffin wax-embedded tissue. We have found that microwave antigen unmasking in Tris buffer produces an optimum immunoreaction. Other laboratories have found the strongest staining intensity with lowest background staining was achieved when both microwave and sonication-induced epitope retrieval methods were used.

Background

The G1 cyclin gene, cyclin D1 (PRAD-1, CCND-1), located on chromosome 11q13 (Motokura et al, 1991), exhibits characteristics of known cellular oncogenes (Schuuring et al, 1992). It plays an integral role in normal cell growth control and a complementary role in the in vitro transformation of cultured cells (Hinds et al, 1994; Hirama & Koeffler, 1995). Mechanisms of abnormal 11q13 regulation leading to cyclin D1 overexpression include genomic amplification in a variety of carcinomas (Proctor et al, 1991; Foulkes et al, 1993; Karlseder et al, 1994), characteristic t(11;14) (q13; q32) reciprocal chromosomal translocations in mantle cell lymphoma (Williams et al, 1991; Ott et al, 1996; Brynes et al, 1997) and chromosome 11 pericentric inversions in parathyroid adenomas. Together, cyclin D1 and cyclin-dependent kinase (Cdk) activities are required for completion of the G1/S transition in the normal mammalian cell cycle (Bartkova et al, 1994). Further, cyclin D1 inhibits the growth suppressive function of retinoblastoma tumorsuppressor protein (Ewen et al, 1993). Cyclin D1 is a 36 kD protein with a maximum expression of cyclin D1 occurring at a critical point in mid to late G1 phase of the cell cycle. Recombinant prokaryotic fusion protein is used as the antigen to raise antibody to cyclin D1 (class IgG2a). In normal tissues, cyclin D1 expression is restricted to the proliferative zone of epithelial tissues and is absent from several other tissues such as lymph node, spleen and tonsil.

Applications

Many neoplasms, including mantle cell lymphoma, parathyroid adenomas and a spectrum of carcinomas including breast, supradiaphragmatic squamous cell, ovarian and bladder transitional cell carcinomas demonstrate overexpression of cyclin D1 antibody on paraffin sections. Immunohistochemical demonstration of nuclear cyclin D1 protein was observed in 75% of mantle cell lymphoma and was not found in normal B cells and other B-cell lymphomas (including follicle center cell lymphoma, diffuse large B-cell lymphoma, lymphocytic lymphoma and MALT lymphoma) (Ott et al, 1996).

Comments

Cyclin D1 is useful to distinguish mantle cell lymphoma from the other low grade B-cell lymphomas. Breast cancer tissue may be used as a positive control.

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Cytokeratins

Cytokeratins (CKs) belong to a group of proteins known as intermediate filaments that constitute the cytoskeletal structure of virtually all epithelial cells. Being intermediate between microfilaments (6nm) and microtubules (25nm), the intermediate filaments comprise five characteristic groups based on cellular origin: CKs (epithelium), glial (astrocytes), neurofilaments (nerve cells), desmin (muscle) and vimentin (mesenchymal cells). More recently, this family of cytoskeletal proteins the intermediate filaments — have been reclassified into six subtypes (Table 1) (Miettinen, 1993). Intermediate filament proteins are composed of a 310 amino acid residue central region known as the rod domain. This is flanked by end domains of varying length and sequence, known as the head and tail. It is these flanking sequences that are the most immunogenic, responsible for the different properties and functions of the intermediate filament proteins. Being exposed, these molecules are also sensitive to fixation artefact due to the formation of crosslinkages. It is also important to note that due to the 30-50% sequence homology between the amino acid sequences of intermediate filaments of different types, monoclonal antibodies may crossreact with different intermediate filament types (Battifora, 1988).

Table 1 Classification of intermediate filaments

I Acidic cytokeratin (CK9-CK20)
II Basic cytokeratin (CK1-CK8)
III Vimentin (mesenchymal cells), desmin (muscle), glial fibrillary acid protein (glial cells and astrocytes), peripherin (neuronal cells)
IV Neurofilaments protein triplet (neurons)
V Nuclear laminin proteins (nuclear lamina)
VI Nestin (CNS stem cells)

CKs are present in both benign and malignant epithelial cells, independent of cellular differentiation. However, CK immunohistochemistry utilizing subset selective antibodies has extended beyond the typing of epithelial tumors, with recent descriptions of non-epithelial cells and tumors expressing CK.

The CKs are a family of proteins coded by different genes and the expression in epithelial cells is dependent on the embryonic development and degree of cellular differentiation. Practically, the most important CKs have been classified and numbered, based on the catalog of Moll et al (numbered 1 to 20) (Moll et al, 1982). These CKs were identified by the biochemical properties in two-dimensional gel electrophoresis of tissue extracts with their identification based on their isoelectric points and molecular weight. Hence, two groups of CKs emerge: type I/A (CK 9-20) with an acidic isoelectric point and type II/B (keratins 1-8) with a basic-neutral isoelectric point. Apart from a few exceptions, CKs are numbered from the highest to the lowest molecular weight in each group (Table 2) (Miettinen, 1993).

An interesting phenomenon is the existence of the keratin intermediate filaments as pairs. With some exceptions, all other CKs form polymers with their corresponding member from each type (Table 2). Hence, it follows that all epithelial cells contain at least two CKs. For example, whilst hepatocytes harbor a single pair of CK 8 and 18, keratinocytes may contain as many as ten CKs.

Thus, these laws governing the expression of various CKs are observed in part by neoplastic cells, forming the basis for the application of antibodies to CKs within neoplastic cells (indicating epithelial differentiation) using immunohistochemical methods (Schaafsma & Ramaekers, 1994).

Table 2 Keratins 1-20 with their molecular weight and most important distribution (Modified from Miettinen, 1993)

TYPE II	MW(kd)	Distribution	Type I	MW(kd)
		Epidermis掌alms and soles	9	64
1	67	Epidermis, keratinizing squamous epithelia	10	56.5
2	65		11	56
3	63	Cornea	12	55
4	59	Non-keratinizing squamous epithelia (internal organs)	13	51
5	58	Basal cells squamous and glandular epithelia, myoepithelium, mesothelium	14	50
		Squamous epithelia	15	50
6	56	Squamous epithelia (hyperproliferative)	16	48
7	54	Simple epithelial	17	46
		Basal cells glandular epithelia, myoepithelium		
8	52	Simple epithelia	18	45
		Simple epithelia, most glandular, some squamous epithelia (basal)	19	40
		Simple epithelia intestines and stomach, Merkel cells	20	46

Keratin pairs in the same line, MW, molecular weight, KD, kilodalton

The emergence of selective monoclonal antibodies identifying individual CKs now offers the advantage of immunohistochemical detection with morphological correlation (Heatley, 1996). Monoclonal antibodies to CKs may be divided into two categories: (i) a broad group that recognizes many members of the keratin family (see later) and (ii) a selective group that reacts with isolated CKs; in this regard, only CKs 7 and 20 will be considered in detail. Nevertheless, Table 3 provides a list of the most important CK subtypes in some epithelial neoplasms (Miettinen, 1993). In addition, popular commercially available antibodies to broad groups of CKs (Table 4) will also be detailed individually. False negativity due to masking of keratin epitopes and loss of

Table 3 Summary of the most important keratin subtypes of some epithelial tumors (Modified from Miettinen, 1993)

Carcinoma type	Keratin composition (Moll's catalog)									
	4	**5**	**7**	**8**	**13**	**14**	**17**	**18**	**19**	**20**
Squamous cell carcinoma, skin		+				+			+*	
Squamous cell Ca of esophagus	+				+	+			+	
Ductal carcinoma of breast			+	+		+*	+*	+	+	
Malignant mesothelioma		+	+	+		+		+	+	
Adenocarcinoma, lung			+	+				+	+	
Adenocarcinoma, colon				+				+	+	+
Adenocarcinoma, pancreas			+	+				+	+	+*
Hepatocellular carcinoma				+				+	+*	
Carcinoid tumor/small cell carcinoma				+				+	+**	

Merkel cell carcinoma			+			+	+	+	
Renal (cell) adenocarcinoma			+			+	+**		
Transitional cell carcinoma, low gr.	+	+	+	+		+	+	+*	
Transitional cell carcinoma, high gr.		+	+	+*		+	+		
Thyroid carcinoma, papillary			+			+	+		
Thyroid carcinoma, follicular			+			+	+*		
Adenocarcinoma of prostate		+*	+			+	+		
Adenocarcinoma of ovary		+	+			+	+	+*	

* Occasionally present/minor component

** Often but inconsistently present

Table 4 Specificities of selected-cytokeratin antibodies [**]

MW (kD)	35βH11	34βE12	AE1	AE3	*Anti bovine keratin	*Anti callus keratin	Cam 5.2	KL1	MNF 116
39							+		+
40			+					+	
45							+	+	+
48			+		+				
50		+	+					+	
51					+				
52			+		+				+
52.5							+	+	
54	+								
56					+	+		+	+
56.5		+	+	+					
57		+							
58		+		+	+		+	+	
60					+				
64						+			
65				+					
65.5								+	
66				+					
67				+					
68		+							

Antibody sources: 35βH11 and 34βE12 (Dakopatts, California, USA); antibovine keratin, anticallus keratin (Dakopatts, California, USA); AE1, AE3 (available as AE1/3 cocktail) Boehringer, Sydney, Australia; Dakopatts, California, USA); Cam 5.2 (Becton Dickinson, California, USA); KL1 (Immunotech, Marseilles, France); MNF116 (Dakopatts, California, USA).

[*]polyclonal antisera

[+]specificities as supplied by manufacturers

antigenicity warrants antigen retrieval in most instances. Hence, the need for extensive and carefully controlled optimization of every new antibody before diagnostic application cannot be overemphasized.

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Cytokeratin 20 (CK 20)

Sources/Clones

American Research Products (IT-Ks 20.10, IT-Ks 20.3, IT-Ks 20.5), Biodesign, Cymbus Bioscience (Ks 20.8, Ks 20.3, Ks 20.5), Dako (K$_s$ 20.8) and Progen (IT-Ks 20.3, IT-Ks 20.5, IT-Ks 20.8).

Fixation/Preparation

Formalin-fixed, paraffin-embedded tissue is ideally suited for this antibody. Immunoreactivity requires pretreatment with a sodium citrate buffer with heated antigen retrieval. Enzyme pretreatment (trypsin or pronase) should not be used as it abolishes signal. The antibody is not recommended for cryostat sections or cell smears due to crossreactivity with cytokeratin 20-epithelia.

Background

CK 20 is a low molecular weight cytokeratin, that was originally identified by Moll et al (1990) as protein IT in two-dimensional gel electrophoresis of cytoskeletal extracts of intestinal epithelia. The antibody reacts with the 46 kD cytokeratin intermediate filament isolated from villi of duodenal mucosa. CK 20 is less acidic than other type 1 cytokeratins and is particularly interesting because of its restricted range of expression.

In normal tissues it is expressed only in gastrointestinal epithelium, urothelium and Merkel cell. Other epithelial cells, including breast epithelia, do not react with CD20, nor does it recognize other intermediate filament proteins.

Applications

Following extensive testing on both primary and metastatic carcinomas, it was concluded that tumors expressing CK 20 were derived from normal epithelia expressing CK 20 (Moll et al, 1992). Hence, colorectal carcinomas consistently express CK 20 while gastric adenocarcinomas and other carcinomas of the gastrointestinal tract express this cytokeratin isotype less frequently. In addition, adenocarcinomas of the biliary tree and pancreatic duct, mucinous ovarian tumors and transitional cell carcinomas also demonstrate positive immunoreaction. Hence, the application of CK 20 antibody for determining the site of origin of carcinomas has been recently mooted largely due to absence of CK 20 expression in adenocarcinomas of the breast, lung, endometrium and nonmucinous tumors of the ovary (Appendices 1.13, 1.19, 1.28). In fact, CK 20 has recently contributed to immunohistochemical evidence supporting the appendiceal origin of pseudomyxoma peritonei in women (Ronnett et al, 1997).

Immunostaining for CK 7 and CK 20 has been shown to be useful in the differentiation of ovarian metastases from colonic carcinoma and primary ovarian carcinoma (Loy et al, 1996). A CK 7-/CK 20+ immunophenotype was seen in 94% of metastatic colonic carcinomas to the ovary, 5% of primary ovarian mucinous carcinomas and none of the primary ovarian endometrioid or serous carcinomas.

The almost consistent staining of Merkel cell carcinoma for CK 20 and the very low frequency of CK 20 reactivity in other small cell carcinomas (except those of salivary gland origin) can help to resolve the diagnostic dilemma between Merkel cell carcinoma and metastatic small cell carcinoma presenting in the skin (Chan et al, 1997) (Appendix 1.19). In fact, it was recently shown that CK 20 positivity in a small cell carcinoma of uncertain origin is strongly predictive of Merkel cell carcinoma, especially when the majority of tumor cells are positive. In contrast, a negative

CK 20 reaction practically rules out Merkel cell carcinoma, provided an effective antigen retrieval technique is used and appropriate immunoreaction obtained with other cytokeratin antibodies.

Finally, CK 20 positivity is often encountered in transitional cell carcinomas of the bladder but is rare in squamous carcinomas of that organ or adenocarcinoma of prostate (Moll et al, 1992).

Comments

CK 20 works extremely well in paraffin sections with microwave antigen retrieval in citrate buffer. It is extremely useful for the distinction between colonic and non-mucinous ovarian adenocarcinomas. Identifying Merkel cell carcinoma from metastatic small cell carcinoma to the skin is also easily accomplished with CK 20. Colonic carcinoma tissue sections should be used as positive control tissue.

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Cytokeratin 7 (CK 7)

Sources/Clones

Accurate (LP5K), American Research Products (RCK105), Biodesign, Biogenesis (C35, C18), Biogenex (OV-TL 12/30), Bioprobe (C-35, C68), Boehringer Mannheim (KS7-18), Chemicon, Dako (OV-TL 12/30), Cymbus Bioscience (C46, LP5K), Dako (OV-TL 12/30), Intracell Corp (RCK105), Japan Tanner (C35, C68), Milab (RCK 105), Novocastra (LP5K), Sanbio (OVTL 12/30, RCK105), Saxon (RCB105), Seralab (CK7) and Sigma (LD5 68).

Fixation/Preparation

Cytokeratin 7 (CK 7) can be used on formalin-fixed, paraffin-embedded tissue sections. Enzymatic digestion with proteolytic enzymes such as trypsin should be performed before staining. Pronase digestion has been found to be harsh on CK 7. This antibody may also be used on acetone-and/or methanol-fixed cryostat sections or fixed-cell smears. It enjoys the additional advantage of being used on cytological preparations already stained by the Papanicolaou stain. For cell smears, the APAAP technique is recommended.

Background

CK 7 antibody reacts with the 54 kD cytokeratin intermediate filament protein isolated from human OTN II ovarian carcinoma cells and other cell lines. Identified as CK 7 according to Moll's catalog, it is a basic cytokeratin found in most glandular and transitional epithelia (Moll et al, 1982).

In normal tissue CK 7 reacts with many ductal and glandular epithelia, but not stratified squamous epithelia. It is also reactive with transitional epithelium of urinary tract. Hepatocytes are negative whilst bile ducts are positive. In addition, lung and breast epithelia are positive with this antibody, whilst colon and prostate epithelial cells are negative (Van Niekerk et al, 1991).

Applications

CK is expressed in specific subtypes of ovarian, breast and lung adenocarcinoma, whilst carcinomas of the colon are negative (Ramackers et al, 1990). Recent studies have indicated that a CK 7+CK 20- immunophenotype is helpful in distinguishing metastatic colonic adenocarcinoma from primary ovarian carcinomas, particularly the endometrioid type (and with the exception of the mucinous type) (Loy et al, 1996) (Appendices 1.13, 1.28). Occasional ovarian mucinous carcinomas may show the same immunophenotype as metastatic colonic carcinomas (CK 7-/CK 20+). Using the same immunophenotypic profile (together with CK 18), CK 7 was recently shown to assist in determining that most ovarian mucinous tumors in pseudomyxoma peritonei in woman are secondary to appendiceal adenoma (Ronnett et al, 1997).

CK 7 is also useful to distinguish transitional cell carcinomas (+ve) from prostate cancer (-ve). The failure of CK 7 to interact with squamous cell carcinomas presents the potential for specificity for adenocarcinoma and transitional cell carcinoma.

Comments

We have found the combination of CK 7 and CK 20 to be extremely useful in distinguishing ovarian carcinomas (except mucinous) from colonic adenocarcinomas. Serous ovarian carcinoma tissue is recommended for positive control tissue.

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Cytokeratins-MNF 116

Sources/Clones

Dako (MNF 116), Immunotech (MNF 116).

Fixation/Preparation

MNF 116 performs well on formalin-fixed, paraffin-embedded tissue sections. Enzymatic predigestion with proteolytic enzymes such as trypsin and pronase is essential prior to immunodetection, trypsin being superior for MNF 116. This antibody may also be applied to acetone-fixed cryostat sections or fixed-cell smears. Incubation of the primary antibody for 1 h at 37 C yields better immunoreaction.

Background

MNF 116 antibody detects an epitope that is present in a wide range of keratins. These comprise a number of discrete polypeptides, whose molecular weights range from 45 to 56.5 kD. These correspond to Moll's keratin numbers 5, 6, 8, 17 and probably 19 (Moll et al, 1982). The MNF 116 immunogen was derived from a crude extract of splenic cells in a nude mouse engrafted with MCF-7 cells.

In normal tissue, the MNF 116 antibody shows a broad pattern of reactivity with epithelial cells from simple glandular to stratified squamous epithelium. Epithelial cells are labeled irrespective of ectodermal, mesodermal or endodermal origin. However, due to the crossreactivity with the other members of the family of intermediate filaments, this antibody (not unlike other monoclonal anti-keratin antibodies) crossreacts with non-epithelial cells including smooth muscle, dendritic cells in lymph nodes, syncytiotrophoblasts, some cortical neurons and a minority of plasma cells.

Applications

MNF 116 demonstrates excellent immunopositivity with a wide range of benign and malignant epithelial neoplasms. A strong pattern of staining is observed in squamous cell carcinoma (including nasopharyngeal carcinoma), small cell carcinoma, sarcomatoid carcinoma, spindle cell carcinoma, adenocarcinoma and mesotheliomas. In small cell carcinomas, a characteristic paranuclear accentuation pattern of staining has been found to be extremely useful in identifying these neoplasms. Both epithelioid and spindle cell components of mesotheliomas react with this antibody (Miettinen, 1993).

MNF 116 is also useful in confirming the diagnosis in a wide range of soft tissue neoplasms. Monophasic and biphasic synovial sarcomas demonstrate strong positivity (albeit focal in the spindle cells). Vascular neoplasms that react with this broad range cytokeratin antibody include epithelioid hemangioendothelioma (focal), epithelioid angiosarcoma and sinonasal hemangiopericytoma (Mentzel et al, 1997). Epithelioid sarcoma (and the recently described proximal variant) require cytokeratin positivity for diagnosis (Evans & Baer, 1993; Guillou et al, 1997). Other tumors in which cytokeratin positivity is essential for diagnosis include desmoplastic small round cell tumors, chordomas and extrarenal rhabdoid tumors that are consistently positive. Mixed tumors and myoepitheliomas arising in soft tissue were recently described and shown to express pankeratin (Kilpatrick et al, 1997).

Among germ cell tumors, embryonal carcinoma and yolk sac tumors are consistently positive with MNF 116.

The following neoplasms may demonstrate aberrant staining with MNF 116. The coexpression of cytokeratins in smooth muscle tumors is well described

(Ramackers et al, 1988). Cytokeratin-positive cells have been revealed in plasmacytoma (Wotherspoon et al, 1989). A few primitive neuroectodermal tumors may show focal cytokeratin expression. Rarely, myofibroblasts may demonstrate focal cytokeratin positivity (Jones et al, 1993; Hojo et al, 1995). Quite logically, all of these potential diagnostic pitfalls may clearly be avoided if relevant panels of immunohistochemical antibodies are applied.

Comments

MNF 116 has developed into a first-line antibody in the application of cytokeratins to surgical pathology. It is, however, necessary to be aware of the aberrant immunoreactions in order to avoid misdiagnosis. It is therefore unwise to arrive at a diagnosis based on the assessment of a single cytokeratin marker without the application of other relevant antibodies used in a diagnostic panel to exclude other possibilities. Any epithelial tissue - glandular or squamous is suitable for use as positive control for MNF 116.

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Cytokeratins-CAM 5.2

Sources/Clones

Becton Dickinson (CAM 5.2).

Fixation/Preparation

CAM 5.2 can be applied to both frozen or formalin-fixed, paraffin-embedded tissue. Trypsin enzyme pretreatment for antigen retrieval is essential for paraffin sections.

Background

CAM 5.2 was derived from hybridization of mouse P3/NS1/1-Ag4-1 cells with spleen cells from BALB/c mice immunized with a human colorectal carcinoma line, HT29 (Makin et al, 1984). It comprises mouse IgG2a heavy chain and κ light chains from spleen parent and myeloma cell lines. The antibody CAM 5.2 detects human cytokeratin epitopes with molecular weights 52 kD and 45 kD corresponding to Moll's catalog numbers 8 and 18 respectively (Moll et al, 1982). In normal tissue CAM 5.2 reacts with secretory epithelia but not stratified squamous epithelium.

Applications

Anticytokeratin antibody CAM 5.2 is useful for the detection of adenocarcinomas, mesotheliomas and certain carcinomas derived from squamous epithelia, the latter including spindle cell carcinomas (Gatter et al, 1985; Battifora, 1988). It should, however, be noted that some squamous cell carcinomas do not stain with CAM 5.2, e.g. those in the cervix, vagina and esophagus. The ability of CAM 5.2 to detect epithelial neoplasms but not normal stratified squamous epithelium (e.g. skin) can be exploited to distinguish Paget's disease (both mammary and extramammary) from superficial spreading melanoma. CAM 5.2 is especially useful in the demonstration of subtle metastatic deposits of breast carcinoma cells in lymph nodes (Raymond & Leong, 1989) and bone marrow. It also successfully reacts with renal cell carcinomas, hepatocellular carcinomas and cholangiocarcinomas (Johnson et al, 1987). CAM 5.2 also detects neuroendocrine carcinomas (including small cell carcinoma and Merkel cell carcinomas), germ cell tumors (with the exception of seminoma), synovial and epithelioid sarcomas (Leader et al, 1986). This antibody is also useful for the detection of epithelial cells in thymomas, particularly when masked by lymphocytes. It is reputed not to label melanomas (except in cryostat sections), (Leader et al, 1986).

Non-epithelial tissues which react with anticytokeratin CAM 5.2 include smooth muscle, rare sarcomas of breast (Pitts et al, 1987), meningiomas (hyaline bodies or malignant variants) (Theaker et al, 1986) and rosettes of neuroblastomas. B-cell anaplastic large cell lymphoma, confirmed by immunohistochemistry and immunoglobulin gene rearrangements, has been shown to be immunoreactive with CAM 5.2 (Frierson et al, 1994).

It should also be noted that large-cell lymphoma of B-cell lineage (verified with PCR) has been shown to be rarely reactive for cytokeratin 8 (Lasota et al, 1996)

Comments

Although CAM 5.2 has a narrow range of cytokeratin immunodetection in surgical pathology, it has proved useful as a second-line marker in specific circumstances such as the identification of spindle cell carcinoma of the skin, subtle metastatic deposits of carcinoma in lymph nodes and to distinguish Paget's disease from superficial spreading melanoma. It also shows

strong staining reaction with-neuroendocrine carcinomas.

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Cytokeratins-AE1/AE3

Sources/Clones

Dako (AE1/AE3), Zymed (AE1, AE3).

Fixation/Preparation

This antibody is suitable for immunohistochemical staining of formalin-fixed, paraffin-embedded or frozen tissue sections. Trypsin or pepsin digestion/antigen retrieval is necessary before staining of formalin-fixed, paraffin-embedded tissue sections, although pepsin has been found to be superior to trypsin. The Zymed antibody is prediluted and ready to use. However, if DAB is used as a chromogen for immunodetection, then a further dilution of the primary antibody may be required.

Background

The antibody AE1/AE3 is a mixture of two monoclonal antibodies, raised against human epidermal keratins (Woodcock-Mitchell et al, 1982). AE1 recognizes most of the acidic (type 1) keratins with molecular weights 56.5, 50, 50', 48 and 40 kD.AE3 recognizes all known basic (type II) cytokeratins (Moll et al, 1982). This combination shows broad reactivity and is claimed to stain almost all epithelia and their neoplasms. It is also reputed not to crossreact with other members of the intermediate filaments.

Applications

The wide reactivity of AE1/AE3 expressed in simple epithelia and their tumors, including cytokeratins expressed in complex stratified squamous epithelia, permits identification of a wide range of epithelial-derived tumors. Hence, strong staining of AE1/AE3 has been demonstrated in adenocarcinomas (e.g. colorectal, gastric, breast, prostate), renal cell carcinoma, hepatocellular carcinoma, transitional cell carcinoma, small cell carcinoma, carcinoid tumors, epithelial component of pleomorphic adenoma and squamous cell carcinoma of the skin (including the spindle cell variant), cervix and bronchus. Thymomas, mesotheliomas (including the sarcomatoid component) and chordomas consistently stain with AE1/AE3. Non-epithelial tumors that demonstrate AE1/AE3 positivity include germ cell tumors (except seminomas), synovial sarcoma and epithelioid sarcoma. Crossreactivity in some leiomyosarcomas has been documented (Tseng et al, 1982; Spagnolo et al, 1983; Battifora, 1988; Goddard et al, 1991).

In a recent study of 290 cases of hepatocellular carcinoma, immunohistochemical evidence of biliary differentiation (reactivity with AE1/AE3 or cytokeratin 19) was found in 29.3% of cases. These hepatocellular carcinomas with biliary differentiation showed clinical features of greater aggressiveness with poorer cellular differentiation and higher expression of proliferation markers (Wu et al, 1996).

Comments

The pankeratin marking potential of antibody AE1/AE3 places it in an ideal position to screen for neoplasms of epithelial origin, especially poorly differentiated carcinomas of diverse origin, and to distinguish these from melanoma and lymphoma. Another useful role is the identification of micrometastases, e.g. breast secondaries in lymph nodes and bone marrow.

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Cytokeratins-MAK-6 ®

Sources/Clones

Triton Diagnostics and Zymed (MAK-6 - clones KA4 and UCD/PR10.11).

Fixation/Preparation

MAK-6 works well in routinely fixed, paraffin-embedded tissue sections. Trypsin pretreatment is necessary for antigen unmasking. Incubation of the primary antibody for 1 h at 37 C or overnight incubation at room temperature yields superior immunostaining. Preincubated with blocking reagents to reduce non-specific background staining has been recommended, but we have found this to be unnecessary.

Background

MAK-6 antibody cocktail contains an optimized mixture of two murine monoclonal antibodies of IgG1 isotype. Antibody kA 4 recognizes human cytokeratin types, 14, 15, 16 and 19 while antibody UCD/PR-10.11 recognizes human cytokeratins 8 and 18.

Antibody UCD/PR 10.11 was produced using shed extracellular antigen purified from MCF-7 tissue culture media and was selected for its specificity to cytokeratin types 8 and 18 (Chan et al, 1986). Antibody kA4 was produced against human sole epidermis and was selected for its specificity to cytokeratin types 14, 15, 16 and 19.

Applications

MAK-6 is reputed to stain all cases of squamous cell carcinomas and the majority of adenocarcinomas, carcinoid tumors and undifferentiated carcinomas. Lymphomas, melanomas, gliomas/astrocytomas and the majority of sarcomas do not demonstrate MAK-6 positivity. The latter is related to the expected cytokeratin expression in synovial sarcomas and epithelioid sarcomas. It should be noted that the majority of ependymomas and basal cell carcinomas of the skin also do not express MAK-6 Caution should be observed in assessing metastatic carcinomas to the brain, since MAK-6 may rarely show crossreactivity with neural tissue. In these instances application of antibody to glial fibrillary acidic protein (GRAP) would be helpful (Cooper et al, 1985; McNutt et al, 1988).

Comments

We have found MAK-6 to be a useful pankeratin marker. Strong immunoreaction is demonstrated in tissue of epithelial origin. Used in conjunction with other pankeratin markers, the majority of neoplasms showing cytokeratin expression may be identified.

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Cytokeratins-34βE12

Sources/Clones

Dako and Enzo Diagnostics.

Fixation/Preparation

34βE12 may be used on formalin-fixed, paraffin-embedded tissue sections. Although reactivity on formalin-fixed tissue is obtainable, better consistency is observed on Carnoy's or methacarn-fixed material. Proteolytic treatment with pronase (for prostatic basal cells) and microwave antigen retrieval (for papillary carcinoma of thyroid) is essential for formaldehyde-fixed material. This antibody may also be used on acetone-fixed cryostat sections and fixed-cell smears. Incubation of the primary antibody for 1 h at room temperature is sufficient for prostatic basal cells. However, incubation of primary antibody at 4 C overnight is necessary for papillary carcinoma of the thyroid gland.

Background

34βE12 identifies keratins of approximately 66 kD and 57 kD in extracts of stratum corneum. The antibody reacts with keratins 1, 5, 10 and 14 in Moll's catalog (molecular weight 68 kD, 58 kD, 56.5 kD, 50 kD) respectively (Moll et al, 1982). In normal tissue the antibody labels squamous, ductal and other complex epithelia.

Applications

Perhaps the most useful application for 34βE12 is in the detection of basal cells of the prostatic acini (O'Malley et al, 1990; Amin, 1995). Demonstration of this high molecular weight cytokeratin in the basal cells of prostatic acini is indicative of benignity. Further, 34βE12 is negative in adenocarcinoma of the prostate. In this context, it is also useful to demonstrate the basal cells in basal cell hyperplasia (partial or atypical) and atypical adenomatous hyperplasia of the prostate, the latter being difficult to distinguish morphologically from prostatic adenocarcinoma.

More recently the role of 34βE12 in diagnostic thyroid pathology was highlighted (Appendix 1.26). It was shown that 34βE12 positivity was confined to papillary carcinoma of the thyroid, whereas follicular neoplasms and hyperplastic nodules were either negative or showed focal staining (Raphael et al, 1995).

34βE12 is also consistently positive in squamous cell carcinomas, ductal carcinoma of breast, pancreas, bile duct and salivary gland. It has also been demonstrated in transitional cell carcinomas of the bladder, nasopharyngeal carcinoma, thymomas and epithelioid mesotheliomas (Gown & Vogel, 1985).

Whilst this antibody has a variable positivity with adenocarcinomas, it is negative in hepatocellular carcinoma, renal cell carcinoma and endometrial carcinoma. Mesenchymal tumors, lymphomas, melanomas, neural tumors and neuroendocrine tumors are negative.

Comments

We have found 34βE12 to be extremely useful in both diagnostic prostatic and thyroid pathology. It should be noted that different incubation protocols need to be followed for these two applications of 34βE12.

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Cytomegalovirus (CMV)

Sources/Clones

Accurate (E13, CCH2), American Research Products (1692-18), Axcel (CCH2), Biodesign (084, BM204, BM219, polyclonal), Biogenesis (BM204, polyclonal), Biogenex (BM204, polyclonal), Chemicon, Dako (AAC10, CCH2), EY Labs, Fitzgerald (M2103126, M210312), International Enzymes (polyclonal), Seralab (E13) and Zymed (DDG9/CCH2).

Fixation/Preparation

These antibodies are suitable for immunohistochemical staining of paraffin-embedded tissue sections. Enzymatic predigestion with trypsin or pepsin is required for clone CCH2. These antibodies may also be used to detect CMV early nuclear proteins in infected human embryonic fibroblasts 24 h following inoculation of clinical specimens on cell culture.

Background

The CCH2 clone recognizes a 43 kD protein, whilst the DDG9 clone recognizes a 76kD protein, both having been demonstrated in glycine-extracted CMV antigen. These proteins are expressed in the immediate early and early stage of CMV replication in infected cells (Zweygberg et al, 1986). Early viral proteins are expressed in the nucleus of infected cells within 6-24 h of infection and prior to viral DNA replication. Several late viral proteins may be demonstrated in the nucleus and the cytoplasm of infected cells. The different viral proteins can be demonstrated in infected cell cultures as well as in infected tissue (Swenson & Kaplan, 1985). These antibodies do not crossreact with adenoviruses or other herpesviruses.

Applications

These antibodies to CMV demonstrate the virus in infected cells, producing a nuclear immunopositive reaction. However, at a later stage, both a nuclear and cytoplasmic immunoreaction with the early CMV antigen is produced, especially with the Zymed product. Antibodies to CMV have a wide application to diagnostic surgical pathology, especially when characteristic CMV inclusions are not clearly evident. CMV infection (latent or active) may be seen in salivary glands, lungs, kidneys, GIT and lymph nodes. Awareness of CMV as an opportunistic infection in the context of immunosuppression suggests the use of CMV immunohistochemistry for definitive diagnosis (Schwartz & Wilcox, 1992). Recently, CMV esophagitis has been observed as a florid aggregate of macrophages without typical inclusions (Greenson, 1997). Small biopsy specimens with such a morphological picture warrant further immunohistochemical study to identify CMV. Conversely, chemotherapy toxicity may mimic CMV gastritis, necessitating CMV immunohistochemistry to exclude false positives (Canioni et al, 1995). Antibodies to CMV may also be applied for the identification of atypical CMV inclusions in gastrointestinal mucosal biopsy specimens, where classic inclusions are rarely found (Schwartz & Wilcox, 1992). The proper recognition of CMV-infected cells in the context of immunosuppression is critical, so that effective therapy is not delayed, preventing further viral dissemination.

Comments

It has been shown that immunohistochemistry with CCH2 detects a higher number of CMV-infected cells than in situ

hybridization (Niedobitek et al, 1988). Hence, for routine diagnostic purposes at least, CMV immunohistochemistry would appear to be the method of choice for a rapid, sensitive and specific method of CMV detection.

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Cytotoxic Molecules (TIA-1, Granzyme B, Perforin)

Sources/Clones

TIA-1

Coulter (2G9)

Granzyme B

Coulter (GB7), Sanbio/Monosan (GrB7), clone GB9 (Dr Kummer, Amsterdam, The Netherlands).

Perforin

Kaimya (KM583), Sumitomo Denko, Osaka, Japan (1B4), T cell Diagnostics (polyclonal).

Fixation/Preparation

All antibodies against cytotoxic molecules can be used in formalin-fixed, paraffin-embedded tissues. Microwave or high-pressure cooking pretreatment in citrate buffer is essential for antigen retrieval.

Background

Natural killer cells and cytotoxic T lymphocytes are characterized by their inclusion of cytoplasmic granules that are released in response to target cell recognition. Among the wealth of cytotoxic molecules found in cytotoxic cells, perforin and granzyme B are two well-characterized proteins involved in one major pathway leading to apoptosis in target cells (Smyth & Trapani, 1995; Liu et al, 1995). Perforin allows for the entry of granzyme molecules into the target cells, which then activate the apoptotic protease CPP32 (Darmon et al, 1995). The genes for perforin (Lichtenheld and Podack, 1989) and granzyme B (Smyth & Trapani, 1995) have been cloned and antibodies directed against these molecules have been generated (Kummer et al, 1993). T cell-restricted intracellular antigen (TIA-1), another molecule found in cytotoxic cells, is recognized by the antibody 2G9 (Anderson et al, 1990). The exact function of TIA-1 has not been elucidated. Since it induces DNA fragmentation of digitonin-permeabilized thymocytes (Tian et al, 1991), it may be implicated in the killing induced by cytotoxic lymphocytes. TIA-1 has been demonstrated in many intestinal intraepithelial lymphocytes of normal proximal small intestine and a corresponding increase of TIA-1 positive cells in active celiac disease (Russell et al, 1993).

Applications

The expression of all three above-mentioned molecules appears to be largely restricted to cytotoxic cells. In addition, in vitro findings have also suggested that, with rare exceptions, expression of perforin and granzyme B is restricted to cytotoxic cells, including natural killer cells and cytotoxic T cells (Liu et al, 1995; Smyth & Trapani, 1995). Analysis of these antigens in conjunction with other marker molecules can therefore further specify the cellular origin of lymphocytes and lymphoid malignancies (Daums et al, 1997). In this regard, granzyme B, TIA-1 and perforin have been demonstrated in the majority of intestinal T-cell lymphomas but not in intestinal B-cell lymphomas and CD8- peripheral nodal T-cell lymphomas (Daums et al, 1997). Antibody 2G9, which recognizes TIA-1, proved to be the most sensitive immunohistological marker, being demonstrated in the highest number of cases and also in high numbers of neoplastic cells in positive cases (Daums et al, 1997). Hence, the cytotoxic differentiation in intestinal T-cell lymphoma was clearly shown, supporting derivation from intraepithelial cytotoxic T lymphocytes.

Anaplastic large cell lymphomas of T cell (T-ALCL) have also been shown to express cytotoxic molecules with antibody

GB9 to granzyme B, whilst being absent in B-cell anaplastic large cell lymphomas, proving that T-ALCL are derived from activated cytotoxic T cells (Foss et al, 1996). Granzyme B+ T-cell lymphomas have also been mainly found in mucosa-associated lymphoid tissue, being more often associated with angioinvasion: nasal, gastrointestinal tract and lung (De Bruin et al, 1994). It has also been shown that immunohistochemical staining with anti-TIA-1 can be used to identify cytolytic T lymphocytes in epidermal lesions of human graft-versus-host disease (Sale et al, 1992). Recent work with antibodies to cytotoxic molecules has shown that the predominant mechanism of cellular destruction in Kikuchi's lymphadenitis was apoptosis mediated by cytolytic lymphocytes (Takakuwa et al, 1996; Felgar et al, 1997).

Comments

Until recently, it was impossible to differentiate most functional T-cell subsets, e.g. suppressor and cytotoxic T cell, by membrane characteristics on paraffin-embedded tissue. The production of monoclonal antibodies against cytotoxic molecules has enabled the identification of the major components of the cytotoxic granules found in the cytoplasm of activated cytotoxic and natural killer cells. Intestinal T-cell lymphomas provide an ideal positive control for antibodies to cytotoxic molecules.

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DBA.44 (Hairy Cell Leukemia)

Sources/Clones

Dako and Immunotech

Fixation/Preparation

The antibody is immunoreactive in formalin-fixed, paraffin-embedded tissues with immunoreactivity enhanced by proteolytic digestion but not by HIER.

Background

DBA.44 recognizes an unknown fixation-resistant B-cell differentiation antigen expressed by mantle zone lymphocytes, reactive immunoblasts, monocytoid B cells and a small proportion of high- and low-grade lymphomas (Al Saati et al, 1989; Hounieu et al, 1992). The monoclonal antibody was one of four generated against a B-lymphoma cell line (DEAU-cell line) grafted in athymic nude mice. Within the group of low-grade B-cell lymphomas, DBA.44 reacted principally with hairy cell leukemia. Among node-based lymphomas, the strongest membrane staining was observed in centroblastic, immunoblastic and monocytoid B-cell lymphomas.

Applications

In a study of bone marrow specimens from 166 patients with hairy cell leukemia, strong positive staining of the "hairy" surface membranes was observed in routinely fixed and decalcified bone marrow biopsies of nearly all cases (Hounieu et al, 1992). Subsequent studies have proven the usefulness of DBA.44 in the identification of hairy cell leukemia, particularly in the detection of minimal residual disease following treatment (Wheaton et al, 1996). DBA.44 has been successfully applied to peripheral blood cytospin preparations and for ultrastructural labeling (Cordone et al, 1995).

The antibody has also been successfully applied to methyl-methacrylate embedded bone marrow biopsies (Kreft et al, 1997). DBA.44 appears to be a more sensitive marker of hairy cells than the traditional tartrate-resistant acid phosphatase (TRAP) activity which has long been a cornerstone in the diagnosis of hairy cell leukemia (Hoyer et al, 1997). Mantle zone lymphocytes and their corresponding lymphoma were DBA.44 and CD 44+, with a weaker reaction for CD w75 than marginal zone lymphocytes and monocytoid B-cells whereas monocytoid B cell lymphoma showed positivity for CD 74 and CD w75 with positivity for DBA.44 observed in only occasional cases. Hairy cell leukemias, in contrast, were all positive for DBA.44, with a weak reaction for CD 74 and a stronger positivity for CD w75 than either mantle cell lymphoma or monocytoid B-cell lymphoma specimens (Ohsawa et al, 1994).

Comments

While useful as a diagnostic marker, DBA.44 is not specific for hairy cells and should be used in a panel of antibodies to separate other B-cell lymphomas which may express the antigen.

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Wheaton S, Tallman MS, Hakimian D, Peterson L 1996. Minimal residual disease may predict bone marrow relapse in patients with hairy cell leukaemia treated with 2-chlorodeoxyadenosine. Blood 87: 1556-1560.

Desmin

Sources/Clones

Accurate (DEU10, 4B4B2, 33), American Research Products/Research Diagnostics (DEU10), Biodesign (33), Biogenesis (BIO-41H), Boehringer (DEB5), Dako (DER-11, D33), Eurodiagnostica (D9), EY Labs, Immunotech (D33, HHF35), Shandon Lipshaw (D33), Sigma (DEU10) and Zymed (ZSD1).

Fixation/Preparation

Most of the available antibodies are immunoreactive in paraffin sections and are enhanced by HIER (Pollock et al, 1995). Enzyme digestion is not required if HIER is employed. Clone D33 can be used without enzyme predigestion.

Background

Desmin belongs to the class of "intermediate" (10 nm) filaments and is a cytoplasmic protein, which is characteristically found in myogenic cells. It has a molecular weight of 53 kD and is composed of an N-terminal "headpiece" and a C-terminal "tailpiece", both of which are non-helical in conformation. The two pieces bracket an α-helical middle domain of about 300 amino acid residues which is highly conserved from species to species, with striking interspecies homology. This homology is even more than that exhibited between intermediate filament proteins in the same species, with cytokeratin, vimentin, glial fibrillary acidic protein, neurofilaments and desmin exhibiting sequence homology of about 30% (Nagai et al, 1985; Li et al, 1993).

In smooth muscle cells, desmin is associated with cytoplasmic dense bodies and subplasmalemmal dense plaques and in striated muscle it is linked to sarcomeric Z disks. Muscle cells depleted of desmin (skeletin) are still able to contract in response to adenosine triphosphate and calcium suggesting that desmin played no role in contractility but rather serves to maintain the relationship and orientation of actin and myosin filaments and to anchor them to the plasmalemmal. More recent findings suggest that, like other intermediate filaments of non-epithelial cells, desmin also serves a nucleic acid-binding function, is susceptible to processing by calcium-activated proteases and is a substrate for cyclic adenosine monophosphate-dependent protein kinases. With its shared structural homology to lamins, the proteins of the nuclear envelope, desmin may also serve as a modulator between extracellular influences governing calcium flux into the cell and may have a role in nuclear transcription and translation. These newer roles of the intermediate filaments, including desmin, relegate the supportive cytoskeletal function of intermediate filaments to a secondary role (Goldman et al, 1985).

Applications

The development of sensitive and specific antibodies to the intermediate filaments, including desmin, heralded a new era in diagnostic immunohistochemistry as they allow the subtyping of many seemingly undifferentiated and pleomorphic tumors through intermediate filament analysis. Through the application of judiciously selected panels of antibodies directed to the differential diagnoses derived from the histologic and clinical findings, it is possible to separate the different entities in the diagnostic categories of pleomorphic spindle cell tumors and round cell tumors (Leong et al, 1989). The former group includes rhabdomyosarcoma, leiomyosarcomas and tumors with focal myogenic differentiation

such as Triton tumors and malignant mixed M黠lerian tumors. The latter group includes embryonal rhabdomyosarcoma, epithelioid leiomyoma and leiomyosarcoma and focal myogenic differentiation in small round cell tumors such as desmoplastic small round cell tumors and primitive/peripheral neuroepithelial tumors. All of these tumors may express desmin (Azumi et al, 1988; Leong & Wannatkrairot, 1992; Parham et al, 1992). In this context, it is important to remember that although myogenous cells often express desmin, it is also seen in myofibroblasts. Focal staining for desmin will be observed in tumors of myofibroblastic differentiation such as the fibromatosis, dermatofibrosarcoma protuberans (Leong et al, 1997) and in reactive conditions with abundant myofibroblasts such as inflammatory pseudotumor and postoperative spindle cell nodule (Hojo et al, 1995). Equally important is the observation that not all muscle cells contain desmin. For example, among mammalian vascular smooth muscle, three immunophenotypes have been observed. Those that display vimentin only, those coexpressing vimentin and desmin and a third group which expresses desmin only (Coindre et al, 1988).

Focal staining for desmin may also be seen in tumors with a background of reactive myofibroblasts or with focal myofibroblastic differentiation such as malignant fibrous histiocytoma.

Comments

While initial antibodies to desmin lacked sensitivity and specificity, current commercial antibodies are more reliable. Both monoclonal and polyclonal antibodies are useful but as desmin shares some common epitopes with actin and myosin, it should be ensured that the antibody employed does not show crossreactivity. We employ clones DE-R-11 and D33, both antibodies being enhanced by HIER.

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Desmoplakins

Sources/Clones

American Research Products, Biodesign (DP2.15), Boehringer Mannheim (2.15), Chemicon, Cymbus Bioscience (DP2.15), ICI (DP2.17), Progen (DP2.15) and Research Diagnostic Inc (DP2.15).

Fixation/Preparation

Current antibodies are immunoreactive only in fresh-frozen sections or cell preparations.

Background

Epithelial cells contain complexes of cytokeratin filaments (tonofilaments) associated with specific domains of the plasma membrane that appear as symmetrical junctions known as desmosomes or as asymmetrical hemidesmosomes. These regions of filament-membrane-attachment are characterized by 14-20 nm-thick dense plaque; these desmosomal plaques comprise a dense mixture of intracellular attachment proteins including plakoglobin and desmoplakins (Mueller & Franke, 1983). Transmembrane linker proteins, which belong to the cadherin family of cell-cell adhesion molecules, bind to the plaques and interact through their extracellular domains to hold the adjacent membranes together by a Ca^{2+} dependent mechanism. Desmoplakins I and II (DPI and DPII) are two polypeptides which make up the desmoplakins and are of molecular masses 46 and 24 kD respectively, suggesting that DPI may be a dimer in solution and DPII a monomer (O'Keefe et al, 1989).

Applications

The widespread presence of desmosomes in epithelial cells and their corresponding tumors makes the presence of desmoplakins a specific marker of epithelial differentiation. Unfortunately, these proteins are fixative sensitive, restricting the use of antibodies to desmoplakins to fresh cellular preparations or frozen sections. Applications in diagnostic pathology have therefore been limited to some studies in bullous skin diseases (Burge & Garrod, 1991; Setoyama et al, 1991). In autoimmune acantholytic diseases such as pemphigus vulgaris and pemphigus erythematosus, desmoplakins are intact even in acantholytic cells, whereas in Hailey-Haileys' disease and Dariers' disease the normal plasma membrane localization of desmoplakins is lost and the protein is internalized and present diffusely in the cytoplasm (Setoyama et al, 1991).

Desmoplakins have been demonstrated in follicular dendritic cells and their corresponding tumors (Chan et al 1997).

Comments

Acetone fixation followed by plastic embedding allows the immunostaining of the desmoplakins in permanent sections. Trypsin digestion needs to be employed (Carmichael et al, 1991).

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Epidermal Growth Factors: TGF-α AND EGFR

Sources/Clones

EGFR

Accurate (21-1, F4), American Qualex, Biodesign (EGFR 1, 2E9, L-4451, F5, E5), Biogenesis (C11, EGFR1), Biogenex (E30), Caltag Laboratories (2E9), Chemicon (polyclonal), Cymbus Bioscience (EGFR1), Dako (EGFR1), Fitzgerald (polyclonal), Immunotech (F4), Novocastra (polyclonal), Oncogene (R.1,225, 455), Pharmingen (c11), Sigma (29.1, F4) and Zymed (Z025).

TGF-α

Biodesign, Biogenesis (2D7/44, 2D7/45,, 8A5/7, Rt, TB21), Chemicon (polyclonal) and Oncogene (134A-2B3, 213. 4-4, 189-2130.1).

Fixation/Preparation

Applicable to formalin-fixed, paraffin-embedded tissue, although an antigen retrieval technique should be used prior to immunostaining, e.g. citrate buffer and microwave oven unmasking. May also be applied to cryostat sections or cell smears.

Background

Transforming growth factors (TGF) were discovered due to their ability to transform fibroblasts to a malignant phenotype (DeLarco and Todaro, 1978). Two distinct polypeptides were subsequently isolated: TGF-α and TGF-β. TGF-α is a polypeptide of 50 amino acids and is acid and heat stable (Prigent and Lemoine, 1992). TGF-α belongs to the epidermal growth factor family, members of which share a common amino acid sequence and biological activities. They also bind to a common receptor, epidermal growth factor receptor (EGFR), on target cells (Carpenter, 1984).

EGFR is a 170 kD protein comprising a cell surface ligand-binding transmembrane domain and a highly conserved cytoplasmic tyrosine kinase domain. When TGFα binds to EGFR, tyrosine kinase of the receptor is activated. This is followed by phosphorylation and an increase in cytosolic calcium ions within target cells. The resultant effect is an increased DNA synthesis with proliferation and differentiation of the cell (Chen et al, 1989).

TGF-α is a potent growth stimulator and is distributed in both fetal and adult tissues, playing a role in the physiological regulation of normal growth and differentiation (Yasui et al, 1992).

Applications

There is sufficient evidence showing that TGFα is an important growth factor for transformation of various cell types to a malignant phenotype (Pusztai et al, 1993).The coexpression of both the ligand (TGF-α) and its receptor (EGFR) has been documented in a variety of carcinomas both gastrointestinal and non-gastrointestinal. This bond is thought to confer autonomy to tumor cells by autocrine or paracrine mechanisms (Sporn & Roberts, 1985). Whilst coexistent expression of a growth factor and its receptor would be expected to confer increased growth advantage to tumor cells, the ability of certain tumors to express both growth factor and/or the respective receptor may be lost during the carcinogenic transformation.

The EGFR antibody reacts with the majority of squamous cell carcinomas arising from both squamous epithelium and metaplastic squamous epithelium (Ozanne & Richards, 1986). Studies on breast cancer

have shown that the expression of the EGF receptor may also be of prognostic value (Nicholson et al, 1991).

Comments

Growth factors and their receptors participate in the process of tumorigenesis by promoting the growth of tumor cells. During this process, tumor cells acquire an increasingly aggressive phenotype with loss of the physiological control for growth and differentiation. At the present time the availability of antibodies to growth factors/receptors can only contribute to our understanding of the complex mechanisms involved in tumorigenesis.

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Epithelial Membrane Antigen (EMA)

Sources/Clones

Accurate (E29), Biodesign, Biogenesis (2D5/11), Biogenex (E29, Mc-5), Bioprobe (HMFGP1.4), Chemicon, Dako (E29), Diagnostic Biosystems (E29), Immunon (polyclonal, E29), Immunotech (E29, E348KP), Medac, Novocastra, Oncogene (MC5), Seralab (HMFG/5/11IC, polyclonal), Serotec and Zymed (ZCE113).

Fixation/Preparation

Most antibodies are immunoreactive in fixed paraffin-embedded sections. Immunostaining is enhanced by proteolytic digestion or HIER, the latter producing less background staining.

Background

Antiepithelial membrane antigen (EMA) antibodies recognize a group of closely related high molecular weight transmembrane glycoproteins with a high carbohydrate content. The MUC1 gene, located on chromosome 1 in 1q21-24 region, encodes EMA. EMA is very similar to the human milk fat globule (HMFG). A heterogeneous population of HMFG proteins can be recovered from the aqueous phase of skimmed milk following extraction in chloroform and methanol. EMA is related to the high molecular weight glycoproteins of HMFG, especially to HMFG2 (Heyderman et al, 1985). Preparations of EMA reacted with polyclonal antibodies raised to delipidized HMFG with avid binding to wheat germ agglutinin and peanut agglutinin. A similar mucin-containing glycoprotein was solubilized from HMFG and labeled PAS-O because of reactivity for PAS (Shimuzu & Yamauchi, 1982). PAS-O and EMA represent closely allied glycoprotein moieties, with common antigenic determinants on both proteins. From a practical standpoint, patterns of immunoreactivity for EMA and HMFG are very similar (Strickler et al, 1987).

EMA reactivity is found in a wide variety of epithelial cells and their corresponding tumors. When present, immunoreactivity is usually limited to apical cell membranes in benign secretory epithelium and well-differentiated carcinomas such as those of the breast, but in poorly differentiated carcinomas, cytoplasmic staining is seen and there is loss of staining polarity in the cell membranes. Secretory epithelia and their fetal anlage that show EMA include eccrine sweat glands, sebaceous and apocrine glands. It is also expressed in salivary gland, exocrine pancreas, gastric and endometrium, bronchial glands, alveolar cells and the epithelium of bile ducts, stomach, bronchi, fallopian tube and vas deferens. In addition to glandular epithelium, EMA has also been demonstrated in non-secretory epithelia such as urothelium, renal distal and collecting tubules and syncytiotrophoblasts.

Applications

Despite the ready availability of anticytokeratin as a marker of epithelial differentiation, there is still widespread use of EMA as a marker of epithelial cells. This is fraught with inconsistencies. While EMA is generally not expressed by germ cells, normal hematolymphoid, mesenchymal, neural and neuroectodermal, it may be expressed by certain non-epithelial tissues such as fetal notochord, arachnoid granulations, ependyma, choroid plexus, epineural and perineural fibroblasts, histiocytes and plasma cells and their corresponding neoplasms. EMA is normally expressed by plasma cells and is conserved and even increased in

plasma cell neoplasms and, by ultrastructural examination, has been located diffusely on the cell membranes and focally within rough endoplasmic reticulum. Neoplasms from earlier stage B-cell differentiation do not usually express EMA and in lymph node-based B-cell lymphomas, EMA is found mainly in diffuse large cell lymphomas and T cell-rich B-cell lymphomas. EMA is more frequently seen in T-cell neoplasms, occurring in about 20% of all T-cell lymphomas (Chittal et al, 1997). EMA expression in Reed-Sternberg cells is unusual although it is frequently found in the L&H cells of nodular lymphocyte-predominant Hodgkin's disease. EMA is also found in almost 50% of cases of anaplastic large cell lymphoma of CD 30 phenotype.

In our practice, staining for EMA is not generally employed as a marker of epithelial cells but more often for the identification of certain mesenchymal tumors including synovial sarcoma (Leong et al, 1997), anaplastic large cell lymphoma (CD 30+) and perineurioma (Li et al, 1996). The expression of EMA in chordoma serves to distinguish it from chondroma and chondrosarcoma (Gown & Leong, 1993; Jeffrey et al, 1995) and EMA is expressed in solitary fibrous tumors (Carneiro et al, 1996). EMA immunostaining may help identify ovarian granulosa cell tumors from tumors that mimic their various histological patterns. While keratin may be expressed in granulosa cell tumors, the absence of EMA and immunoreactivity for smooth muscle actin allows distinction from primary and metastatic carcinomas (Costa et al, 1994).

Immunostaining for EMA is a valuable adjunct to the examination of effusions and biopsies for malignant mesothelioma. By ultrastructural examination EMA has been demonstrated exclusively on the long microvillous surfaces of the tumors cells with virtually no cytoplasmic labeling (Van Der Kwast et al, 1987). These findings have been transposed to cytologic preparations and biopsies and careful staining for EMA employing clone E29 shows membranous labeling of malignant mesothelial cells and demonstrates the long microvilli characteristic of the tumor. In contrast, adenocarcinomas display diffuse cytoplasmic staining, with or without membranous enhancement, but long microvilli are not seen (Leong et al, 1990).

Comments

For diagnostic applications we prefer to use anti-EMA (clone E29) instead of HMFG, especially as both antigens have very similar tissue distribution.

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Epstein-Barr Virus, LMP

Sources/Clones

Accurate (CS1-4), Biodesign (polyclonal), Dako (CS1-4), EY Labs, Novocastra (polyclonal) and Research Diagnostics (4C5).

Fixation/Preparation

Applicable to formalin-fixed, paraffin-embedded tissue sections. Enzymatic digestion (e.g. trypsin) is essential to enhance immunopositivity. The application of microwave irradiation for antigen retrieval has also been used to good effect (Kaczorowski et al, 1994). This antibody may also be used for labeling acetone-fixed cryostat sections or fixed-cell smears.

Background

The antibody (isotype: IgG1, κ) has been raised against recombinant fusion protein containing sequences of bacterial β -galactosidase and the EBV-encoded latent membrane protein (LMP-1). LMP is one of the few viral proteins that are expressed in a latent infection. The antibody reacts with a 60 kD latent membrane protein encoded by the BNLF gene of the Epstein-Barr virus. Being a cocktail of clones CS1, CS2, CS3 and CS4, all four anti-LMP antibodies recognize distinct epitopes on the hydrophilic carboxyl region of LMP (Rowe et al, 1987). These four epitopes are present on the internal aspect of the membrane-associated viral LMP. Therefore the antibody does not react with viable cells, but with fixed cells in paraffin sections, cytological preparations and cryostat sections and in immunoblotting.

Applications

The antibody is characterized by its strong positivity with EBV+ lymphoblastoid cell lines and EBV-infected B-cell immunoblasts in infectious mononucleosis. Although EBV is consistently present in nasopharyngeal undifferentiated carcinoma among Oriental patients, LMP-1 antibody is only positive in about 60% of cases (Hording et al, 1993; Lopategui et al, 1994). LMP protein expression is especially useful in identifying these cancers in cervical lymph node metastases. This antibody may also be useful in the diagnosis of lymphoepithelioma-like carcinoma of the lung, mediastinum, stomach and paranasal sinuses (Dimery et al, 1988; Weiss et al, 1989; Shibata et al, 1991).

Posttransplantation lymphoproliferative disorders arising in patients treated with a variety of immunosuppressive regimens after organ transplantation usually show a type III latency pattern with LMP-1 expression (Delecluse et al, 1995). The EBV+ AIDS-associated B-cell lymphomas usually demonstrate a latency type III in the large cell lymphomas, permitting the use of antibody to LMP-1 (Hamilton-Du Toit et al, 1993).

Nasal T/NK-cell lymphoma is strongly associated with EBV (Kanavaros et al, 1993). However, LMP-1 protein expression has been inconsistent on paraffin sections, although one study consistently demonstrated LMP-1 protein in frozen sections, suggesting a low level of protein expression. LMP-1 immunohistochemistry is positive in 17% of adult T-cell leukemia/lymphoma (Tokunaga et al, 1993). LMP-1 expression has also been associated with an aggressive clinical course and hepatosplenomegaly in nodal T-cell lymphomas (Bruin, 1993). About 20-30% of CD 30 (Ki-1)+ anaplastic large cell lymphoma show LMP-1 immunoreaction (Herbst et al, 1991).

Approximately 50% of

Hodgkin's disease cases are associated with EBV. In almost all of these positive cases, nearly all the Reed-Sternberg cells are positive for EBV. Using modern epitope retrieval techniques, an almost 1:1 correlation between the results of LMP-1 paraffin-based immunohistochemistry studies and EBER in situ hybridization studies has been demonstrated in Reed-Sternberg cells and Hodgkin's cells of EBV-associated Hodgkin's disease (Delsol et al, 1992; Pinkus et al, 1994; Oudejans et al 1997). With respect to antibodies against LMP, a note of caution is advised: strong staining of normal early myeloid and erythroid precursors may be seen despite a total absence of evidence of EBV by PCR (Hammer et al, 1996).

Comments

As a research tool, EBV immunohistochemical investigation is superior to PCR, in that the latter does not exclude background/resident lymphocytes harboring EBV.

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Estrogen Receptor (ER)

Sources/Clones

Abbott (H222), Accurate (CC4.5), Biogenesis (ERLH1), Dako (1D5), Eurodiagnostics/Accurate (polyclonal), Immunotech (1D5), Novocastra (CC4.5, LH1), Oncogene (TE111), Vector (6F11) and Zymed (iD5).

Fixation/Preparation

The method of preparation is very much dependent on the antibody clone employed. The ERICA antibody (clone H222) is mostly only immunoreactive in fresh-frozen tissues although some laboratories had success with tissues which had only short exposure to formaldehyde (Raymond & Leong, 1988, 1990) or following the careful use of specific antigen retrieval agents such as DNAse. The development of clone 1D5 made it possible for the immunostaining of routinely fixed paraffin-embedded sections but only following heat-induced epitope retrieval (HIER) (Leong & Milios, 1993; Balaton et al, 1996). The latter procedure has no effect with the H222 antibody.

Background

The first monoclonal antibodies to the estrogen receptor (ER) protein (estrophilin) were produced from a human breast cancer cell line, MCF-7, subjected to affinity column processing and elution (Greene et al, 1980). The antibodies were produced by immunization of rats with this partially purified estradiol-estrophilin complex. Fusion of splenic lymphocytes from the immunized animals with myeloma cells yielded three hybridoma cells lines after cloning by limited dilution techniques, the antibodies thus produced recognized estrogen-occupied as well as unoccupied receptors (Pousen et al, 1985).

The human ER is a member of a family of nuclear receptors for small hydrophobic ligands such as thyroid hormone, vitamin D, retinoic acid and the steroid hormones. Each receptor has a ligand-binding domain, a hinge region, a DNA-binding domain and a variable or regulatory domain. The ER gene is located on the long arm of chromosome 6 (q24-27) and comprises eight exons and intervening introns spanning at least 140 kilobases. Binding of the ligand to the receptor is thought to result in an allosteric alteration that allows the hormone-receptor complex to bind to its DNA response element in the promoter region of a target gene. In the absence of hormone binding, the domain appears to be inhibitory in function, preventing transcriptional activation. Besides establishing the allosteric association of the hormone binding and the regulatory domains, the sequences contained in the hinge region are critical in directing the ER and progesterone (PR) proteins to the nucleus after they are synthesized in the cytoplasm. The DNA-binding domain has many basic amino acids, some of which are repeating units folded into a "fingered" structure coordinated by a zinc ion, known as the "zinc finger" (Schwabe et al, 1990). The ER and PR appear to enhance the transcriptional activity of selected genes. The actual mechanism is not known but probably involves interactions between receptors and other transcriptional factors with the promoter regions of the respective genes. In the current model of ER action, estradiol diffuses into the cell and binds to the receptor, leading to its dimerization and tight binding to its specific DNA target. Following this binding to the estrogen response element in target genes, there is stimulation to increase the transcription of target genes, some directly or indirectly leading to the establishment of both autocrine and paracrine growth

stimulatory loops (Leong & Lee, 1995).

Early studies employing monoclonal antibodies to estrophilin reported both cytoplasmic and nuclear staining, the former being stronger. However, subsequent studies have established an exclusive nuclear localization for ER proteins in both human breast carcinomas as well as other steroid-responsive tissues. This has been confirmed by autoradiographic studies and immunoelectron microscopy has shown the ER protein to be present in the euchromatin portion of the nucleus in breast, endometrial and ovarian cancers as well as in benign endometrium. The cytoplasm in all cases did not reveal presence of the receptor although there was some reaction product in the ribosomes. This latter reactivity was considered to be non-specific although the possibility of synthesis of ER at the ribosomal level was not completely ruled out. Studies demonstrating cytoplasmic localization of ER protein in addition to nuclear localization have mostly employed fluoresceintagged estrogen analogs, whereas modern immunoenzyme techniques utilizing monoclonal antibodies to ER protein have shown only nuclear reactivity.

Applications

Over 100 years ago it was recognized that oophorectomy was associated with clinical remission in women with metastatic breast cancer. Despite the usefulness of hormonal manipulation in some women, only approximately 30% of unselected women with metastatic breast cancer responded to such treatment. There was therefore a need to distinguish those women whose breast cancers are hormone dependent from those whose tumors are hormone independent. Employing cytosol-based ligand-binding assays, it was shown that about 50-60% of women with ER-rich breast cancers responded to hormone treatment, while less than 10% with ER-poor tumors showed a similar response. The relevance of ER status and hormonal treatment in node negative tumors, however, is less clear. It is also clinically recognized that a small proportion of patients whose tumors are receptor negative by cytosol-based assays will show a positive response to hormone treatment and as many as one-third of those with ER-positive tumors may fail to respond to such treatment. On the basis of comparative immunohistologic studies, it is believed that some of these discrepancies are caused by inherent errors of the biochemical method which assays homogenized tissue samples with resultant errors introduced by the inclusion of benign epithelium, the dilutional effect of abundant stroma and inadequate tumor sampling. Indeed, there is recent persuasive evidence, based on hormonal response as the ultimate yardstick, that immunostaining in frozen or paraffin sections is the more accurate measurement of ER status (Pertschuk et al, 1996) and this and many other advantages make immunostaining the "gold standard" to replace cytosol assays (Taylor, 1997).

It has been suggested that ER may be used to identify metastatic breast carcinoma but a variety of other lesions with epithelioid features may also express ER.

These include epithelioid smooth muscle tumors, malignant melanoma, meningioma, sclerosing hemangioma (Leong et al, 1997), desmoid tumors, thyroid neoplasms and cervical, endometrial and ovarian cancers, rendering the marker less useful as a diagnostic discriminant.

Comments

Since the development of clone 1D5, it has become possible to perform immunostaining for ER in routinely fixed and processed tissues inexpensively, accurately and consistently. Some form of HIER procedure is essential when using clone 1D5; in contrast, H222 fails to stain following HIER, indicating that only one epitope of the ER, and not the entire antigen, is retrieved by the heating process. For this reason, it has been suggested that the procedure be called heat-induced epitope retrieval rather than the original term "heat-induced antigen retrieval". It is possible to obtain consistent staining of ER in cytologic preparations by employing clone 1D5 with HIER on smears which are initially completely air-dried before fixation in 10% buffered formalin.

It is important, as with most other antibodies employed for immunohistology, that each laboratory determines its optimal time for epitope retrieval and does not purely rely on the procedures developed for other laboratories.

A concordance of 77-100% exists between immunostaining in paraffin and frozen sections with the dextran-coated charcoal assay (DCC) and we have adopted 10% staining of tumors cells as the cut-off value as it corresponded with 10 fmol/mg of proteins by DCC

assay. There is strong correlation-of the percentage of positive cells with the intensity of staining. The results of immunostaining may be expressed subjectively as positive or negative, as a percentage of positive tumor cells, or as a score derived by adding grades of staining (1, 2 and 3, corresponding to mild, intermediate and strong staining) and the percentage as scores of 0, 1, 2, 3 and 4 (corresponding to 10%, 11-25%, 26-50%, 51-75% and > 75%). Computerized image analysis has been claimed to produce an increasing specificity and sensitivity relative to biochemical assays but other studies have shown identical results by image analysis and visual examination and significantly similar agreement between the two and biochemical values.

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Factor VIII RA (von Willebrand factor)

Sources/Clones

Accurate (KG7/30), Axell/Accurate (F8/86, polyclonal), Biodesign (101, 102, 103), Biogenesis (37-56/3, 21-43, WF7, polyclonal), Biogenex (polyclonal), Biomeda (polyclonal), Chemicon, Diagnostic Biosystems (polyclonal), Dako (F8/86, polyclonal), Sanbio (KG7/30), Serotec (F8, F8/86) and Zymed (Z002, polyclonal).

Fixation/Preparation

The antigen is fixation resistant. Proteolytic digestion or HIER enhances immunoreactivity.

Background

Factor VIII-related antigen is more appropriately known as the von Willebrand factor (Marder et al, 1985). Factor VIII is a glycoprotein and is complexed with factor VIII-related antigen in plasma. Factor VIII is also present in endothelial cells, where it shows a granular pattern of reactivity, and in the cytoplasm of megakaryocytes.

Applications

Factor VIII-related antigen or von Willebrand factor was one of the first markers employed for endothelial cell differentiation in angiosarcomas (Sehested & Hon-Jensen, 1981), but it soon became apparent that the von Willebrand factor is seldom expressed in poorly differentiated vascular tumors (Swanson & Wick, 1993). Other markers of endothelial cells provide a higher diagnostic yield and they include CD 34, CD 31 (Appendix 1.23) and *Ulex europaeus* agglutinin I. There is also considerable overlap between the expression of von Willebrand factor in vascular and lymphatic endothelium (Suthipintawong et al, 1995).

Von Willebrand factor remains a sensitive marker of benign blood vessels and has been used for the study of angiogenesis in neoplasms such as breast cancer (Weidner et al, 1991).

Comments

Von Willlebrand factor must be used in conjunction with other more sensitive markers of endothelial cells when identifying angiosarcomas. It should be noted that seepage of the antigen may occur from surrounding blood, particularly in hemorrhagic or vascular lesions, and interpretation should be made with caution in such situations.

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Factor XIIIa

Sources/Clones

Beringwerke (polyclonal), Calbiochem (polyclonal) and Novocastra (polyclonal).

Fixation/Preparation

The antibodies are immunoreactive in routinely fixed, paraffin-embedded sections. Staining is enhanced by HIER.

Background

Factor XIIIa is a blood proenzyme found in plasma and platelets. The reaction of Factor XIIIa with fibrin is the last enzyme-catalyzed step on the coagulation cascade, leading to the formation of a normal blood clot stabilized as a result of fibrin crosslinkage. This transglutaminase exists in two forms, as an extracellular or plasma factor XIIIa subunit attached to a dimer of the carrier protein or factor XIIIb and an intracellular factor XIII, which is exclusively the dimer of subunit "a" only. Intracellular factor XIIIa has been identified in a variety of cells including human dendritic reticulum cells in reactive lymphoid follicles, fibroblast-like mesenchymal cells in connective tissue, and neoplastic fibroblastic and fibrohistiocytic lesions (Cerio et al, 1990). The dermal dendrocytes have been characterized as factor XIIIa+ dendritic cells of bone marrow origin that are typically found in the adventitia of dermal blood vessels and in the interstitial dermal connective tissues. In one study of dermal dendritic cells using CD 34 and factor XIIIa, it was found that antigenic profiles differed among the dendritic cell types (Nestle & Nickoloff, 1995). At ultrastructural level, subepidermal dendritic cells (probably identical with lining macrophages) expressed factor XIIIa only, perivascular dermal dendritic cells reacted with both factor XIIIa and CD 34, and reticular dermal dendritic cells were negative for factor XIIIa but positive for CD 34. However, at light microscopic level, perivascular dermal dendritic cells also expressed CD 34.

Applications

The current diagnostic applications of factor XIIIa pertain largely to the identification of dermal dendritic cells and their presence and role in various cutaneous and soft tissue tumors (Takata et al, 1994). Factor XIIIa has been described in various so-called fibrohistiocytic tumors including aneurysmal fibrous histiocytoma (Zelger et al, 1996), malignant fibrous histiocytoma (Nemes & Thomaszy, 1988), dermatofibroma (Nestle et al 1995) and dermatofibrosarcoma protuberans (Leong & Lim, 1994). In the latter two conditions, factor XIIIa expression appears to be associated with early lesions, with loss of expression in late or "mature" lesions. The marker has been reported to show promise as a diagnostic discriminator between hepatocellular carcinoma and its morphologic mimics cholangiocarcinoma and metastatic carcinoma in the liver (Fucich et al, 1994) although this has not been our experience (unpublished data).

Comments

Reactivity in frozen sections is generally weak. We employ the polyclonal antibody from Calbiochem. We understand that Beringwerke has discontinued distributing the antiserum recently.

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Fas (CD 95) and Fas-ligand (CD 95L)

Sources/Clones

Fas (CD 95)

Alexis Corp., San Diego, California (SM1/17, SM1/1, SM1/23, APO1-3), Dako (APO-1, DX2) and Pharmingen (DX2, G254-274).

Fas-Ligand (CD 95L, Anti-Fas)

Immunotech (4A5, 4H9) and Pharmingen (NOK-1, NOK-2, G247-4).

Fixation/Preparation

Several of the antibodies (clones APO-1, DX2) are immunoreactive in fixed, paraffin-embedded tissue sections as well as frozen sections and cell preparations.

Background

Fas (CD 95) is a cell surface protein that belongs to the tumor necrosis factor family. Crosslinking of Fas and Fas-ligand (FasL) transduces signals, which culminate in apoptosis in sensitive cells. These proteins therefore have a role in the genesis of neoplasms and have been extensively studied in this context. Their expression in certain malignancies has been implicated as a possible key mechanism in the immune privilege of such tumors. FasL is also expressed in immunologically privileged sites in the nonneoplastic state. The induction of apoptosis by FasL in invading lymphocytes acts as a mechanism of immune-privilege and is important in preventing graft rejection. The placenta, another immune-privileged site, has also been shown to express high levels of FasL. The induction of apoptosis in lymphocytes by invading trophoblasts may account for the immune tolerance of the fetal semiallograft (Hunt et al, 1997; Bamberger et al, 1997; Uckan et al, 1997). Experimentally, FasL can be employed to induce apoptosis in Fas-bearing cells.

Applications

Fas and FasL expression have been studied in a wide variety of tissues and in other diseases besides neoplasms (Nonomura et al, 1996; Tachibana et al, 1996; Nichans et al, 1997; Muller et al, 1997; Hellquist et al, 1997). These include idiopathic pulmonary fibrosis (Kazufumi et al, 1997), human cancers following ionizing radiation (Sheard et al, 1997), Alzheimers' disease (De La Monte et al, 1997), chronic hepatitis (Luo et al, 1997), alveolar type II pneumocytes (Fine et al, 1997), colonic epithelial cells (Strater et al, 1997), inflammatory myopathies (Behrens et al, 1997), diabetes (Chervonsky et al, 1997) and germ cells of the testis (Lee et al, 1997).

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Ferritin

Sources/Clones

American Qualex (polyclonal), American Research Products (047A1703), Axcel/Accurate (polyclonal), Biodesign (ME.110, S1, S2, 501, 502, 503, 504, polyclonal), Biogenesis (05, 7D3/7, polyclonal), Biogenex (M3.170, polyclonal), Chemicon (polyclonal), Dako (polyclonal), Fitzgerald (M94156, M94157, M94159, M94160, M94212, M94258, polyclonal), Serotec (polyclonal) and Zymed (ZMFE1).

Fixation/Preparation

Ferritin is resistant to formalin fixation and immunoreactivity is enhanced following HIER.

Background

Ferritin, the iron storage protein, plays a key role in iron metabolism and its ability to sequester iron gives ferritin the dual functions of iron detoxification and iron reserve. The distribution of ferritin is ubiquitous among living species and its three-dimensional structure is highly conserved. All ferritins have 24 protein subunits arranged in 432 symmetry to give a hollow shell with an 80 A diameter cavity capable of storing up to 45000 Fe (III) atoms as an inorganic complex. Subunits are folded as four-helix bundles each having a fifth short helix at roughly 60 to the bundle axis (Harrison & Arosio, 1996).

Applications

Ferritin was one of the first markers employed for the identification of hepatocytes and their neoplastic counterparts (Imoto et al, 1985; Johnson et al, 1992), but it proved to be of low sensitivity and low specificity, being found in a wide range of benign and neoplastic tissues (Fleming, 1987; Pennys & Zlatkiss, 1990; Tuccari et al, 1992; Momotani et al, 1992). Ferritin is expressed in hepatoid tumors such as those in the ovary (Nogales et al, 1993) and hepatoblastomas (Abenoza et al, 1987). It is employed as a marker of hemorrhage in the brain (Carter et al, 1991; Ozawa et al, 1994) and as a marker of microglia (Kaneko et al, 1989). In bone marrow biopsies, ferritin has been found to correlate with marrow hemosiderin as detected by the Perl's stain and is advocated as a more sensitive tool for the evaluation of body iron stores (Navone et al, 1988). In the skin, ferritin is localized to the outer layer of the eccrine duct and in sweat gland neoplasms, two distinct patterns were noted. In syringoma the antibody decorated the outermost layer of cells in the epithelial cords of the tumor so that a characteristic ring was produced in cross-sections whereas only sparse staining was observed with other eccrine duct tumors such as dermal duct tumor and eccrine poroma. Syringoma showed diffuse staining, as did acrospiroma and a number of other adnexal carcinomas (Penneys & Zlatkiss, 1990).

Comments

The diagnostic applications of this marker are limited and, except perhaps for the assessment of bone marrow iron stores, ferritin is never employed alone.

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Fibrin

Sources/Clones

Accurate (T2G1), American Diagnostica (3B622, 352, 350), Biodesign (polyclonal), Biogenesis (2F7) and Serotec (E8).

Fixation/Preparation

The antigen is resistant to formalin fixation and proteolytic digestion or HIER enhances immunoreactivity.

Background

Proteolytic conversion of fibrinogen to fibrin results in selfassembly to form a clot matrix that subsequently becomes crosslinked by factor XIIIa to form the main structural element of the thrombus in vivo. The roles of fibrin and its precursor have been extensively studied both in vitro and in vivo (Lorand, 1965 Mosessan, 1992, 1997; Blomback, 1994, 1996; Gaffney, 1997).

Applications

Diagnostic applications of fibrin are mainly limited to the study of glomerulopathy (Dowling, 1993) with sporadic use of antifibrin to identify fibrin deposits and thrombi in extrarenal sites (Bini & Kudryk, 1994; Takahashi et al, 1996; Imokawa et al, 1997).

Comments

The diagnostic applications of antifibrin are limited to specific situations.

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Takahashi H, Shibata Y, Fujita S, Okabe H 1996. Immunohistochemical findings of arterial fibrinoid necrosis in major and lingual minor salivary glands of primary Sjogren's syndrome. Anatomical and Cellular Pathology 12: 145-157.

Fibronectin

Sources/Clones

Accurate (2B6F9, 568), American Research Products (568), Axcel/Accurate (polyclonal), Biodesign (1601, 1602, 120-5), Biogenesis (BIO-FIBTN-001, Bo, Rt, polyclonal), Biogenex (2755-8), Calbiochem (3E1, polyclonal), Caltag Laboratories, Cymbus Bioscience (FN4), Dako (polyclonal), EY Labs, Fitzgerald (polyclonal), Harlan Seralab/Accurate (2.3F9), Novocastra (polyclonal), Serotec (polyclonal), Sigma (FN-15, FN3-E2) and Zymed (Z068, FN12-8).

Fixation/Preparation

The antibody is well suited for both formalin-fixed, paraffin embedded sections and cryostat sections. Proteolytic predigestion with protease or pepsin of formalin-fixed tissue is recommended (Kirkpatrick & D'Ardenne, 1984).

Background

Fibronectin is a non-collagenous connective tissue glycoprotein found in association with both basement membranes and interstitial connective tissue (Stenman & Vaheri, 1978). The exact ultrastructural localization of fibronectin within the basement membrane is still controversial (Laurie et al, 1982). Fibronectin is a β-glycoprotein with a molecular weight of 44 kD, comprising two nearly identical subchains. It is widely distributed throughout many normal tissues including connective tissues, blood vessel walls and basement membranes. Some of the properties of fibronectin include forming crosslinks with fibrin in blood clots through factor XIII and binding to heparin and collagen. It is also thought to play a role in cellular adhesion, wound healing and tissue repair (Mosher & Fiocht, 1981). Antiserum to human fibronectin was produced from purified human material isolated from a pool of normal human plasma.

Applications

Fibronectin (and laminin) has been demonstrated to line cystic lumina and around tumor islands in adenoid cystic breast and salivary gland carcinomas (D'Ardenne et al, 1986). This pattern of distribution has been recommended as an aid to the diagnosis of these tumors, whilst the absence may have important prognostic implications with an aggressive outcome. Fibronectin immunoreactivity in breast adenoid cystic carcinomas is also useful to distinguish them from cribriform carcinoma, the latter being negative.

In a comparative study of epithelial neoplasms of gastrointestinal and salivary gland origin, the difficulty in distinguishing between fibronectin of epithelial and fibroblastic origin was emphasized (D'Ardenne et al, 1983). In addition, carcinoma fibronectin was sometimes but not invariably lost from epithelial cell surfaces, suggesting that loss of cell surface fibronectin was unlikely to serve as a useful diagnostic marker for malignancy. In soft tissue tumors, fibronectin was found to be most abundant in the stroma, both benign and malignant (D'Ardenne et al, 1984).

Comments

The major role of fibronectin is in the diagnosis of adenoid cystic carcinoma of the salivary gland and breast, with the latter being distinguished from cribriform carcinoma. Either adenoid cystic carcinoma or connective tissue stroma may be used as a positive control.

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Stenman S, Vaheri A 1978. Distribution of a major connective tissue protein, fibronectin, in normal human tissues. Journal of Experimental Medicine 147: 1054-1064.

Fibrinogen

Sources/Clones

Accurate (2C2G7, 85D4), Axcel/Accurate (polyclonal), American Qualex (polyclonal), Biodesign (PA), Biogenesis (2D1-2, polyclonal), Biogenex (2D1-2, polyclonal), Calbiochem (polyclonal), Caltag Laboratories, Chemicon, Coulter (D1G10VL2, E3F8E5), Dako (polyclonal), EY Labs, Immunotech (D1G10VL2, E3F8E5), Seralab (polyclonal) and Sigma (85D4, FG21).

Fixation/Preparation

Fibrinogen is fixative resistant.

Background

Fibrinogen is a 340 kD multisubunit glycoprotein present in plasma and tissue of all classes of vertebrates. It has a variety of physiologically important functions, most of which, if not all, are assigned to certain structures of fibrin including double-stranded fibrin protofibrils and highly crosslinked fibrin networks (Shafer & Higgins, 1988; Mosessan 1997). Its role in hemostasis and thrombosis has been extensively studied (Henschen, 1983; Mosessan, 1992; Blomback, 1996; Gaffney, 1997).

Applications

Diagnostic applications of fibrinogen are largely limited to the identification of fibrinogen deposition and breakdown products in glomerular diseases (Dowling 1993).

Comments

None.

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Glial Fibrillary Acidic Protein (GFAP)

Sources/Clones

Accurate (GA-5, 6F2, polyclonal), Amersham, Biodesign (DP46.10, GF-01), Biogenesis (GF-01, polyclonal), Biogenex (GA-5, polyclonal), Chemicon (monoclonal, polyclonal), Cymbus Bioscience (polyclonal), Dako (6F2, polyclonal), Enzo, EY Labs, ICN (polyclonal), Immunotech (DP46.10), Milab (polyclonal), Novocastra, Sanbio (6F2, polyclonal), Saxo (polyclonal), Seralab (GA-5), Serotec (GA5, MIG-G2), Sigma (GA-5, polyclonal), Signet and Zymed (ZSGFAP2, ZCG29).

Fixation/Preparation

Glial fibrillary acidic protein (GFAP) is relatively resilient to fixation and most antibodies are immunoreactive in routinely fixed and processed tissue sections. GFAP staining seems more consistent after fixation in Bouin's fixative. Monoclonal antibodies are more fixative sensitive and polyclonal antibodies show more intense and more extensive staining. GFAP immunoreactivity is mildly enhanced by HIER.

Background

GFAP is an intermediate filament (IF) protein of astroglia and belongs to the type III subclass of IF proteins. Like other IF proteins, GFAP is composed of an amino terminal head domain, a central rod domain and a carboxy terminal tail domain. GFAP, with a molecular mass of 50 kD, has the smallest head domain among the class III IF proteins. Despite its insolubility, GFAP is in dynamic equilibrium between assembled filaments and unassembled subunits. As with other IF proteins, assembly of GFAP is regulated by phosphorylation-dephosphorylation of the head domain by alteration of its charge. The frequent copolymerization of GFAP with vimentin IF in immature, reactive or radial glia indicates that vimentin has an important role in the build-up of the glial architecture (Inagaki et al, 1994). The human GFAP gene is localized to chromosome 17.

Applications

In the central nervous system, astrocytes, rare ependymal cells and cerebellar radial glia express GFAP (Appendix 1.2). While mature oligodendrocytes do not. GFAP or a GFAP-like protein is also found in Schwann cells, enteric glia, cells in all portions of the pituitary, cartilage, the iris and lens epithelium and the fat-storing cells of the liver. While monoclonal antibodies are said to recognize the GFAP epitope exclusively, there may be crossreactivity with common epitopes shared by other IFs like neurofilaments and vimentin.

Immunohistochemical staining of GFAP has proven use in the identification of benign astrocytes and neoplastic cells of glial lineage (Sillevis-Smitt et al, 1993). Its application to the developing nervous system has contributed to our understanding of the histogenesis of neural tissue and its identification in various forms of injury and neoplasia has helped in the understanding of the role of astrocytes in these processes.

While it was initially thought that the GFAP expression in salivary gland tissues and pleomorphic adenomas was in myoepithelial cells (Lee et al, 1993), more recent evidence from developmental and cell culture studies indicates that GFAP is expressed in the epithelial cells, the myoepithelial cells being uniformly negative for the antigen (Okura et al, 1996). GFAP has been demonstrated in cartilage cells in culture (Benjamin et al, 1994) but do not appear to occur in chondrosarcomas and mesenchymal chondrosarcomas (Swanson et al, 1990) and in vivo

and immunohistochemical detection of GFAP is used to identify chordomas. Choroid plexus tumors (Radotra et al, 1994) and ependymomas express GFAP in addition to S100 protein and, occasionally, cytokeratin and epithelial membrane antigen. In the setting of vacuolated clear cell tumors occurring in the retroperitoneal space, GFAP positivity would serve to identify chordoma and ependymoma from other mimics, including renal cell carcinoma and colorectal carcinoma (Coffin et al, 1993).

Comments

Polyclonal antibodies to GFAP produce more intense and more extensive staining than monoclonal antibodies (Wittchow & Landas, 1991).

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Gross Cystic Disease Fluid Protein-15 (GCDFP-15, BRST-2)

Sources/Clones

Biogenex (GCDFP-15) and Signet (GCDFP-15)

Fixation/Preparation

The antigen is fixation stable and can be detected in paraffin-embedded sections as well as fresh-frozen sections and cell preparations. HIER enhances immunostaining and proteolytic digestion is unnecessary. Cytologic preparations should be fixed in 10% formalin or Bouin's solution. Alcohol-fixed preparations are not immunoreactive.

Background

Gross cystic disease fluid protein-15 (GCDFP-15) is one of four major component proteins found in the cystic fluid obtained from patients with fibrocystic changes of the breast. GCDFP-15 is a marker of apocrine glandular differentiation in both benign and malignant mammary epithelium (Haagensen et al, 1990). This protein has widespread distribution in apocrine glands elsewhere in the axillary and perianal tissues, as well as in the sublingual and submaxillary salivary glands. The GCDFP-15 protein is a 15 kD glycoprotein shown to be prolactin inducible, the GCDFP-15 gene having been recently cloned (Myal et al, 1991). Ultrastructurally, the GCDFP-15 protein has been localized in Golgi vesicles and cytoplasmic granules. The protein is released by exocytosis at the apices of the mammary epithelial cells (Mazoujian et al, 1984).

Applications

Carcinoma of the breast is a treatable disease with a variable prognostic outcome. Its recognition is therefore of great therapeutic importance but in metastatic sites, identification of breast carcinoma can often be difficult. A marker of mammary epithelial differentiation would be of diagnostic importance. GCDFP-15 goes some way towards fulfiling this role and is currently the best marker yet to identify breast cancer metastases. GCDFP-15 was identified by immunostaining in 55-74% of cases of breast carcinoma (Mazoujian et al, 1989; Wick et al, 1989) and has a higher rate of sensitivity and specificity than α-lactalbumin as a marker of both primary and metastatic breast cancer. Besides mammary carcinomas, the major tumor types that expressed GCDFP-15 were salivary glands, sweat glands and prostate (Wick et al, 1989). It is also a marker of apocrine differentiation in the skin (particularly in combination with lysozyme) and can be suitably applied for the separation of cutaneous adnexal tumors (Appendix 1.19). It is worth noting that the expression of GCDFP-15 varies among the histologic subtypes of breast carcinoma, with highest incidence in infiltrating lobular carcinoma with signet ring cell differentiation (90%), compared to 70% in ordinary infiltrating ductal carcinoma and 75% in those subtypes showing apocrine differentiation (Mazoujian et al, 1989). Expression of the GCDFP-15 gene was significantly associated with relapse-free survival and was suggested to represent a marker of prognostic relevance (Pagani et al, 1994).

Antibodies to GCDFP-15 have been used successfully to identify metastases from breast carcinoma in the brain (Perry et al, 1997), ovary (Monteagudo et al, 1991) and other sites (Chaubert & Hurlimann, 1992). Immunodistinction of metastasis from breast cancer and eccrine and apocrine tumors in the skin can be difficult as the latter tumors also express this antigen (Tsubura

et al, 1992; Wallace et al, 1995; Wallace & Smoller, 1996). However, as with other metastatic sites, the highest diagnostic yield was obtained when anti-GCDFP-15 was employed together with other antibodies in a diagnostic panel.

GCDFP-15 is also a suitable marker in cytological specimens and the best results are obtained following fixation in 10% formalin or Bouin's solution, alcohol-fixed samples showing no immunoreactivity for this antigen (Fiel et al, 1996).

Comments

The immunoreactivity of monoclonal antibodies and polyclonal antisera to GCDFP-15 appears to be the same (Mazoujian et al, 1988), HIER enhancing immunoreactivity of both antibodies.

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HAM 56 (Macrophage marker)

Sources/Clones

Dako (HAM 56).

Fixation/Preparation

This antibody is applicable to both formalin-fixed paraffin sections and frozen sections. Either enzyme or microwave pretreatment is beneficial to the immunoreactivity with the HAM 56 antibody.

Background

Human alveolar macrophage-56 (IgM, k) is a monoclonal antibody developed against human alveolar macrophages (Gown et al, 1986). The antigen recognized by HAM 56 has not yet been identified. This antibody was developed specifically for the study of human atherosclerotic plaques, to identify tissue macrophages and monocyte-derived cells (Gown et al, 1986).

Applications

HAM 56 has wide immunoreactivity including tissue macrophages, germinal center macrophages, interdigitating reticulum cells, subsets of monocytes, a small population of lymphocytes and endothelial cells. Variable reactivity with a small number of B-cell lymphomas has also been reported. Recent attempts using HAM 56 to distinguish between ovarian and gastrointestinal carcinomas have proved inconclusive (Fowler et al, 1994; Cheung et al, 1997).

Comments

Apart from its initial usefulness in identifying macrophages in atherosclerotic plaques, HAM 56 has yet to establish a niche in diagnostic surgical pathology. Tissue rich in macrophages is the recommended positive control.

References

Cheung ANY, Chiu P-M, Khoo U-S 1997. Is immunostaining with HAM 56 antibody useful in identifying ovarian origin of metastatic adenocarcinomas? Human Pathology 28:91-94.

Fowler LJ, Maygarden SJ, Novotny DB 1994. Human alveolar macrophage-56 and carcinoembryonic antigen monoclonal antibodies in the differential diagnosis between primary ovarian and metastatic gastrointestinal carcinomas. Human Pathology 25:666-670.

Gown AM, Tsukadat, Ross R 1986. Human atherosclerosis. II. Immunocytochemical analysis of the cellular composition of human atherosclerotic lesions. American Journal of Pathology 125:191-207.

HBME-1 (Mesothelial Cell)

Sources/Clones

Dako (HBME-1).

Fixation/Preparation

The antibody is immunoreactive in formalin-fixed, paraffin-embedded tissue sections and in frozen sections and cell preparations.

Background

The antibody reacts with an antigen present in the membrane of mesothelial cells and their neoplastic counterparts, particularly epithelioid mesotheliomas. In initial testing the antibody failed to decorate epithelial cells of the kidney, lung, liver, ovary and pancreas. The antibody was derived from human epithelioid mesothelioma cells.

Applications

The antibody was designed primarily for the identification of normal and neoplastic mesothelial cells from metastatic carcinoma and is useful in this context. However, like many previous attempts to produce a mesothelial cell-specific antibody, HBME-1 has met with limited success. In one study, HBME-1 labeled all 17 cases of mesothelioma but also adenocarcinoma cells in ten of 14 cases (Bateman et al, 1997). Among other markers employed in the same study, the authors found that CA 125 labeled 15 of 17 mesotheliomas and seven of 14 adenocarcinomas. They concluded that both HBME-1 and CA 125 were not sufficiently specific to be employed on their own as mesothelial markers but made a contribution when used in an appropriate panel. Similar results have been previously reported (Attanoos et al, 1996). Negative staining for HBME-1 makes the diagnosis of mesothelioma unlikely. A study of serous effusions revealed HBME-1 reactivity on the membranes of all reactive and malignant mesothelial cells but also in 24% of metastatic carcinomas and as many as 83% of ovarian carcinomas (Ascoli et al, 1997). HBME-1 does not label sarcomatous malignant mesothelioma (Donna et al, 1997).

HBME-1 produces a "thick pattern of immunoreactivity of the cell surfaces, often including the intracytoplasmic lumina" and is said to show excellent correlation with the presence of abundant long microvilli with electron microscopy (Battifora & McCaughey, 1995). There is usually no cytoplasmic labeling and although adenocarcinoma cells may show membrane staining, they do not display the characteristic "thick" membranes and may show cytoplasmic staining. A similar pattern of immunoreactivity with antiepithelial membrane antigen (anti-EMA) was described earlier by Leong et al (1990) and corresponds to labeling of the cell membranes and long microvilli characteristic of mesothelioma cells (Van Der Kwast et al, 1987). It was emphasized that the microvillous processes which are visible with EMA immunostaining are not only abnormally long but their circumferential distribution around the cell is aberrant in nature and signifies malignancy (Leong & Vermin-Roberts, 1994).

Comments

As with EMA, immunostaining with HBME-1 is aimed at highlighting the cell membranes and long microvilli. Optimal dilutions of the antibody have to be ascertained before use in diagnostic panels as high concentrations will result in cytoplasmic staining of both mesothelioma and adenocarcinoma cells, reducing the usefulness of HBME-1 as a diagnostic discriminator between the two entities.

References

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Ascoli V, Carnovale-Scalzo C, Taccogna S, Nardi F 1997. Utility of HBME-1 immunostaining in serous effusions. Cytopathology 8: 328-335.

Bateman AC, Al-Talib RK, Newman T, Williams, Herbert A 1997. Immunohistochemical phenotype of malignant mesothelioma: predictive value of CA 125 and HBME-1 expression. Histopathology 30: 49-56.

Battifora H, McCaughey WTE 1995. Tumors of the serosal membranes. Atlas of tumor pathology, 3rd series, fascicle 15. Washington DC: Armed Forces Institute of Pathology, p 73.

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Van Der Kwast TH, Versnel MA, Delahaye M et al 1987. Expression of epithelial membrane antigen on malignant mesothelial cells. An immunocytochemical and immunoelectron microscopic study. Acta Cytologica 32: 169-174.

Heat shock proteins (Hsps)

Sources/Clones

Only clones of Hsp 27, Hsp 60 and Hsp 70 are listed.

Hsp 27

Biogenex (G3.1), Immunotech (G3.1) and Stress Gen (G3.1)

Hsp 60

Accurate (LK1, LK2), Affinity Bio (4B9/89, 2E1/53), Sanbio (LK1, LK2), Sigma (LK2) and Stress Gen (LK1, LK2, polyclonal).

Hsp 70

Affinity Bio (3a3, 5A5, 4G4, 7.10), Amersham, Biogenex (BRM22), Dako (polyclonal), Diagnostic Biosystems (polyclonal), Pharmingen (5G10), Sigma (BRM11) and Stress Gen (N27F3-4, 1B5, C92F3A-5).

Fixation/Preparation

Some of the antibodies are immunoreactive in fixed paraffin-embedded tissue sections, others are immunoreactive only in cryostat sections and fresh cell preparations. HIER produces enhancement of immunoreactivity.

Background

When prokaryotic or eukaryotic cells are submitted to a transient rise in temperature or to other proteolytic treatments, the synthesis of a set of proteins called heat shock proteins (Hsps) is induced. The structure of these proteins has been highly conserved during evolution. The signal leading to the transcriptional activation of the corresponding genes is the accumulation of denatured and/or aggregated proteins inside the cells after being subjected to stress. The expression of a subset of Hsp is also induced during early embryogenesis and many differentiation processes. Two different functions have been ascribed to Hsps: a molecular chaperone function whereby they mediate the folding, assembly or translocation across the intracellular membranes of other polypeptides, and a role in protein degradation. Some of the essential components of the cytoplasmic ubiquitin-dependent degradative pathway are Hsps (Mayer et al, 1991). These functions of Hsps are essential in every living cell and are required for repairing the damage that results from stress. In addition, the Hsps may also have a number of biological functions apparently distinct from their role during stress, such as in tyrosine kinase and steroid hormone function (Welch 1987; Pratt & Welsh 1994).

Applications

Current interest in Hsps lies in their role as prognostic markers in various tumors and in tumor resistance to chemotherapy, overexpression of Hsps allowing tumor cells to resist stressful situations and agents, including cytotoxic drugs. In endometrial cancers, expression of Hsp 27 has been correlated with the degree of tumor differentiation as well as with the presence of estrogen and progesterone receptors. In patients with cervical cancer, Hsp 27 is predominantly expressed in well-differentiated and moderately differentiated squamous cell carcinomas but the expression of this protein seems to be a negative prognostic factor for gastric cancer (Ciocca et al, 1993b). In the case of malignant fibrous histiocytoma, the expression of Hsp 27 was found to be the strongest prognostic factor, correlating with longer

disease-free intervals and overall survival, independent of tumor size, necrosis and histological subtype (Tetu et al, 1992). Different isoforms of Hsp

27 have been found in lymphoid tissue of patients with acute lymphoblastic leukemia and the protein has been associated with viral infections.

The presence of Hsp 70 appears to be associated with breast cancers of high histological grades (Lazaris et al, 1997) and it has been suggested that high levels of the protein identify a subset of patients with node-negative breast cancer who show a high risk for disease recurrence (Ciocca et al, 1993a). Increased Hsp 70 expression has also been correlated with low levels of differentiation in colorectal cancer (Lazaris et al, 1995).

Immunostaining for Hsp 27 and Hsp 90 has been studied in a variety of central nervous system tumors (Kato et al, 1992, 1995).

Comments

The Hsps show immunolocalization in the cytoplasm as diffuse or finely granular staining.

References

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Kato M, Herz F, Kato S, Hirano A 1992. Expression of stress-response (heat-shock) protein 27 in human brain tumors: an immunohistochemical study. Acta Neuropathologica (Berlin) 83: 420-422.

Kato S, Morita T, Takenaka T et al 1995. Stress-response (heat-shock) protein 90 expression in tumors of the central nervous system: an immunohistochemical study. Acta Neuropathologica (Berlin) 89: 184-188.

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Tetu B, Lacasse B, Bouchard HL et al 1992. Prognostic influence of HSP-27 expression in malignant fibrous histiocytoma: a clinicopathological and immunohistochemical study. Cancer Research 52:2325-2328.

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Helicobacter Pylori

Sources/Clones

Biodesign (51-13), Biogenesis (1G6, CP15), Biogenex (UM01), Dako (polyclonal) and Sanbio/Monosan (51-13).

Fixation/Preparation

Applicable to 10% neutral buffered formalin or Bouin's fixed tissue.

Background

Helicobacter pylori (HP) is a spiral bacillus that can colonize the human gastric mucosa and induce a specific humoral immunologic reaction in the host. Colonization of the gastric mucosa by HP is a very common finding in gastric ulcers and active chronic gastritis. HP is increasingly recognized as one of the most prevalent human pathogens worldwide and possibly plays a pathogenetic role in gastric carcinogenesis and primary gastric lymphogenesis. The details of the interaction between bacteria, epithelial cells and inflammatory cells are currently being explored. As effective specific treatment for HP-associated gastroduodenal disorders emerges, surgical pathologists are requested to identify the organism in endoscopic biopsies. Histologic identification of HP (with special staining methods) has been shown to be as accurate as microbiologic culture techniques (Hui et al, 1992; Genta et al, 1994).

The Signet rabbit anti-HP polyclonal antisera was raised against *H.pylori* strain CH-20429 and detects antigens of the whole organism in formalin-fixed, paraffin-embedded, frozen and cytologic specimens.

Applications

Bacteria lying within the mucus and on the epithelial surface can be seen on sections stained with hematoxylin and eosin (H&E). However, organisms closely adherent to cells, insinuated in intercellular spaces or intimately associated with and perhaps phagocytosed by inflammatory cells are frequently difficult to identify.

There are several published special stains that demonstrate HP efficiently in the histologic sections (Genta et al, 1994). However, the use of immunohistochemical methods is highly specific and has an important role in selected situations (Cartun et al, 1991). For example, small gastric biopsies with a very low density of *H.pylori*, posttreatment biopsy specimens to assess therapeutic success or when abundant debris or mucus is present on gastric surface and pits, may benefit from identification of *H.pylori* with immunohistochemistry.

Comments

Immunohistochemical methods for the detection of *H.pylori* are highly specific and play an important role in selected situations, but cannot be advocated for the routine diagnosis of *H.pylori* gastritis. HP-infected gastric tissue is recommended as positive control tissue.

References

Cartun RW, Kryzmowski GA, Pedersen CA et al 1991. Immunocytochemical identification of *H.pylori* in formalin-fixed gastric biopsies. Modern Pathology 4: 498-502.

Genta RM, Robason GO, Graham DY 1994. Simultaneous visualization of Helicobacter pylori and gastric morphology: a new stain. Human Pathology 25: 221-226.

Hui PK, Chan WY, Cheung PS, Chan JKC, Ng CS 1992. Pathologic changes of gastric mucosa colonized by H.pylori. Human Pathology 23: 548-556.

Hep Par 1 (Hepatocyte Marker)

Sources/Clones

Dako (OCH 1E5).

Fixation/Preparation

The antibody is immunoreactive in fixed paraffin-embedded sections and immunoreactivity is slightly enhanced following HIER.

Background

Hep Par 1 (hepatocyte paraffin 1) is an IgGK antibody to both normal and neoplastic hepatocytes raised at the Pittsburgh Cancer Institute. Hep Par 1 detects an antigen that is localized to the hepatocyte cytoplasm and produces no staining of bile ducts or other non-parenchymal cells. The staining is granular, occasionally ring-like and is seen diffusely throughout the hepatocyte cytoplasm, without canalicular accentuation. There is no apparent zonal preference in normal liver. In the first paper documenting its specificity in human tissue sections, the antibody labeled 37 of 38 cases of hepatocellular carcinoma (HCC), although four tumors had only rare positivity. The negative case was an example of the sclerosing variant of hepatocellular carcinoma. Five examples of fibrolamellar variant were positive. Two of 31 cases of cholangiocarcinoma (CC) were positive for Hep Par 1 and the antibody also decorated three of ten cases of gastric carcinoma (Wennerberg et al, 1993). In our own recent study, Hep Par 1 labelled 31 of 32 HCCs as well as four of 27 cases of CC (Leong et al, 1998), but was not found in metastatic adenocarcinomas. One other study has employed Hep Par 1 and showed staining in 289 of 290 HCCs (Wu et al, 1996).

Applications

The highest diagnostic yield is obtained with Hep Par 1 when it is employed in a panel of antibodies in the context of the differential diagnosis (Appendix 1.8). Its main diagnostic application would be for the distinction of HCC from CC and metastatic adenocarcinoma in the liver. When employed with CK 19 and CK 20, it is able to provide useful diagnostic information to allow the separation of these three entities (Leong et al, 1998). CK 19 is largely limited to bile duct epitheliam and their corresponding neoplasms, including CC (Balaton et al, 1988; Terada et al, 1995), whereas CK 20 is a marker of gastrointestinal carcinomas, particularly those from the colon and, less consistently, the upper gastrointestinal tract and pancreas (Mietinen, 1995).

Comments

As the staining of Hep Par 1 is heterogeneous and may be focal within HCCs, caution should be exercised in interpretation as small biopsies such as needle cores may produce false-negative results. Despite its limitations, Hep Par 1 is still the best antibody yet for use in the differential diagnosis of liver carcinomas.

References

Balaton AJ, Nehama-Sibony M, Gotheil C et al 1988. Distinction between hepatocellular carcinoma, cholangiocarcinoma, and metastatic carcinoma based on immunohistochemical staining for carcinoembryonic antigen and for cytokeratin 19 on paraffin sections. Journal of Pathology 156: 305-310.

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Terada T, Hoso M, Nakanuma Y 1995. Distribution of cytokeratin 19-positive biliary cells in cirrhotic nodules, hepatic borderline nodules (atypical adenomatous hyperplasia), and small hepatocellular carcinomas. Modern Pathology 8: 371-379.

Wennerberg AE, Nalesnik MA, Coleman WB 1993. Hepatocyte paraffin 1: a monoclonal antibody that reacts with hepatocytes and can be used for differential diagnosis of hepatic tumors. American Journal of Pathology 143: 1050-1054.

Wu P-c, Fand JW-S, Lau VK-T et al 1996. Classification of hepatocellular carcinoma according to hepatocellular and biliary differentiation markers. Clinical and biological implications. American Journal of Pathology 149: 1167-1175.

Hepatitis B Core Antigen (HBcAg)

Sources/Clones

Accurate/Axcel (polyclonal), American Research Products (1734-17), Biodesign (1841), Biogenesis (polyclonal), Biogenex (ESP512, polyclonal), Boehringer Mannheim (BW35A/312), Dako (polyclonal, B586), Fitzgerald (M29091, M22131), Immunon (polyclonal), Novocastra (polyclonal) and Zymed (polyclonal).

Fixation/Preparation

These antibodies are applicable to formalin-fixed, paraffin embedded tissue. No antigen unmasking is required. However, caution is advised when using the ABC immunodetection as hepatocytes contain biotin that may crossreact with the ABC system.

Background

The complete HB virus (Dane particle) is a 42 nm double-stranded DNA virus (Hepadna virus), composed of a 27 nm core particle and envelope, 7 nm in thickness, and immunolocalized within the endoplasmic reticulum of liver cells. The HB core protein of 183 amino acids is encoded by the gene C. It is self-assembling and has binding sites for HBV-RNA which is encapsulated together with viral polymerase. Immunolocalization of HBcAg is cytoplasmic, cytoplasmic membranous and nuclear (Kakumu et al, 1989). Antibodies are raised against HBcAg obtained from recombinant core DNA of HB virus, purified from lysates of*E.coli* clones. HBcAg is expressed predominantly in the nuclei of liver cells, although variable immunoreaction may also be seen in the perinuclear cytoplasm (Burns, 1975; Chu & Liaw, 1990).

Applications

Antibody to HBcAg detects the replicative form of the virus found in the nucleus of HB-infected cells. Perinuclear cytoplasmic immunolocalization is sometimes observed. In very actively replicating infections, cells with cytoplasmic reactivity may outnumber those with nuclear labeling. The presence of HBcAg on immunohistochemistry is usually correlated with complete viral synthesis as proved by positivity for viral DNA in both liver and blood, as well as circulating Dane particles in blood (Ballare et al, 1989). Demonstration of HBc in liver cells therefore reflects failure to eliminate cells with active viral replication. This is often associated with signs of active disease (piecemeal necrosis or chronic lobular hepatitis) with a membranous pattern of HBsAg (Bianchi & Dudat, 1994). HBcAg is seen with the greatest frequency in immunosuppressed patients with chronic hepatitis (Tapp & Jones, 1977). Excess accumulation of core particles can be recognized in an H&E stain in rare cases as `sanded' nuclei (Bianchi & Gudat, 1976).

Comments

It is assumed that viral DNA active in HBcAg production is episomal and not integrated into the host genome.

References

Ballare M, Lavarini C, Brunetto MR et al 1989. Relationship between the intrahepatic expression of e and c epitopes of the nucleocapsid protein of hepatitis B virus and viraemia. Clinical Experimental Immunology 75: 64-69.

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Chu CM, Liaw YF 1990. Intrahepatic expression of HBcAg in chronic HBV hepatitis: lessons from molecular biology. Hepatology 12: 1443-1445.

Kakumu S, Arao M, Yoshioka K, Tsutsumi Y, Inoue M 1989. Distribution of HBcAg in hepatitis B detected by immunoperoxidase staining with three different preparations of anti-HBc antibodies. Journal of Clinical Pathology 42: 284-288.

Tapp E, Jones DM 1977. HBsAg and HBcAg in the livers of asymptomatic hepatitis B antigen carriers. Journal of Clinical Pathology 30: 671-677.

Hepatitis B Surface Antigen (HBsAG)

Sources/Clones

Accurate (BM51), American Research Products (03A3403), American Research Products/EY Labs, Axcel/Accurate (polyclonal), Becton Dickinson, Biogenesis (1044-329, polyclonal), Biogenex (SI201), Calbiochem, Dako (polyclonal, 3E7), Fitzgerald (M94172, M94173, M94253, M94254, polyclonal), Harlan Seralab/Accurate (V2.5G4, V2.6E4), Novocastra (1044/341), Pharmingen (S1-210) and Zymed (ZCH16, ZMHB5).

Fixation/Preparation

These antibodies are applicable to formalin-fixed, paraffin embedded tissues. No antigen unmasking is required. However, caution is advised when using the ABC immunodetection method, as liver cells contain biotin and may cross react with the ABC system.

Background

The complete hepatitis B virus (Dane particle) is a 42 nm double-stranded DNA virus (Hepadna virus), composed of a 27 nm core particle and envelope, 7 nm in thickness, and immunolocalized within the endoplasmic reticulum of liver cells. The glycosylated surface protein of hepatitis B (HB) virus is composed of three gene products: the small, middle and large HBs protein, governed by the S, pre S2 and pre S1 domains respectively (Gudat & Bianchi, 1977).

Applications

These antibodies react with antigen-positive cells in patients with type B viral hepatitis, cirrhosis and hepatocellular carcinoma. Immunoreaction may occur in seropositive as well as seronegative patients. HBsAg in human liver biopsies has two expression patterns with apparently different biological implications.

Membranous HBsAg is strongly associated with HBc expression and is an indirect indication of replicative HBV infection.

Intracytoplasmic HBsAg in excess is visible by H&E staining as a homogeneous ground-glass appearance of the cytoplasm (Hadziyannis et al, 1973), and is an indicator of chronic elimination insufficiency for this antigen but is an unreliable marker of active replication. In contrast, membrane-associated HBsAg should always raise suspicion of active viral replication.

Comments

Liver tissue from known patients with hepatitis may be used as control tissue for both HbcAg and HbsAg.

References

Gudat F, Bianchi L 1977. HGsAg: a target antigen on the liver cell? In: Popper H, Bianchi L, Reutter W, eds. Membrane alterations as basis of liver injury. Lancaster: MTP Press, 171-178.

Hadziyannis S, Gerber MA, Vissoulis C, Popper H 1973. Cytoplasmic hepatitis B antigen in "ground glass" hepatocytes of carriers. Archives of Pathology 96: 327-330.

Herpes Simplex Virus I and II (HSV I and II)

Sources/Clones

Polyclonal HSV I and HSV II.

Biodesign, Biogenesis, Biogenex, Chemicon, Dako (polyclonal), Fitzgerald, Immunon and Pharmingen.

Monoclonal Antibody

Accurate (A321, M22253A, HP2M222M53A), American Research Products (1697-151, 1589-136, 1645-18), Biodesign (203, 206, 016, 017), Biogenesis (CHA437, 10527, H62), Biogenex (G16, E10, 023A1909, 045A1930B), EY Labs, Fitzgerald (M22254, M22255, M2110155, M2110156) and Seralab (CHA437).

Fixation/Preparation

Both antibodies 302M and 303M are applicable to frozen cryostat sections as well as fixed paraffin-embedded tissue sections. The latter require microwave pretreatment to eliminate non-specific background staining.

Background

The antigens used in the production of these antibodies comprise detergent-solubilized HSV I- and HSV II -infected whole rabbit cornea cells respectively. The 302M antibody reacts with HSV I- specific antigens whilst the 303M antibody reacts with HSV II- specific antigens. Both antibodies react with antigens common for HSV I and II: all major glycoproteins present in the viral envelope and at least one core protein. There is no demonstrable crossreactivity with varicella zoster virus, cytomegalovirus or Epstein-Barr virus.

Applications

Antibodies 302M and 303M detect the presence of HSV I and HSV II, respectively, in tissue sections, e.g. skin and brain. A diffuse intranuclear signal is produced, often coinciding with the ground-glass intranuclear inclusions of HSV. Similar intranuclear inclusions associated with biotin accumulation have been observed in glandular epithelia of gestational endometrium (Shigeo et al, 1993; Sickel & Di Sant'Agnese 1994). Hence, for the unwary any attempt to demonstrate HSV in these biotin inclusions may produce a false-positive immunoreaction, especially when the avidin-biotin immunodetection system is utilized. The recommended use of prewashing with 0.05% free avidin and 0.05% free biotin does *not* eliminate this crossimmunoreactivity. It is therefore recommended that the PAP or APAAP immunodetection system be used for any HSV immunohistochemical investigation of gestational endometrium (Cooper et al, 1997).

Comments

Biotin-like activity has been observed in thyroid lesions as well (Kashima et al, 1997). Hence, awareness of this interference is crucial to avoid misinterpretation of immunohistochemical investigations, especially with the ABC immunodetection system. Genital lesions with typical multinucleated giant cells with "ground" glass-intranuclear inclusions should be used as positive control tissue.

References

Cooper K, Haffajee Z, Taylor L 1997. Comparative analysis of biotin intranuclear inclusions of gestational endometrium using the APAAP, ABC and the PAP immunodetection systems. Journal of Clinical Pathology 50: 153-156.

Kashima K, Yokoyama S, Tsutomu Da, Nakayama I, Nickerson PA,

Noguchi S 1997. Cytoplasmic biotin-like activity interferes with immunohistochemical analysis of thyroid lesions: a comparison of antigen retrieval methods. Modern Pathology 10: 515-519.

Shigeo Y, Kenji K, Souichi I, Daa T, Nakamaya I, Moriushi A 1993. Biotin-containing intranuclear inclusions in endometrial glands during gestation and puerperium. American Journal of Clinical Pathology 99: 13-17.

Sickel JZ, Di Sant' Agnese A 1994. Anomalous immunostaining of `optically clear' nuclei in gestational endometrium. Archives of Pathology and Laboratory Medicine 118: 831-833.

HLA-DR

Sources/Clones

Accurate (917D7, CLBHLADR, DR), American Research Products (Dra, LDR), Axcel/Accurate (DK22), Biogenesis (HL-12, polyclonal), Biogenex (Q513), Biosource (BF1), Boehringer Mannheim (CR3-43), Caltag Laboratories (7.5.10.1, HL38, MIG-H13), Coulter (12, 13), Cymbus Bioscience (DDII, IQU9 TAL1B), Dako (DK22, TAL.1B5), Exalpha Co. (423L), Harlan Seralab/Accurate (MID3, YD1-63.4.10), Immunotech (B8.12.2), Novocastra (polyclonal), Pharmingen (TU36), Research Diagnostics (CLB-HLA-DR), Sanbio/Monosan (HL39), Sanbio/Monosan/Accurate (HL39), Sigma (HA14) and Zymed (LN3).

Fixation/Preparation

These antibodies are applicable to B5 or formalin-fixed, paraffin-embedded tissue section. In addition, cryostat sections and cell smears may also be used.

Background

HLA molecules are highly polymorphic glycoproteins with a single binding site for immunogenic peptides. The complex formed by HLA-DR molecules and peptides is the entity specifically recognized by the antigen receptor of CD 4+ helper T lymphocytes. This biological function has been linked to the constitutive cell surface expression of HLA molecules on antigen-presenting cells, which provide immunogenic peptides through denaturation or fragmentation of antigen (Jendro et al, 1991).

The HLA-DR is a member of the 11β subclass of HLA (the other member is HLA-DQ). The HLA-DR antigen is expressed by B cells of the germinal centers and mantle zones. It is also expressed by macrophages, monocytes and antigen-presenting cells like interdigitating reticulum cells and Langerhans cells of the skin. Activated T cells may express the HLA-DR antigen, but not inactive T cells. Some endothelial and epithelial tissues may also express HLA-DR.

Applications

Anti-HLA-DR may be useful in distinguishing B-cell follicle center lymphomas from T-cell lymphomas. The antibody also detects class II antigens which may be expressed de novo or increased in certain pathological states, e.g. autoimmune diseases. Similarly, it will demonstrate aberrant expression of class II antigen in various malignant cell types (Crumpton et al, 1984).

Comments

Tonsil or skin may be used as positive control tissue.

References

Crumpton MJ, Bodmer JC, Bodmer WF et al 1984. Biochemistry of class II antigens: workshop report. In: Albert ED, Mayr WR, (eds). Histocompatibility testing. Berlin: Springer-Verlag pp 29-37.

Jendro M, Goronzy JJ, Weyand CM 1991. Structural and functional characterization of HLA-DR molecules circulating in the serum. Autoimmunity 8:289-296.

HMB-45 (Melanoma Marker)

Sources/Clones

Axcel/Accurate, Biodesign, Biogenesis, Biogenex, Dako, Enzo and Immunotech.

Fixation/Preparation

The antibody is immunoreactive in paraffin-embedded tissue as well as frozen sections. Immunoreactivity is stronger in ethanol-fixed tissues than following formalin fixation, with immunoreactivity diminishing significantly following prolonged fixation in the latter. Sensitivity is enhanced following heat-induced antigen retrieval. Mercury-based fixatives result in a high degree of non-specific staining.

Background

The HMB-45 monoclonal antibody was generated to a whole-cell extract of a heavily pigmented lymph node deposit of human melanoma and has been shown to be a highly specific and sensitive reagent for the identification of melanoma. The designation HMB is derived from the immunogen employed, i.e. Human Melanoma, Black. The antigen is intracytoplasmic and ultrastructural studies suggest that the antibody reacts with melanosomes before melanin deposition with HMB-45 binding to stage 1 and 2 melanosomes and to the non-melanized portion of stage 3, whereas stage 4 melanosomes and melanosome complexes found in macrophages and keratinocytes have been negative. The antibody appears to label premature and immature melanosomes in retinal pigment epithelium from fetuses and neonates but not from adults, leading to the suggestion that this "oncofetal" pattern of expression may indicate a role in melanocytic cell proliferation. This thesis has not been confirmed and the sequential expression of the HMB-45 antigen in melanocytes may relate to the activation by specific growth factors, resulting in alterations in protein glycosylation during various ontogenic and pathologic states of melanocytes. The epitope recognized by HMB-45 appears to be, in part, the oligosaccharide side chain of a sialated glycoconjugate as the immunoreactivity can be abolished with neuraminidase treatment (Bacchi et al, 1996).

The gene corresponding to the HMB-45 defined proteins has recently been cloned and designated gp 100-cl. This gene encodes the melanocyte lineage-specific antigens recognized by HMB-45 and HMB-50 (one of two other monoclonal antibodies to melanocytes initially obtained with HMB-45) as well as another monoclonal antibody, NKI-beteb. These three antibodies appear to recognize different epitopes of the same antigen, the melanosomal matrix protein or pmel 17 gene product defined by them being apparently related by differential splicing.

Applications

Immunoreactivity for HMB-45 is seen in normal fetal and neonatal melanocytes but not in adult resting melanocytes. Reactive or proliferating melanocytes in inflamed adult skin, wound healing, increased vascularity and in skin overlying certain dermal neoplasms may label for HMB-45 as a result of activation and stimulation by growth factors and "reexpression" of the antigen. HMB-45+ melanocytes have been demonstrated in the anal squamous zone and transitional zone but not in the colorectal zone. Increased numbers of such melanocytes can be present adjacent to primary anal melanomas.

The staining for HMB-45 in melanocytic nevi depends on their location within the skin.

Junctional nevi and the junctional components of compound nevi are HMB-45+. In contrast, intradermal nevi and the dermal components of compound nevi are consistently negative. Thus, HMB-45 does not provide distinction between benign and malignant melanocytic proliferations and the difference in reactivity supports the concept, based on differences in morphology, enzyme activity and other immunological reactivity, that junctional and dermal cells are not identical. Junctional nevus cells are in an activated or proliferative state compared to their quiescent dermal counterparts and their immunoreactivity with HMB-45 is analogous to the proliferating fetal melanocytes which are positive for the antigen whilst quiescent, adult melanocytes are non-reactive.

Dysplastic nevi, in contrast, usually express HMB-45 in both the junctional nevus cells as well as in the dysplastic cells in the superficial dermis. Nevus cells within the deeper dermis do not usually react with HMB-45. In one study, minimally dysplastic nevi displayed intense immunolabeling of the junctional melanocytes but no staining of dermal nevus cells whereas, with the moderately and severely dysplastic nevi, the dermal melanocytes showed focal cytoplasmic immunoreactivity. The likelihood of expression of HMB-45 paralleled the degree of dysplasia of the nevi. Common blue nevi and cellular blue nevi are generally HMB-45+, as are malignant blue nevi. Other nevi such as spindle and epithelioid cell nevi, congenital nevi and other nevi occurring in hormonally reactive sites show immunostaining in nevus cells in the deep dermis as well as those near the dermoepidermal junction. Less common benign melanocytic proliferations such as plexiform spindle cell nevi, Spitz nevi and atypical melanocytic hyperplasias are also HMB-45+ (Bacchi et al, 1996).

Malignant melanoma show strong cytoplasmic positivity for HMB-45 in the majority of cases (65-95%), with the proportion of positive tumor cells ranging from a few to 100%. When the expression of the antigen is weak, staining may appear as a fine granularity similar to that seen in cytologic preparations. The positivity for HMB-45 is seen in almost all types of primary and metastatic melanoma including amelanotic melanoma, spindle cell melanoma and acral lentiginous melanoma (Appendix 1.10). One important exception is desmoplastic malignant melanoma, which consistently displays a much lower rate of positivity and may be completely negative. When positive, reactivity is usually seen in the superficial epithelioid cell rather than the dermal spindle cells, which only rarely stain for HMB-45 (Leong & Milios, 1989).

Attesting to the specificity of the antigen, HMB-45 reactivity has been demonstrated in malignant melanomas of diverse morphology such as signet ring melanoma, myxoid melanoma, small cell melanoma, balloon cell melanoma and in melanomas of different anatomic sites such as the gallbladder, urinary bladder, anorectal region, vulva, sinonasal region, uterine cervix, other mucosal sites and bone. Melanomas and melanocytic proliferations occurring in complex tumors such as pulmonary blastoma have also been HMB-45+ (Ordonez et al, 1988; Bacchi et al, 1996).

HMB-45 staining also has application in the separation of melanin-containing macrophages from melanoma cells, allowing the accurate determination of tumor thickness and depth of invasion. Similarly, labeling for the antigen helps the distinction of recurrence or residual spindle melanoma cells from desmoplastic fibroblasts at resection sites.

As HMB-45 immunoreactivity is melanocyte specific, positivity can be encountered in lesions with melanin production such as adrenal pheochromocytoma, melanotic neuroectodermal tumor of infancy (progonoma), melanin-containing hepatoblastoma, malignant epithelioid schwannoma of the skin, pigmented carcinoid tumor and esthesioneuroblastoma.

More recently, HMB-45 positivity has been reported in a variety of lesions, which may have implications for their differentiation or histogenesis. These include angiomyolipoma, lymphangiomyomatosis and sugar tumor of the lung. While these tumors consistently manifest HMB-45 immunoreactivity, they do not display obvious pigmentation. However, recent ultrastructural studies confirm the presence of premelanosomes and all three lesions also manifest evidence of smooth muscle differentiation. The reactivity for HMB-45 can be a useful diagnostic discriminant, especially in the case

of clear cell or sugar tumor which resembles metastatic renal cell carcinoma and clear cell carcinoma of the lung (Appendix

1.9). Similarly, immunoreactivity for HMB-45 can be helpful in the identification of lymphangiomyomatosis in transbronchial biopsies, obviating the need for an open biopsy for definitive diagnosis.

The apparent expression of this antigen in angiomyolipoma and lymphangiomyomatosis, both manifestations of the tuberous sclerosis complex, has been linked by the recent demonstration of HMB-45 immunoreactivity in cardiac rhabdomyoma, brain lesions and other mesenchymal as well as neural lesions found in the tuberous sclerosis complex (Weeks et al, 1994). These lesions have also shown ultrastructural granules suggestive of melanosome formation and are in agreement with previous suggestions that a smooth muscle cell with unusual features links the various lesions of tuberous sclerosis. HMB-45 immunoreactivity in these lesions now provides another common denominator.

Comments

Immunoreactivity of formalin-fixed tissue is enhanced following heat-induced antigen retrieval. Enzyme pretreatment does not significantly improve immunostaining for HMB-45. As in other diagnostic situations, heavily pigmented melanocytic lesions may pose a problem in differentiating melanin in other cells, such as macrophages, from tumor cells with true brown immunoreactivity when 3,3'-diaminobenzidine (DAB) is used as the chromogen. This problem can be simply eliminated by employing azure B as a substitute for hematoxylin as the counterstain. Azure B renders melanin granules blue-green, contrasting against the brown granules resulting from positive immunoreactivity.

The HMB-45 antibody has been reported to rarely show false-positive staining in non-melanomatous tumors and some normal tissues. These include breast carcinoma and normal breast epithelium, sweat gland tumors and normal counterparts, pheochromocytomas, hepatocellular carcinoma, chordoma, adenocarcinomas, lymphoma, plasmacytoma and plasma cells (Leong & Milios, 1989; Bonetti et al, 1991). This spurious staining is usually apical or perinuclear in location and granular in nature. This false positivity has been attributed to contamination of commercial ascites fluid preparations with non-specific antibodies and the culture supernatant fluid of the hybridoma cell line, now available from Dako, has been shown to eliminate this falsepositivity with HMB-45 (Bonetti et al, 1991; Bacchi et al, 1996).

Mercury-based fixatives such as B5 should be avoided as they result in extensive false-positive staining of mesenchymal cells including vessels, fibroblasts and inflammatory cells.

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Human Immunodeficiency Virus (HIV)

Sources/Clones

American Research Products (HIV1-1, HIV1-2), Biosource (LOHIV1-1), Dako (Kal-1) and Harlan SeraLab/Accurate (1HIVp24).

Fixation Preparation

Applicable to formalin-fixed, paraffin-embedded tissue sections. Pretreatment with proteolytic enzymes such as pronase improves immunoreactivity. May also be used for labeling cryostat sections and fixed-cell smears. Although the manufacturers provide working dilutions, optimization in individual laboratories is necessary. We have discovered that sections require a dual pretreatment with 0.5% trypsin (37 C, 15 min) followed by microwave treatment in citrate buffer.

Background

Kal-1 reacts with the HIV type 1 capsid protein p24 and its precursor p55 as demonstrated by immunohistochemistry, immunoprecipitation, ELISA and immunoblotting using lysates of purified virus and lysates of HIV type 1 infected cells (Daugharty et al, 1990). The antibody detects an epitope of the p24 protein, which is resistant to fixation and paraffin embedding (Kaluza et al, 1992). It does not crossreact with HIV type 2 or simian immunodeficiency virus (SIV), as shown by immunoblotting. During the phase of persistent generalized lymphadenopathy and subsequent stages of disease leading to the development of AIDS, follicular dendritic cells forming the framework of lymphoid follicles degenerate (Tenner-Racz et al, 1986). The expression of HIV-1 proteins by follicular dendritic cells (FDC) in germinal centers in situ and the presence of HIV-1 mRNA+ cells in germinal follicles suggest that FDC are infected and able to produce HIV-1 (Parmentier et al, 1990). Such infection may contribute significantly to the destruction of the FDC network during the lymphadenopathy phase after HIV-1 infection. Kal-1 reacts with the p24 protein in cells infected with HIV type 1, i.e. lymphocytes, monocytes and macrophages, Langerhans cells of the skin, follicular dendritic cells and brain cells of monocyte-macrophage or microglia lineage.

On formalin-fixed, paraffin-embedded tissue, Kal-1 antibody produces a positive immunoreaction of HIV-infected dendritic reticulum cells in the germinal centers of lymph nodes. The dendritic processes are highlighted, producing the typical network pattern within germinal centers. Occasional positive mononuclear cells and lymphocytes may be observed in the interfollicular areas of the lymph node. However, only immunopositivity confined to the follicular dendritic cells in lymph nodes should be considered as specific.

Comments

Interpretation of a positive lymph node biopsy with this antibody should always be confirmed with a serological assay or Western blot. In some countries an informed consent is required from the patient before testing for HIV status. Hence, histopathologists should be cautious in the reporting of p24 + lymph node biopsies.

References

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Human Milk Fat Globule (HMFG)

Sources/Clones

Biodesign, Biogenesis (3.14.A3), Biogenex (115D8), Immunotech (KC4, 1.10.F3, 3.14.A3), Novocastra (1.10.F3, 3.14.A3) and Unipath (1.10.F3, 3.14.A3).

Fixation/Preparation

Both antibody clones available are immunoreactive in fixed, paraffin-embedded section. HIER enhances staining.

Background

The human milk fat globule (HMFG) is a complex secretory product of mammary epithelium. HMFG is a relatively pure cell membrane product and is partially covered by a typical unit membrane that is extruded from the luminal surface of breast epithelial cells by reverse pinocytosis (Freudenstein et al, 1979). Besides the covering unit membrane, filamentous membrane structures, including cytoplasm-associated glycoproteins, can be detected on the inner coat of the HMFG. Similar to the plasma membrane, HMFG expresses considerable enzymatic activity, including that of glucose-6-phosphate dehydrogenase, acid and alkaline phosphatases, magnesium-dependent ATPase, aldolase, galactosyl transferase and xanthine oxidase.

A heterogeneous population of HMFG proteins can be recovered from the aqueous phase of skimmed milk following extraction in chloroform and methanol. This pool of solubilized glycoproteins is derived from a human epithelial membrane and referred to as epithelial membrane antigen (EMA). HMFG is thus very similar to EMA and from a practical standpoint, antibodies to these proteins have very similar patterns of immunoreactivity. A polyclonal antibody was initially shown to react with EMA and related HMFG protein determinants in formalin-fixed, paraffin-embedded sections and has been extensively used in normal and neoplastic tissues (Heyderman et al, 1979; Sloane et al, 1983).

Applications

The expression of HMFG is heterogeneous in both normal and neoplastic epithelium and its distribution bears no relationship to cellular morphology. The heterogeneity appears to be the result of normal cellular glycosylation patterns and appears to be reproducible in clonal proliferations of all epithelial cells (Edwards, 1985). HMFG proteins are widely distributed in secretory epithelia and their corresponding tumors and fetal anlage. These include sweat glands, sebaceous, apocrine and salivary glands, epithelium of the intestines, bile ducts, endometrium and endosalpinx, pulmonary alveolar cells and exocrine pancreas. HMFG is also expressed by some non-secretory epithelia such as the distal and collecting tubules of the kidney, and urothelium. Syncytiotrophoblasts, glandular cells of the endocervix, prostate, epididymis, rete testes and thyroid may also be reactive for HMFG. Generally, hepatocytes and proximal tubular epithelia are negative.

Comments

We employ anti-EMA in preference to anti-HMFG but do not use it as a generic marker of epithelial differentiation as some mesenchymal cells such as mesothelial cells, plasma cells and their corresponding tumors may express HMFG/EMA. In addition, soft tissue tumors such as synovial sarcoma, epithelioid sarcoma, peripheral nerve sheath tumor, smooth muscle tumor, rhabdomyosarcoma, chordoma,

ependymoma and choroid plexus tumors may express HMFG/EMA.

References

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Sloane JP, Hughes F, Ormerod MG 1983 An assessment of the value of epithelial membrane antigen and other epithelial markers in solving diagnostic problems in tumour histology. Histochemistry Journal 15:645-654.

Human Papilloma Virus (HPV)

Sources/Clones

Accurate (polyclonal), American Research Products (1535-18, 1501-17, 1502-17, 1505-17), Biodesign, Biogenesis (H11B, 16L1, C1P5), Biogenex (CHO613), Cymbus Bioscience (BF7), Cymbus Bioscience/Pharmingen (CAM-VIR1), Dako (polyclonal to HPV 1), Novocastra (4C4/F10/H7/83, 5A3/C8) and Pharmingen (7H7, TVG401, TVG402)

Fixation/Preparation

Antibodies to HPV are applicable to formalin-fixed, paraffin-embedded tissues and frozen cryostat sections.

Background

The most extensively studied area of HPV infection has been in epithelia of the anogenital tract, particularly the uterine cervix. Over 25 HPV genotypes have been isolated to date from the female genital tract. HPV genotypes have enabled specific types to be correlated with morphological lesions, e.g. HPV 6/11 is commonly associated with condylomata whilst HPV 16/18 are frequently associated with high-grade cervical intraepithelial neoplasia (CIN) and invasive squamous cell carcinoma (for review, see Cooper & McGee, 1997). It has recently been demonstrated that over 90% of cervical squamous cell carcinomas harbor a high-risk HPV (Bosch et al, 1995), the genome of which is usually integrated into the host DNA. Hence, in conjunction with epidemiological data showing that HPV infection and cervical squamous cell carcinoma share several risk factors, the association between high-risk HPV and cervical cancer is now firmly established. Although only a small proportion of high-grade CIN progress to invasive carcinoma, it is thought that HPV detection may assist in predicting the invasive potential of high grade CIN.

Applications

The detection of HPV in clinical samples depends on the demonstration of viral components within cells and tissues. This entails the detection of either protein or nucleic acid. Viral proteins may be visualized with immunohistochemical techniques using either polyclonal or monoclonal antibodies (Graham et al, 1991). Antibodies directed to viral proteins are dependent on the expression/synthesis of the latter by the virus, which is dependent on transcription/translation of the viral genome within the nucleus. Polyclonal antibodies raised to bovine papillomavirus capsid protein are applicable to HPV types in human biopsy specimens as they crossreact with several human subtypes (Jenkins et al, 1986). The synthesis of bacterial fusion proteins used as immunogens in mice has led to the generation of monoclonal antibodies to specific viral proteins to achieve viral specificity (Patel et al, 1989). The use of the HPV 16 L1 (capsid) protein has led to the production of several antibodies of varying specificity. The immunoreactivity of antibodies to HPV capsid protein is dependent on active viral replication which is closely correlated with keratin production. This therefore produces an intranuclear signal in the upper third of the squamous epithelia harboring the virus. Apart from the cervix, the use of antibodies to HPV is applicable to the vulva, penis, anus, oral cavity, larynx and esophagus.

Comments

With the advent of advanced in situ hybridization technology for

the detection of HPV DNA, the demand for HPV immunohistochemistry has fallen. Non-isotope in situ hybridization techniques are easily accessible and readily applicable to the routine diagnostic histopathology laboratory. Squamous epithelia showing the typical morphological features of HPV infection are recommended for use as positive controls. Staining should be mainly intranuclear, with some perinuclear staining of koilocytes.

References

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Human Parvovirus B19

Sources/Clones

Chemicon, Dako (polyclonal), Novocastra (R92F6) and Vector Laboratories (R92F6).

Fixation/Preparation

Applicable to archival formalin-fixed paraffin-embedded tissue sections. Before immunostaining, sections should be subjected to heat-induced epitope retrieval buffer at 100 C for 20 min (Liu et al, 1997).

Background

Human parvovirus B19 was accidentally discovered in 1975 in human serum being screened for hepatitis B surface antigen (Cossart et al, 1975). Since discovery, this virus has been found to be the causative agent in erythema infectiosum, chronic anemia in immunosuppressed patients, fetal death associated with hydrops and acute arthralgia/arthritis in adults (Liu et al, 1997).

Parvovirus B19, which is cytotoxic to erythroid progenitor cells in vivo and in vitro, enters the erythroid precursor cell via the blood group P antigen (Mortimer et al, 1983; Brown et al, 1993). In the past several years, human parvovirus B19 has been reported as a cause of severe and persistent anemia in patients immunocompromised from organ transplantation, autoimmune disease, hematologic malignancies, chemotherapy and congenital or acquired immunodeficiency states including HIV infection (Liu et al, 1997).

The R92F6 monoclonal antibody is directed against the VP1 and VP2 capsid protein of parvovirus B19.

Applications

On bone marrow smears and trephine biopsies, finding the giant erythroblast and small erythroid precursors with nuclear inclusions (lantern cells) establishes the diagnosis. However, these cells may easily be overlooked by the inexperienced observer. Hence, the use of antibody to parvovirus B19 may be useful in establishing the diagnosis. Therefore, a high index of suspicion when assessing bone marrow smears/biopsies in patients with chronic severe anemia in the immunocompromised will alert the observer to the possibility of parvovirus infection. Although Liu et al found antiparvovirus B19 antibody to be less sensitive than in situ hybridization (ISH), others have not made the same observation. Investigating fatal non-immune hydrops fetalis, Morey et al (1992) found good correlation between R92F6 antibody staining and B19 DNA in 19 cases. Further, these workers demonstrated the virus with immunohistochemistry (and ISH) in two cases that lacked parvovirus B19 inclusions on H&E stains indicating that low-grade or resolving infections may be missed on simple morphological examination alone.

Comments

Parvovirus B19 infection should be considered in any unexplained chronic persistent anemia in an immunocompromised patient.

References

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Mortimer PP, Humphries RK, Moore JG et al 1983 A human parvovirus-like virus inhibits hematopoietic colony formation in vitro. Nature 302: 426-429.

Human Placental Lactogen (hPL)

Sources/Clones

Accurate (KIHPL3-489D5F3, polyclonal), American Research Products (polyclonal), Biogenesis (LIP603), Chemicon (polyclonal), Dako (polyclonal), Fitzgerald (M310198, M310199, polyclonal), Seralab (polyclonal) and Zymed (polyclonal).

Fixation/Preparation

The antigen is resistant to formalin fixation and immunoreactivity is enhanced by proteolytic digestion.

Background

Human placental lactogen (hPL) is a member of an evolutionarily related gene family that includes human growth hormone (hGH) and human prolactin. hPL human chorionic gonadotropin and pregnancy-specific β 1 glycoprotein (SP1) are the three major proteins produced by the placenta. Although its expression is limited to the placenta, the physiological actions of hPL are far reaching: it has a direct somatotropic effect on fetal tissues it alters maternal carbohydrate and lipid metabolism to provide for fetal nutrient requirements and aids in the stimulation of mammary cell proliferation. Two hPL genes (hPL3 and hPL4), encoding identical proteins, are responsible for the production of up to 1 — 3 g hPL hormone/day (Walker et al, 1991; Wright et all, 1991).

Applications

Several studies have employed hPL and other placental markers for the distinction of intrauterine from extrauterine pregnancies. The presence of cytokeratin and hPL was found to be useful in identifying trophoblastic elements in endometrial curettings (Sorensen et al, 1991; Khong et al, 1994), with a sensitivity of 73% in one study (Kaspar et al, 1991). hPL can also be employed in a panel for the distinction of trophoblastic proliferations (see Appendices 1.5, 1.29, 1.30, 1.31). Complete hydatidiform mole showed strong expression of human chorionic gonadotropin (hCG) and weak expression of placental alkaline phosphatase (PLAP), whereas partial mole showed weak hCG and strong PLAP.

Choriocarcinoma, on the other hand, showed strong hCG and weak hPL and PLAP. All tissues were positive for cytokeratin but negative for vimentin (Losch & Kainz, 1996). Focal expression of hCG and diffuse expressions of hPL and PLAP was a profile not observed in complete moles (Brescia et al, 1987; Cheah & Looi, 1994).

hPL has also been employed as a marker of intermediate trophoblasts although the expression of pregnancy-specific glycoprotein, cytokeratin and vimentin is a more reliable marker (Yeh et al, 1990; Shibata & Rutgers 1994).

Comments

Various tumors which show trophoblastic differentiation may express hPL (Boucher & Yoneda, 1995; Ulbright et al, 1997).

References

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Immunoglobulins: Igκ, Igλ, IgA, IgD, IgE, IgG, IgM

Sources/Clones

Both monoclonal and polyclonal antibodies to immunoglobulins of the various types are available from a wide variety of sources. Affinity-isolated F (ab')2 fragments to Igκ and Igλ are also available.

Igκ

Accurate (EA2-38), American Research Products (HK3), Becton Dickinson (TB28-2), Biodesign/Pharmingen (polyclonal), Biogenesis (HK3, polyclonal), Biosource (LOHK3), Caltag Laboratories (3B10, HP6062), Calbiochem (HP6062, polyclonal), Central Lab Netherlands Red Cross/Accurate (MH19), Cymbus Bioscience (24K6), Dako (R10-21-F3, A8B5, polyclonal), Eurodiagnostica/Accurate (MH19-1), Harlan Seralab/Accurate (SL.2, KAP3.B3), Immunotech (G6.42), Pharmingen (polyclonal, G20-193), Research Diagnostics (6KA4G7), Sanbio/Monosan/Accurate (2B7) and Zymed (HP6053).

Igλ

Accurate (AG7.47), American Research Products (HL2), Becton Dickinson (1-155-2), Biogenesis (polyclonal), Biosource (LOHL2), Caltag Laboratories/Sigma (HP6054), Central Lab Netherlands Red Cross/Accurate (MH292), Cymbus Bioscience (24L6), Harlan Seralab/Accurate (Lam2.G4), Pharmingen (JDC12, polyclonal), Research Diagnostics (4LA2G9) and Zymed (HP6054).

IgA

Accurate (GA1, SB14, A1-18), Accurate/Sigma (GA112), American Research Products (14A3E3, HA8, 14A1B12), American Research Products/Research Diagnostics (14A2B5), Becton Dickinson (1-155-1), Biodesign (polyclonal), Biogenesis (polyclonal, 15D6, 2E2), Biosource (LOHA8), Calbiochem (HP6130, HP6141), Caltag Laboratories (SB14), Central Lab Netherlands Red Cross/Accurate (MH14-1), Coulter (NIF2), Cymbus Bioscience (M24A), Dako 6E2C1, polyclonal), E-Y Labs (polyclonal), Immunotech/Immunotech SA (NIF2), Pharmingen (polyclonal), Research Diagnostics (polyclonal), Sanbio/Monsan/Accurate (MH14-1), Sigma (A1-18) and Zymed (WAN741).

IgD

American Research Products (HD11), Becton Dickinson (TA4.1), Biogenesis (polyclonal), Biogenex (NI158, polyclonal), Biosource (LOHD11), Dako (IgD26, polyclonal), EY Labs (polyclonal), Harlan SeraLab/Accurate (1AD86), Immunotech (JA11), SeraLab Ltd (12.1), and Sigma (HJ9).

IgE

Accurate (GE1, AMD-E), Accurate/Sigma (GE1), Biodesign (polyclonal), Biogenesis (0257), Dako A-E-7.12, E1, polyclonal), EY Labs (polyclonal), Fitzgerald (M94175, M94176, M94177, M94178, M94179) Research Diagnostics (polyclonal).

IgG

Accurate (polyclonal, 4.22D10, A57H, SL13), Accurate/Sigma (GG4), American Research Products (HE10, HE17, 1619-18, HG20, HG22), Becton Dickinson (C3-124), Becton Dickinson/Biodesign (polyclonal), Biodesign (polyclonal), Biogenesis (polyclonal, 2D7), Biosource (LOHE17), Calbiochem (HP6058, HP6019), Caltag Laboratories (SB15, SB16), Central Lab Netherlands Red Cross/Accurate (MH25-1, MH164), Coulter (679.1MC7, U7.27, LODNP16, MOPC195), Cymbus Bioscience (MR36G),

Dako (A57H, polyclonal), EY Labs (polyclonal, NL16, GB7B), Harlan SeraLab/Accurate (ISE503, C3-8-80, C27-15), Pharmingen (G7-18, G18-145, G18-21, G18-3, polyclonal), Research Diagnostics (10G2C11, 22G5G12, 20G5C7), Sanbio/Monosan/Accurate (MH25-1, BL-G4-1) and Sigma (SH21, SK44).

IgG F(ab)

Accurate/Sigma (SG16) and EY Labs (HP6014).

IgM

Accurate (AMD-u, SB17), Accurate/Sigma (MB11), American Research Products (HM9), American Research Products/Biogenesis (HM7), Becton Dickinson (145-8), Biogenesis (polyclonal), Biosource (LOHM9), Coulter (GC323), Coulter/Immunotech (AF6), Cymbus Bioscience (RVS-M), Dako (R1/69, polyclonal), EY Labs (polyclonal), Pharmingen (G20-127, polyclonal) and Research Diagnostics (MH15-1-3).

Fixation/Preparation

Immunostaining of cytoplasmic immunoglobulin can be performed in formalin-fixed paraffin-embedded sections, fresh frozen sections and cytologic preparations. Other fixatives and processing procedures such as AMEX (Sato et al, 1986) and freeze-drying (Stein et al, 1985) have been suggested to produce effective immunoglobulin staining.

Background

Surface membrane immunoglobulin (SIg) expression is the classic and specific marker of B lymphocytes and serves as the antigen recognition molecule for this lymphocyte population. Each of the heavy chain classes of Ig can be expressed on the B-cell membrane and more than one heavy chain class can be expressed on the same cell, the majority of peripheral B-cells expressing IgM with or without IgD, less than 10% expressing IgM or IgA.

IgM is the first heavy chain class to appear in B-cell ontogeny with the majority of immature B cells expressing IgM in high density. This decreases in density with maturation and increasing amounts of IgD appear on the cell membrane. The IgM and IgD molecules that coexist in the same membrane cap exist independently but share the same idiotype and have the same light chain. Following B-cell activation and differentiation, there is loss of IgM and IgD as the result of a productive isotype gene rearrangement switch. With the progression to antibody-forming plasma cells, different subpopulations of SIgM and/or SIgG-bearing memory B-cells may appear.

Clonality of a given B-cell population can be inferred from the uniformity of light chain class expression as individual B cells can express either κ or λ light chains but not both the ratio of κ to λ bearing B cells being 2:1. A vast predominance of κ or λ light chain-bearing B cells indicates monoclonality, generally implying a neoplastic proliferation, whereas a mixture of light chain-bearing cell types suggests polyclonality and a reactive or non-neoplastic proliferation of B cells.

Direct immunofluorescent staining with heterologous antisera raised against whole or Fab fragments of human Ig molecules is the simplest method of identifying SIg. Class-specific antisera monospecific for individual heavy and light chain determinants (monovalent antisera) may be employed to determine the precise isotype of the SIg but these procedures require fresh cell preparations. Alternatively, immunoenzyme techniques can be used on cytocentrifuge preparations and imprints as well as frozen sections (Banks et al, 1983; Forbes & Leong, 1987). The latter procedures have suffered from the high level of background staining which can make interpretation difficult.

Ideally, the aim would be to be able to perform consistent staining of immunoglobulin in fixed, paraffin-embedded sections, allowing the advantage of retrospectivity as well as optimal

cytomorphology. While many attempts have been made with special fixatives such as B5 and other mercury-based fixatives and the application of various enzymatic digestions, they have not met with much success. Coupled with the recent introduction of HIER, the use of 4 M urea as the retrieval solutions as produced consistent results, with the claim that the procedure allows the demonstration of not only cytoplasmic Ig but also surface Ig (Merz et al, 1993).

Applications

About 80% of non-Hodgkin's lymphoma cases in Western countries are of B cell lineage and the majority express monotypic SIg (Lukes et al, 1978; Tubbs et al, 1983). The examination of

lymphoid proliferations for the presence and clonal nature of SIg expression is a common practice and forms the basis for traditional immunophenotypic analysis (Leong and Forbes, 1982). By convention, it is inferred that monoclonal B-cell proliferations are neoplastic. This analysis has traditionally been carried out by flow cytometry on cell suspensions, in cytospin preparations of disaggregated cells, or in frozen tissue sections. The SIg isotypes expressed by B-cell non-Hodgkin's lymphoma and lymphoid leukemias parallel those of normal B cells. The most common heavy chain class is IgM, with or without associated IgD, and IgG and IgA are expressed much less frequently. The ratio of Igκ to Igκ-bearing lymphomas is about 2:1.

Comments

When staining terminally differentiated B cells such as plasma cells, it is important to remember that, unlike SIg, which is detectable in viable cells in suspension or in minimally fixed frozen sections, the staining of CIg requires permeabilization of the cell membrane by the fixative to allow penetration of the anti-Ig reagents. Therefore, sections fixed by a gentle fixative such as acetone will not allow the demonstration of CIg and plasma cells may show false-negative staining. Alcohol and formalin are suitable fixatives for the demonstration of CIg in cell preparations and tissue sections respectively. We have found that fixation in 10% buffered formalin of freshly prepared or air-dried smears and cell preparations followed by HIER in 4 M urea produces excellent staining of CIg in lymphoid cells through a wide range of differentiation. Formalin-fixed, paraffin-embedded sections also show consistent staining for both CIg as well as SIg following HIER in 4 M urea and trypsin digestion.

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Tubbs RR, Fishleder A, Weiss RA et al 1983. Immunohistologic cellular phenotypes of lymphoproliferative disorders. Comprehensive evaluation of 564 cases including 257 non-Hodgkin's lymphomas classified by the International Formulation. American Journal of Pathology 113: 207-221.

Inhibin

Sources/Clones

Biogenesis and Serotec (R1, E4).

Fixation/Preparation

Antibodies to inhibin may be applied to paraffin-embedded tissues fixed in formalin but microwave pretreatment in citrate buffer is essential for optimum immunostaining.

Background

Inhibin is a peptide hormone produced by ovarian granulosa cells, which selectively inhibits the release of follicle-stimulating hormone (FSH) from the pituitary gland (McLachlan et al, 1987), acting as a modulator of folliculogenesis (Findlay 1993). Peak serum level is reached during the follicular phase of the menstrual cycle; being undetectable in the serum of menopausal women (Lapp鰄n et al, 1989). It is produced and overexpressed by granulosa cell tumors, thus being an early marker for tumor growth. Hence, its usefulness is as a marker of tumor recurrence before clinical manifestation (Lapp鰄n et al, 1989). Several inhibin subunits can be detected by immunostaining in the granulosa cell layers of the human ovary and in neighboring theca cells. Clone R1 was raised against a synthetic peptide corresponding to the 1-32 peptide of theα subunit of 32 kD human inhibin and reacts specifically with this molecule (Isotype: IgG2b) (Groome et al, 1990). Clone E4 was raised against a synthetic peptide corresponding to the 84-114 peptide sequence of theβ A subunit of 32 kD human inhibin A and activin A (isotype 2b) (Groome and Lawrence, 1991). E4 reacts with both theβ A and β B subunits of human inhibin and activin.

Applications

Using monoclonal antibody to human inhibin 32 kDα subunit, follicle epithelia in 6/6 samples of ovarian tissue (under 40 years), 6/6 adult granulosa cell tumors and three late metastases from granulosa cell tumors in females showed positive immunoreaction (Fleming et al, 1995). No reaction was found in hemangiopericytoma, leiomyosarcoma and malignant melanoma. This antibody would be useful in distinguishing the sarcomatoid growth pattern of granulosa cell tumors from soft tissue tumors. Further, no positive reaction was observed in ten ovarian carcinomas whilst in two of these cases, single cells of the specialized ovarian stroma stained positively with inhibin.

In another study inhibin immunostaining was also detected in stromal hyperthecosis, juvenile granulosa cell tumors and Sertoli-Leydig cell tumors (Stewart et al, 1997), proving that inhibin is a sensitive immunohistochemical marker of a wide range of gonadal stromal tumors. Strong cytoplasmic staining of 17/19 cases of hepatocellular carcinoma, including pleomorphic and glandular variants, has been demonstrated (McCluggage et al, 1997). In this study focal weak luminal staining of glands of adenocarcinoma was also present. Hence, immunostaining with antiinhibin antibody may be of value in the differentiation of hepatocellular carcinoma from adenocarcinoma involving the liver.

Comments

Inhibin antibody is useful in confirming the diagnosis of both adult and juvenile granulosa cell tumors, especially tumors with unusual growth patterns and in metastatic sites. It is also helpful in distinguishing hepatocellular carcinoma from adenocarcinomas in the liver.

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Ki-67 (MIB1, Ki-S5)

Sources/Clones

Accurate (MM1), Biogenex (Ki-67, MIB1), Boehringer Mannheim (Ki-67, Ki-S5), Cymbus Bioscience (Ki67), Dako (Ki-67, polyclonal), Diagnostic Biosystems (Ki-67, polyclonal), Immunotech (MIB1), Novocastra (MM1), RDI (Ki67), Serotec (Ki67), and Zymed (7B11).

Fixation/Preparation

Monoclonal Ki-67 is immunoreactive in frozen sections but not in paraffin sections (although there are claims of reactivity following HIER, the results are not consistent). MIB1, Ki-S5 and polyclonal Ki-67 are all immunoreactive in routinely fixed, paraffin-embedded tissues. Immunoreactivity is enhanced following HIER combined with proteolytic digestion. Best results are obtained with retrieval solutions of low pH and at neutral and high pHs.

Background

The Ki-67 antibody was generated against a Hodgkin's disease cell line and was found to identify a nuclear antigen expressed in all non-G_0 phases of the cell cycle, i.e., all proliferating cells. The antigen recognized by Ki-67 is a 345-395 kD non-histone protein complex which is highly susceptible to protease treatment (Gerdes 1991 et al). The gene encoding the Ki-67 protein is localized on chromosome 10 and organized in 15 exons. The center of the gene is formed by an extraordinary 6845 bp exon containing 16 successively repeated homologous segments of 366 bp, the "Ki-67 repeats", each containing a highly conserved new motif of 66 bp, the "Ki-67 motif". The deduced peptide sequence of this central exon is associated with high turnover proteins such as other cell cycle-related proteins, oncogenes and transcription factors. Like the latter, the Ki-67 antigen plays a pivotal role in maintaining cell proliferation because Ki-67 protein antisense oligonucleotides significantly inhibit ^{3}H-thymidine uptake in human tumor cell lines in a dose-dependent manner (Duchrow et al, 1995).

There is a good correlation between the percentage of Ki-67 positive cells in normal tissues and cell kinetic parameters such as ^{3}H-thymidine labeling indices although generally, Ki-67 immunostaining gives a higher proliferative index than the Sphase fraction, as defined by flow cytometric analysis or by ^{3}H-thymidine incorporation.

Until recently, the limitation of the Ki-67 antibody was its requirement for frozen tissue. Several antibodies to the Ki-67 antigen are now available which are immunoreactive in routinely fixed sections, namely MIB1, Ki-S5, polyclonal Ki-67 and Ki-S1 (not commercially available to our knowledge). The proliferation indices obtained with all these five antibodies correlated well with that obtained with monoclonal Ki-67 in frozen sections, indicating that they are suitable substitutes with the advantage of being immunoreactive in fixed paraffin-embedded sections (Leong et al, 1995). This was not the case with the antibodies to proliferating cell nuclear antigens PC10 and 19A2, both of which have been demonstrated to be fixation dependent (Leong et al, 1993).

Applications

Numerous studies have compared the Ki-67 proliferation indices in frozen sections with other prognostic parameters such as tumor grade, hormone receptor status and p53 expression. In general, Ki-67 indices have been shown to be of prognostic

relevance (Brown and Gatter, 1990; Raymond et al, 1988, Raymond and Leong 1989). Similar studies have now been performed in wax-embedded archival tissues with some of the new antibodies, particularly MIB1, confirming their relevance as prognostic markers (Wintzer et al, 1991; Sahin et al, 1991, Healy et al, 1995; Kindblom et al, 1995; Nawa et al, 1996). Ki-67 counts have also been useful in distinguishing between benign and malignant liver proliferations (Grigioni et al, 1995) and predicting progress of granulosa cell tumors (Costa et al 1996), Barrett's dysplasia (Polkowski et al 1995) and ovarian serous tumors (Garzetti et al, 1995).

Comments

In many cells the Ki-67 antigen appears to be localized to the nucleoli or perinucleolar region, with lighter diffuse nuclear staining in both frozen and fixed sections. When assessing proliferation indices, notable intratumoral heterogeneity will be observed and counts should be taken from the areas of highest proliferation, usually at the periphery of the tumor.

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Laminin

Sources/Clones

Becton Dickinson (4C12.8), Biogenesis (2D8-39, 2D8-30, 2D8-33), Biogenex (LAM1), Dako (polyclonal, 4C7), EY Labs (polyclonal), Eurodiagnostics (polyclonal), Immunotech (4C12) and Monosan (polyclonal).

Fixation/Preparation

Most available antibodies are immunoreactive in cryostat sections and fixed, embedded tissues but require antigen retrieval in the form of HIER or proteolytic digestion or both.

Background

Laminin, a glycoprotein of about 900 kD, is secreted by fibroblasts, epithelial, myoepithelial, endothelial and smooth muscle cells and, together with type IV collagen, forms the principal component of basal lamina. There are three genetically distinct chains of laminin: α-, β-, and γ-chains, which are held together by disulfide bonds and by a triple-stranded coiled structure. Ultrastructurally, the basal lamina is composed of a lamina lucida of low electron density, adjacent to the parenchymal cells, and a basal lamina densa of high electron density, adjacent to the connective tissue matrix. By rotary shadowing, laminin has a cruciform shape, consisting of three short arms of 200 kD and one long arm of 400 kD. Laminin is exclusively localized to the basal lamina, predominantly to the lamina lucida, and is invariably present in basal lamina surrounding muscle, nerve, fat and decidua cells and separating epithelial and endothelial cells from adjacent connective tissues. Laminins are potent modulators of numerous biological processes in development, including cell proliferation, migration and differentiation. In adult tissues, laminins influence the maintenance of specific gene expression and are involved in various pathological situations, including fibrosis, carcinogenesis and metastasis.

Clone 4D7 reacts with the terminal globular domain of the A-chain of laminin, whereas polyclonal antibodies were generated to laminin isolated from rat yolk sac tumor cell line.

Applications

Laminin has been shown to play a role in cell adhesion and attachment to the basal lamina, both *in vivo* and *in vitro*. The basal lamina is generally extremely stable but in certain pathological states may undergo local dissolution. This process is likely to play a crucial role in the invasiveness and progression of malignant tumors (Liotta, 1984). Loss or defective organization of the basal lamina matrix in malignant neoplasms may be the result of increased breakdown by tumor-derived degradative enzymes, decreased synthesis or decreased or abnormal assembly of the secreted basal lamina components (Pujuguet et al, 1994). In human breast carcinoma, there is suggestion that overexpression of the nm23-H1 gene, a putative metastasis suppressor gene, leads to the formation of basal lamina and growth arrest (Howlett et al, 1994). Antisera to type IV collagen and laminin, the major components of basal lamina allow the study of the organization of the basal lamina in various benign and malignant tumors (Autio-Harmainen et al, 1988; Havenith et al, 1989, Nair et al, 1997, Kuwano et al, 1997). Laminin immunostaining with the immunogold-silver technique in resin-embedded sections allows exquisite demonstration of the basal lamina in a variety of tissues (Leong, 1993). The majority of

invasive carcinomas are recognized to synthesize varying amounts of basal lamina material, but the basal lamina surrounding the tumor nests are generally fragmented and, in many cases, completely absent. Benign and *in situ* lesions appear to be circumscribed by intact basal lamina.

Diagnostic applications of collagen type IV immunostaining have mostly centered around the demonstration of basal lamina in invasive tumors, particularly epithelial tumors, and their changes with tumor invasion and metastasis. In particular, the demonstration of an intact basal lamina has been used to distinguish benign glandular proliferations such as microglandular adenosis and sclerosing adenosis from well-differentiated carcinoma like tubular carcinoma of the breast (Raymond & Leong, 1991; Tavassoli & Bratthauer, 1993). Distinctive patterns of basal distribution were recently demonstrated in various types of soft tissue tumors, adding to the diagnostic armamentarium for this group of neoplasms which are often difficult to separate histologically and with existing immunological markers (Leong et al, 1997). While the presence of basal lamina cannot be used as an absolute discriminant for blood vessels and lymphatic spaces, the latter do not display the reduplication of the basal lamina characteristic of blood vessels and generally show thin and discontinuous staining of basal lamina (Suthipintawong et al, 1995). The distinctive staining observed around blood vessels has been employed as a marker when performing capillary density measurements (Madsen & Holmskov, 1995).

A recent study suggests that the presence of basal lamina as demonstrated with laminin immunostaining may be a clue to the identification of hepatocellular carcinoma as non-malignant hepatocytes lack basal lamina (Yoshida et al, 1996).

Comments

The use of proteolytic digestion following HIER further enhances immunoreactivity. With the polyclonal antibodies we employ protease at 0.25 mg/ml for 2 min (Leong et al 1996).

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Lysozyme (Muramidase)

Sources/Clones

Axcel/Accurate (polyclonal), Biodesign (polyclonal), Biogenesis (6C6/8, polyclonal), Biogenex (polyclonal), Biomedia (polyclonal), Calbiochem (polyclonal), Chemicon (polyclonal), Dako (polyclonal), Diagnostic Biosystems (polyclonal), Fitzgerald (polyclonal), Milab (polyclonal), and Zymed (polyclonal).

Fixation/Preparation

Lysozyme (muramidase) is resistant to fixation and immunoreactivity is enhanced following proteolytic digestion and HIER. The antibodies are immunoreactive in frozen sections and cytological preparations.

Background

Lysozyme (muramidase) is a 14.5 kD strongly basic protein which is a mucolytic enzyme found in saliva, gastrointestinal secretions, tears, urine and serum. Lysozyme has been localized in granulocytes, histiocytes and some epithelial cells. The protein has been localized ultrastructurally in the secretory granules of Paneth cells and the brush border of granular mucus cells of the small intestine. It has also been localized to the granules of alveolar type II pneumocytes and the lysosomal granules of multinucleated histiocytes. In the lymph node, lysozyme is found in the tingible body macrophages of the germinal centers and in macrophages scattered in the paracortex. Dendritic reticulum cells, interdigitating reticulum cells, lymphocytes and plasma cells generally lack lysozyme. Sinus macrophages may show stainable lysozyme.

Applications

Lysozyme has been employed as a marker of histiocytes/macrophages and of myeloid differentiation. It is a useful marker in both paraffin-embedded trephine biopsies and bone marrow clot preparations allowing distinction of acute myeloid leukemia from acute lymphoblastic leukemia (Davey et al, 1990; Horny et al, 1994), as well as in the identification of extramedullary myeloid cell tumors (Traweek et al, 1993). The combination of myeloperoxidase and lysozyme was found to be a reliable marker of myeloid lineage (Quintanilla-Martinez et al, 1995).

Lysozyme has been described in Langerhans histiocytosis (Thompson et al, 1996), follicular dendritic cell tumors (Masunaga et al, 1997), granular cell tumors, malignant fibrous histiocytoma and various alleged histiocytic tumors.

Lysozyme combined with GCDFP-15 had great specificity for apocrine differentiation in adnexal tumors of the skin whereas eccrine tumors stained only for GCDFP-15 (Ansai et al, 1995; Meyhehm & Fischer, 1997) (Appendix 1.19).

Comments

While a useful marker of lysosomal inclusions in a variety of cell types including histiocytes/monocytes, this protein is not specific and must be employed in a panel which includes other histiocytic markers.

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MAC 387 (Macrophage Marker)

Sources/Clones

Dako (MAC 387).

Fixation/Preparation

Applicable to formalin-fixed paraffin sections, acetone-fixed cryostat sections or fixed-cell smears. Requires enzymatic pretreatment for optimum immunostaining but immunoreactivity is not enhanced by HIER.

Background

MAC 387 (IgG1, K) was raised against purified peripheral blood monocytes (Flavell et al, 1987). The antibody recognizes the leukocyte antigen L1 or calprotectin (Steinbakk et al, 1990). The L1 antigen consists of three non-covalently bound polypeptide chains with a total molecular mass of 365 kD. L1 was discovered more than 15 years ago as a major cytosol protein fraction (50-60%) of neutrophilic granulocytes (Fagerhol et al, 1990). This antigen is expressed in neutrophil granulocytes, monocytes, certain reactive tissue macrophages, squamous epithelia and reactive epidermis (Brandtzaeg et al, 1988). It is also reputed to be expressed in early inflammation and is present only in cells of the mononuclear-phagocyte system and not the dendritic system. In vitro experiments have shown the purified composite molecule to exhibit striking antimicrobial properties (Steinbakk et al, 1990).

Applications

MAC 387 antibody produces a cytoplasmic labeling pattern in many myelomonocytic cells. Apart from identifying reactive macrophages, MAC 387 also highlights macrophages in several histiocytoses including hemophagocytic syndrome, Rosai-Dorfman disease and Langerhans' cell histiocytoses (Malone, 1991; Brandtzaeg et al, 1992). True histiocytic lymphomas should be MAC 387+, whilst a small number of large cell anaplastic lymphomas may show immunopositivity (Norton & Isaacson, 1989). Squamous cell carcinomas of the skin, bronchus, bladder and oral cavity may show immunoreactivity (Brandtzaeg et al, 1992), helping us to distinguish them from other types of carcinoma.

Comments

Although both MAC 387 and KP1 are good broad-spectrum macrophage markers, MAC 387 is less specific for cells of the mononuclear-phagocyte system than KP1. Tissues rich in macrophages are suitable as positive controls.

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MDM-2 Protein

Sources/Clones

Accurate (19E3), Dako (SMP14), Novocastra (1B10, polyclonal), and Oncogene (1F2).

Fixation/Preparation

Several of the antibodies are immunoreactive in fixed paraffin-embedded tissue sections and also fresh tissue and cell preparations.

Background

The MDM-2 protein encodes for a nuclear phosphoprotein that binds p53 and inhibits its ability to activate transcription by concealing the p53 activation domain. It has been suggested that MDM-2 overexpression might represent an alternative mechanism by which p53-mediated pathways are inactivated in human tumors, thus having a possible role in oncogenesis. MDM-2 overexpression as a result of gene amplification and/or increased mRNA expression can be detected by immunohistochemical analysis.

Applications

The ability to stain for MDM-2 protein in fixed tissue sections has stimulated a great deal of interest in its expression in various neoplasms. The correlation of MDM-2 protein levels with p53 may provide insights into oncogenesis and has the potential of providing prognostic information. Several studies have included the detection of p21/waf1 protein together with MDM-2 as both these oncoproteins are downstream effectors of p53, p21 playing a major role in negatively regulating cell cycle progression, while MDM-2 inhibits the effects of p53.

Current results of immunohistochemical analyses of MDM-2 and p53 protein are far from conclusive, although many support an inverse correlation between the two oncoproteins. Such studies have included uterine sarcoma (Seki et al, 1997), breast carcinoma (Jiang et al, 1997), thymoma (Stefanaki et al, 1997), osteogenic sarcoma (Lonardo et al, 1997), glioblastoma (Biernat et al, 1997), lung carcinoma (Higashiyama et al, 1997), oral carcinoma (Matsumura et al, 1996), malignant melanoma (Gelsleichter et al, 1995), thyroid carcinoma (Jennings et al, 1995) and rhabdomyosarcoma (Keletti et al, 1996).

Comments

Immunostaining for MDM-2 has also been successfully conducted on cytological preparations (Dowell et al, 1996).

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Measles

Sources/Clones

Biogenex (1.3, polyclonal), Chemicon (polyclonal), and Seralab.

Fixation/Preparation

This antibody is applicable to formalin-fixed, paraffin wax-embedded tissue. The number of positive cells is increased significantly with microwave pretreatment (McQuaid et al, 1995).

Background

Measles, an acute febrile eruption, has been one of the most common diseases of civilization. Despite the development of an effective vaccine, it remains a worldwide health problem.

The measles virion is composed of a central core of ribonucleic acid with a helically arranged protein coat surrounded by a lipoprotein envelope with spike-like structures. The virion is 120-200 nm in diameter and is classified as a morbillivirus in the paramyxovirus family.

Applications

Subacute sclerosing panencephalitis (SSPE) is a rare, fatal disease of children caused by a persistent measles virus infection of the central nervous system. Immunodetection of viral proteins using antibodies raised to measles is useful to confirm the diagnosis of SSPE in brain biopsies and post mortem CNS tissue (McQuaid et al, 1995).

Using microwave antigen retrieval systems, increased immunoreactivity was seen in neuronal processes, suggesting that this may represent virus spreading from cell to cell (McQuaid et al, 1995). Attempts to demonstrate the M-protein in the brain of an SSPE patient using immunocytochemistry proved futile, even though nucleotide sequences coding for M-protein were detected (Brown et al, 1987). This suggested either diminished synthesis and/or rapid degradation of M-protein in the SSPE brain.

Recently, the capacity for measles to persist and induce chronic inflammation has suggested this virus as a likely candidate for the etiology of Crohn's disease (Wakefield et al, 1995). Immunocytochemistry using measles virus-specific monoclonal and polyclonal antibodies was positive within endothelial cells in areas of granulomatous vasculitis. In situ hybridization for measles virus genomic RNA not only produced positive signals in a similar location but also showed strongly positive cells in the secondary lymphoid follicles (Wakefield et al, 1993). By employing an immunogold method, ultrastructural studies have shown significantly higher levels of anti measles antigen in Crohn's disease compared to ulcerative colitis, tuberculous lymphadenitis and non-granulomatous areas of bowels but no significant difference between Crohn's disease and SSPE (Daszak et al, 1997). An epidemiological association between Crohn's disease and measles virus exposure in early life has been suggested in case-control studies (Ekbom et al, 1996). It is therefore suggested that Crohn's disease may be a chronic granulomatous vasculitis in reaction to a persistent infection with measles virus within the vascular endothelium (Wakefield et al, 1995).

Comments

Application of antibodies to measles virus would be useful in developing countries where SSPE is more frequently seen. Both polyclonal and monoclonal antibodies give good immunoreactivity following

microwave pretreatment (Rahman et al, 1996; Allen et al, 1996).

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Metallothioneins

Sources/Clones

Dako (E9).

Fixation/Preparation

Immunoreactive in fixed paraffin-embedded tissue sections as well as cell preparations and frozen sections. Immunoreactivity is enhanced following HIER.

Background

Metallothioneins (MTs) are low molecular weight, heavy metalbinding proteins whose expression is induced by heavy metals as well as other factors such as stress, glucocorticoids, lymphokines and xenobiotics (Kagi, 1993). MTs have been described in most vertebrate and invertebrate species. Two major isoforms, MT-I and MT-II, are distributed in most adult mammalian tissues. Recently, another isoform, MT-0, has been recognized and genes for MT-III and MT-IV with restriction to brain neurons and stratified epithelium have been described (Jasani & Schmid, 1997). Interest in MTs has focused on their overexpression and susceptibility to carcinogenic and anticarcinogenic effects of cadmium, spontaneous mutagenesis and anticancer drugs, and tumor resistance to chemotherapeutic agent (see Jasani & Schmid 1997).

Applications

The ability to stain for MTs with immunohistochemical methods has produced a large amount of data concerning their expression at different stages of development and progression of a wide variety of tumors. Briefly, overexpression of MT has been associated with the type and grade of some tumors such as ductal breast carcinoma (Oyama et al, 1996), skin carcinoma (Zelger et al, 1994), cervical carcinoma (Lim et al, 1996), pancreatic carcinoma (Ohshio et al, 1996), prostatic carcinoma (Zhang et al 1996), melanoma (Zelger et al, 1993) and acute lymphoblastic leukemia. While overexpression of MTs appears to be mostly associated with locally invasive carcinomas of poor histological type and grade, reduced overall survival and local recurrence of tumor (but not lymph node or distant metastases), this is not true of all tumors. In colonic (Gaiuffre et al, 1996), bladder (Saika et al, 1994; Bahnson et al, 1994) and fibroblastic skin tumors (Zelger et al, 1994), overexpression of MTs is associated with lower grade, better differentiated tumors. The reason for this apparent discrepancy is not clear. It has been suggested that current antibodies for immunostaining are unable to distinguish between MT-I and MT-II isoforms or metal-bound and metal-free (apoMT) forms of the protein. Furthermore, they are also unable to detect overexpression of MT-0, MT-III and MT-IV isoforms, accounting for the apparently conflicting observations (Jasani & Schmid, 1997). The use of MT expression to predict response to chemotherapy is another avenue which requires further study (Saika et al, 1994; Gaiuffre et al, 1996).

A recent paper describes MT as a marker of deep penetrating dermatofibroma, allowing its distinction from dermatofibrosarcoma protuberans, which was consistently negative by immunostaining (Zelger et al, 1994).

Comments

MT staining is found in both nucleus and cytoplasm and the proliferating edges of tumors show most intense staining (Cherian, 1994).

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Muscle-Specific Actin (MSA)

Sources/Clones

Biogenex (HHF35), Dako (HHF35), Diagnostic Biosystems (HHF35), Enzo (HHF35), Biogenesis, Sanbio (SA1C1), and Zymed (ZMSA-5, ZCA34, ZSA-1).

Fixation/Preparation

The antibody HHF35 is immunoreactive in fixed paraffin-embedded tissue sections and staining is enhanced following HIER.

Background

There are at least six different actin isotypes in mammals. They are four isotypes found exclusively in muscular tissues and include α-skeletal, α-cardiac, and α and γ-smooth muscle actins and two other isotypes, β-and γ-cytoplasmic actin, found in most cell types, including non-muscle cells of the body. Early antiactin antibodies were polyclonal and did not distinguish among various actin isotypes and were of low sensitivity and specificity. Various monoclonal antibodies have now been described and the most widely used is clone HHF35, available commercially, which recognizes a common epitope of α-skeletal, α-cardiac and α- and γ-smooth muscle actin isotypes (Tsukada et al, 1987). This antibody labels myoepithelial and smooth muscle cells as well as leiomyomas and leiomyosarcomas. Muscle-specific actins (MSA) have also been described in pericytes, reactive myofibroblasts, and skeletal and cardiac muscle. Positive staining cells have been reported in the deep ovarian cortical stroma and theca externa of secondary ovarian follicles, alveolar soft part sarcoma, epithelioid sarcoma, infantile digital fibromatosis, ovarian sclerosing stromal tumors and Kaposi's sarcoma, representing either myofibroblasts or pericytes in these conditions. Glomus tumors stain positive for MSA, a finding which supports a smooth muscle derivation of these tumors (Dervan et al, 1989), and the variable extent of MSA staining observed in malignant mesothelioma (Kung et al, 1995) and malignant fibrous histiocytoma has been attributed to myofibroblastic differentiation in these tumors. Actin staining of unequivocal tumor cells has been reported in occasional cases of metastatic endometrial stromal sarcoma and malignant peripheral nerve sheath tumor but it has not been ascertained if these findings represent aberrant actin expression of tumor cells or crossreactivity of antiactin antibodies. MSA has also been observed in the cells of the capsule of the liver, kidney and spleen and in decidual cells, some stromal cells of chorionic villi and the so-called fibroblastic reticulum cells of lymph nodes and spleens.

Applications

The increased sensitivity and specificity of newer monoclonal antibodies allow the use of anti-MSA antibodies in the identification of pleomorphic spindle cell tumors. Because of varying sensitivities, it is best to employ MSA with other myogenic markers such as smooth muscle actin and desmin when examining tumors, which potentially confuse with rhabdomyosarcoma (RMS), leiomyosarcoma (LMS) and myofibroblastic tumors (Azumi et al, 1988). Much of the current controversy as to which of these markers is the most sensitive for myogenic differentiation stems from the fact that the expression of the individual markers varies with the site of origin of the tumor (Rangdaeng & Truong, 1991). For example, most soft tissue and uterine LMS contain predominantly α- smooth muscle actin but those from the

gastrointestinal tract show only β and γ non-muscle actins and would thus be negative for HHF35. Myofibroblasts show heterogeneous immunophenotype and may be positive for vimentin only; for vimentin and α- smooth muscle actin; for vimentin and desmin; or for vimentin, desmin and α- smooth muscle actin (Skalli et al, 1989). Myofibroblastic proliferations such as nodular fasciitis may display characteristic peripheral/subplasmalemmal staining for muscle actin, yielding a "tram track" appearance (Leong & Gown, 1993). Increased expression of MSA has been correlated with mesangial cell injury and proliferation in both rats and humans and can be employed as a marker of mesangial cell injury, activation and proliferation (Alpers et al, 1992).

Comments

Zenker's fixative appears to cause a marked decrease in the intensity of MSA staining. HIER enhances immunostaining and simple heating in distilled water at 60 C overnight or in ZnSO4 at 90 C for 10 min produces the best results. False-positive reactivity with clones HHF35 and 1A4 has been reported in non-Hodgkin's lymphoma, a problem attributed to contaminating antibodies, partial antibody degradation or excess antibody concentration which may occur with ascitic fluid preparations of anti-MSA (Sheehan & O'Brian, 1995). The problem was not observed in tissue culture supernatant antibodies and was abolished by the addition of 50 mmol/l of EDTA to the prediluted antibody.

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Myelin Basic Protein (MBP)

Sources/Clones

Axcel/Accurate (polyclonal), Biogenesis, Biogenex (130-137), Biomedia/Accurate (MAB3), Biosource, Biotec, Boehringer Mannheim, Cymbus (MIG-MI9), Dako, Research Diagnostics (MIG-MI9), Serotec and Zymed (polyclonal).

Fixation/Preparation

The antigen is formalin resistant and its immunoreactivity is enhanced by proteolytic digestion or HIER. The antibody can be applied to frozen sections.

Background

Myelin basic protein (MBP) is found in the central and peripheral system. It is found in oligodendrocytes and myelin of white matter in the brain and spinal cord and to a lesser extent in gray matter. It is also found in peripheral nerve.

Applications

MBP is useful in research but has limited applications in diagnostic immunohistochemistry where its use is largely in the diagnosis of soft tissue tumors. It has been demonstrated in neuromas, neurofibromas, ganglioneuromas and tumors with neural differentiation and neural elements but is not present in glial tissues. The protein has been employed in a panel of antibodies to identify palisaded encapsulated neuromas of the skin (Argenyi, 1990) and is useful for the distinction of neurofibromas from neurotized melanocytic nevi. Neurofibromas showed focal staining for CD 57 (Leu 7), glial fibrillary acidic protein and MBP whereas neurotized nevi failed to express these markers (Gray et al, 1990). MBP (together with CD 57) has also been demonstrated in some granular cell tumors (Mazur et al, 1990), suggesting neural differentiation in some of these lesions. MBP is a useful marker of ganglioneuroblastomas, ganglioneuromas and gangliocytic paraganglioma (Furihata et al, 1996; Molenaar et al, 1990).

Comments

The use of MBP as a marker of Schwannomas is well established (Wick et al, 1987) although some studies have failed to find MBP in Scwann cell neoplasms (Clark et al, 1985; Johnson et al, 1988; Sharma et al, 1990) and both immunohistochemical and Western blot analyses have failed to demonstrate MBP in oligodendrogliomas and Schwann cell tumors (Schwechheimer et al, 1992). Other markers such as S100, fibrillary acidic protein and CD 57 are preferred for the characterization of nerve sheath differentiation.

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Wick MR, Swanson PE, Scheithauer BW, Manival JC 1987. Malignant peripheral nerve sheath tumor: an immunohistochemical study of 62 cases. American Journal of Clinical Pathology 87: 425-433.

Myeloperoxidase

Sources/Clones

Accurate (CLBMPO.1), Axcel/Accurate (MPO-7, polyclonal), Biodesign (polyclonal) Caltag Laboratories (H43-5), Dako (MPO-7, polyclonal), and Research Diagnostics (CLB-MPO1-1).

Fixation/Preparation

May be applied to formalin-fixed, paraffin-embedded tissue sections. This antibody may also be used to label acetone-fixed, frozen sections and fixed-cell smears. The rabbit polyclonal antibody reacts with myeloperoxidase in a variety of fixatives including Zenker's acetic acid solution, B5 solution and formalin (Pinkus & Pinkus, 1991). Pretreatment with trypsin is essential before immunostaining. HIER does not appear to enhance immunoreactivity but is not deleterious. The monoclonal antibodies do not work on formalin-fixed tissues and should only be used on frozen sections.

Background

Myeloperoxidase is the major constituent of primary granules of myeloid cells. It therefore serves as a reliable marker for myeloid cells, including early (immature) and mature forms. The appearance of myeloperoxidase precedes neutrophil elastase during myeloid cell differentiation. Further, myeloperoxidase antibody does not react with lymphoid or epithelial cells (Pinkus & Pinkus, 1991). The myeloperoxidase immunogen was isolated from human granulocytes.

Other immunohistochemical markers for myeloid cells, e.g. lysozyme, CD15, Mac 387 and CD 68, despite being sensitive, lack specificity in that they also stain histiocytes and other cell types including epithelium (Mason & Taylor, 1975). CD 43 and CD 45RO also stain myeloid cells frequently, but demonstrate T cells and histiocytes as well (Traweek et al, 1993).

Applications

Immunostaining for myeloperoxidase on paraffin sections is helpful in confirming the myeloid nature of the primitive cells that infiltrate marrow tissue. Positive reaction excludes lymphoblastic leukemia and malignant lymphoma and is therefore crucial for patient management (Van Der Schoot et al, 1990). Skin infiltrated with acute myeloid leukemia, which may be subtle, benefits from the application of antimyeloperoxidase antibody to highlight the neoplastic population (Wong & Chan, 1995).

Granulocytic sarcoma presenting as a tumor mass may occur in isolation or in association with myeloid disorders (Nieman et al, 1981). In the absence of a history of a hematological malignancy, an erroneous diagnosis of lymphoma may lead to an inappropriate treatment being instituted. Hence a high index of suspicion and the use of antibodies (including myeloperoxidase) for the demonstration of the myeloid nature of the cellular proliferation avoids a misdiagnosis. A study of 22 cases of granulocytic sarcoma on archival material proved myeloperoxidase immunostaining to be the most sensitive for demonstrating neoplastic myeloid cells, being positive in all cases (Wong & Chan, 1995). Chloroacetate esterase and lysozyme were positive in only 68% and 86% of case respectively. Lysozyme may show a strong reaction in some cases of granulocytic sarcoma, complicating acute myelomonocytic leukemia. The advantage of myeloperoxidase is the reduced background staining. Various other studies (Pinkus & Pinkus, 1991; Traweek et al, 1993)

have also demonstrated myeloperoxidase to be a highly sensitive tool for the confirmation of neoplastic myeloid cells in granulocytic sarcoma.

Comments

Antimyeloperoxidase should be included in the immunohistochemical panel for lymphoma investigation. Any "lymphoma" that cannot be classified with confidence should raise the suspicion of a granulocytic sarcoma. Furthermore, tumor cells marking with only T-cell markers CD 43 or CD 45RO, but not the specific T-cell marker CD 3 or, alternatively, which stain only for histiocytic markers such as CD 68 or CD 15, should raise the alarm for a possible granulocytic sarcoma (Wong & Chan, 1995).

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Wong KF, Chan JKC 1995 Antimyeloperoxidase: antibody of choice for labeling of myeloid cells including diagnosis of granulocytic sarcoma. Advances in Anatomic Pathology 2: 65-68.

MyoD1

Sources/Clones

Accurate/Novocastra (5.8A) and Dako (5.8A).

Fixation/Preparation

Anti-MyoD1 can be used on formalin-fixed, paraffin-embedded tissue sections. Deparaffinized tissue sections require heat pretreatment in citrate buffer prior to immunohistochemical staining procedure. Sialinized slides are recommended to improve adherence of tissue sections to glass slides. Ideally, this antibody requires fresh-frozen tissue for optimum results.

Background

The differentiation of skeletal muscle at the molecular level requires activation and transcription of genes encoding muscle-specific proteins and enzymes such as desmin and creatine kinase. These activities are controlled by a set of genes including *MyoD1*, myogenin, myf-5 and myf-6 (Funk et al, 1991; Tonin et al, 1991). It is thought that *MyoD1* activation is an early event that commits the cell to skeletal muscle lineage (Hosoi et al, 1992). Transfection of the *MyoD1* gene into non-muscle cells has been shown to induce conversion of fibroblasts into myoblasts (Davis et al, 1987). Similarly, muscle-specific genes in tumor cell lines may be activated by forced expression of exogenously introduced *MyoD1* (Weintraub et al, 1989). The *MyoD1* gene has been localized to the short arm of chromosome 11 (Gressler et al, 1990). Its activation, as reflected in the detection of mRNA or protein product, represents a stage of skeletal muscle differentiation that is earlier than that of currently available immunohistochemical markers, such as desmin and myoglobin.

The MyoD1 protein is a 45 kD nuclear phosphoprotein (5.8A reacts with an epitope between amino acid residues 170 and 209), with nuclear expression restricted to skeletal muscle tissue. Monoclonal anti-MyoD1 strongly stains nuclei of myoblasts in developing skeletal muscle whilst the majority of adult skeletal muscle has been found to be negative (Wang et al, 1995), including a wide variety of normal tissue. However, weak cytoplasmic staining has been observed in non-muscle tissue, including glandular epithelium (Wang et al, 1995).

Applications

MyoD1 nuclear immunostaining has been demonstrated in the majority of rhabdomyosarcomas of various histological subtypes (Appendix 1.24). In fact, it has been shown that the MyoD1 expression in rhabdomyosarcomas is inversely related to the degree of cellular differentiation of tumor cells (Wang et al, 1995). This phenomenon is useful to distinguish embryonal rhabdomyosarcomas from other small blue round cell tumors of childhood, such as, Ewing's sarcoma/peripheral primitive neuroectodermal tumor, neuroblastoma and childhood lymphomas. Wilm's tumors and ectomesenchymoma with rhabdomyosarcomatous foci also show nuclear expression of MyoD1 (Dias et al, 1992) (Appendix 1.3). It has also been shown that the sensitivity and specificity of the MyoD1 antibody in the differential diagnosis of adult pleomorphic soft tissue sarcomas approaches that of pediatric rhabdomyosarcomas (Wesche et al, 1995). The demonstration of MyoD1 protein in four cases of alveolar soft part sarcoma has provided good evidence for its rhabdomyosarcomatous differentiation (Rosai et al, 1991).

Comments

Caution is necessary in that cytoplasmic immunoreactivity with antibody to MyoD1 has been demonstrated in most neuroblastomas and occasional Ewing's sarcomas/PNETs (Wang et al, 1995). Hence, only nuclear staining should be considered as evidence of skeletal myogenic differentiation, although our own experience has been that nuclear expression occurs in the primitive skeletal tumors, whilst tumors with cytoplasmic/myogenic differentiation have demonstrated cytoplasmic immunopositivity. The cytoplasmic immunostaining with anti-MyoD1 (clone 5.8A) has been suggested to represent crossreactivity with an unknown cytoplasmic antigen. In their study of 12 cases of alveolar soft part sarcoma, Wang et al (1996) found positivity for desmin in six tumors but no specimen showed nuclear expression of MyoD1 or myogenin. However, there was considerable cytoplasmic staining with the anti-MyoD1, a phenomenon observed with various non-muscle and neoplastic tissues with this antibody. Biochemical analysis of fresh-frozen tumor tissue showed no specific band corresponding to the 45 kD MyoD1.

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Myogenin

Sources/Clones

Dako (F5D), Pharmingen (5FD) and Santa Cruz (polyclonal).

Fixation/Preparation

F5D is immunoreactive in fixed, paraffin-embedded tissue sections and HIER enhances immunoreactivity.

Background

Myogenin belongs to a family of regulatory proteins essential for muscle development. Studies in mice indicate that myogenin is not required for the initial aspects of myogenesis, including myotome formation and the appearance of myoblasts, but late stages of embryogenesis are more dependent on myogenin (Venuti et al, 1995). Expression of myogenin is restricted to cells of skeletal muscle origin and appears to be inversely related to the degree of cellular differentiation, making it a potentially useful marker for skeletal muscle differentiation in the identification and typing of anaplastic round cell tumors in childhood (Appendix 1.3).

Applications

F5D recognizes an epitope located in the amino acid region 138-158 of the myogenin protein and labels nuclei of the majority of human rhabdomyosarcomas (Folpe et al, 1997). No reactivity is reported with Ewing's sarcoma/peripheral primitive neuroectodermal tumor, neuroblastoma or adult skeletal muscle (Wang et al, 1995).

Comments

Only nuclear staining should be regarded as positive. Clone F5D shows strong reactivity in paraffin sections following HIER. Myogenin may prove to be a better and more sensitive marker of skeletal muscle differentiation in poorly differentiated rhabdomyosarcoma than MyoD1, given that the latter displays non-specific crossreactivity with an unknown cytoplasmic antigen in non-muscle cells and tumors (Wang et al, 1995).

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Myoglobin

Sources/Clones

Accurate (M-2-167, M-3-416), American Research Products (1B4, 1F6, 4G8, 8H5), Axell/Accurate (polyclonal), Biogenesis (DA2, polyclonal), Biodesign/Research Diagnostics, Biogenex (MG-1, polyclonal), Chemicon (polyclonal), Dako (polyclonal), Fitzgerald (M312211, M312212, polyclonal), Immunon (polyclonal), Seralab (polyclonal), Sigma (MG-1) and Zymed (Z001).

Fixation/Preparation

Myoglobin is resistant to formalin fixation. Immunoreactivity is not significantly enhanced by proteolytic digestion and is not responsive to HIER.

Background

Myoglobin, a 17.8 kD protein is the oxygen carrier hemoprotein, a specific marker for striated muscle cells. It is also present in cardiac muscle. The antibodies do not crossreact with hemoglobin. Crossreactivity with myoglobins of other mammalian species may occur with some antibodies.

Applications

Antimyoglobin has been used to indicate early myocardium necrosis and skeletal muscle trauma and necrosis. Myoglobin was one of the earliest markers of striated muscle differentiation but its expression appears to be linked to the differentiation of rhabdomyosarcoma cells, so that a sizable number of such tumors, particularly the poorly differentiated ones, exhibit no staining. In our experience, morphologically recognizable rhabdomyoblasts express myoglobin, whereas poorly differentiated tumors fail to stain so that this marker is not helpful when it is actually required (Leong et al, 1989; Guruchala et al, 1997). Its application as a marker of early ischemic myocardium appears to be less reliable than cytoskeletal proteins such as vinculin, desmin andα-actinin (Zhang & Riddick, 1996). Myoglobin immunostaining has been employed in the study of raggedred fiber of patients with mitochondrial encephalomyopathy (Kunishige et al, 1996).

Staining for myoglobin can also be performed in renal biopsies of patients with myoglobin-containing casts due to conditions such as necrotizing myopathy or rhabdomyolysis (Helliwell et al, 1991).

Comments

Myoglobin is obviously not a dependable marker of striated muscle differentiation, especially in poorly differentiated rhabdomyosarcoma. Other markers such as desmin, muscle-specific actin and MyoD1 should be employed for the identification of striated muscle differentiation. The protein released from necrotic muscle may be phagocytosed by macrophages which should not be mistaken for rhabdomyoblasts.

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and lymphoblastic lymphoma. Surgical Pathology; 2: 5-17.

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Neurofilaments

Sources/Clones

Neurofilament Triplet Proteins

Antibodies are available from Accurate (A286), Biogenex (2F11, NF01), Dako (2F11, NR4), Diagnostic, EY Labs, Enzo, Labsystems and Seralab (BIO-51H, 2F11).

Neurofilament 70 kD

Antibodies are available from Accurate, Biodesign (NR4, DP5-1-12), Biogenesis (NF01), Boehringer Mannheim (N52), Calbiochem, Chemicon, Cymbus Bioscience (NR4), ICN, Immunotech (DP5-1-12), Novocastra, Oncogene (NR4), Seralab (NR4), Serotec (DP5-1-12), Sigma (NR4, N52) and Zymed (RMS12).

Neurofilament 150 kD

Antibodies are available from Accurate (NN18, RNF403), Amersham, American Research (NF403), Chemicon, Cymbus Bioscience (BF10), Biodesign (DP43.16), Biogenesis (BIO-46H, polyclonal), Boehringer Mannheim (BF10, NN18), Immunotech (DP43.16), Medac, Milab (NF403), Novocastra (BF10), Oncogene (NN-18), RDI (BF10), Saxon (403), Seralab (NN18), Sigma (NN18) and Zymed (RM0270, RM0281, FNP7).

Neurofilament 200 kD

Antibodies are available from Accurate (N52.1.7), Amersham, American Research (NF402), Biodesign (RT97), Biogenesis (BIO-66H), Boehringer Mannheim (RT97, NE14), Calbiochem, Chemicon, Cymbus Bioscience (RT97), ICN (402), Immunotech (DP12.10), Medac, Milab, Novocastra (RT97), RDI (RT97), Oncogene (NE-14), Pierce (NE14), Saxon (402), Seralab (NE14), Serotec, Sigma (NE14) and Zymed (RM024, TA51).

Fixation/Preparation

Most antibodies available are immunoreactive in routine processed tissues but the neurofilament triplet proteins are fixation dependent and immunostaining is enhanced following HIER.

Background

Neurofilaments (NF) are distinct from other intermediate filaments (IF) in that they are composed of three different subunits of distinct but related proteins of 70, 150 and 200 kD as compared to other IFs which range from 40 to 70 kD in molecular weight. The antigenic determinants of each of the subunits may be unique or shared and each NF protein is a separate gene product. NFs are found in neurons and the neuronal processes of the central and peripheral nervous tissue. It is likely that nearly all neurons can constitutively express all three NF genes and reports of absence of subunits of NF in certain neurons probably reflect technical limitations, as the proteins are fixation dependent.

Applications

The antibodies to NFs stain all neurons and axonal processes of the central and peripheral nervous system. The only exception seems to be the olfactory sensory neurons, which contain only vimentin IFs and are unique in that they die and are replenished throughout the lifespan of the mammal. The immunostaining of NF is employed for the study of neuronal distribution and innervation in normal and

abnormal tissues (Krammer et al, 1994; Oki et al, 1995) and neuronal differentiation in neoplasms. The detection of NF

helps identify neurons and axonal processes in cases of suspected Hirschsprung's disease. NFs are found in a variety of tumors including neuroblastoma, ganglioglioma, medulloblastoma, retinoblastoma, pineal parenchymal tumors (Appendix 1.7) and in neuroendocrine and neuroepithelial tumors such as Merkel cell carcinoma (Leong et al, 1986), carcinoid (Kimura et al, 1989), esthesioneuroblastoma, ganglioneuroblastoma, ganglioneuroma, neuroblastoma, oat cell carcinoma, paraganglioma, pheochromocytoma and in teratomas with neuronal differentiation. NF may also be expressed in primitive/peripheral neuroectodermal tumors (PNETs) (Llombart-Bosch et al, 1989; Papierz et al, 1995). Anti-NF is useful in the separation of neuroblastoma and PNET from other small round cell tumors in childhood (Leong et al, 1989), which include rhabdomyosarcoma, lymphoblastic leukemia and small cell osteogenic sarcoma.

Comments

As all neurons express all three subunits of NF, antibodies to the triplet protein should be employed in diagnostic workups.

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Neuron-Specific Enolase (NSE)

Sources/Clones

Accurate (BBSNCU1), American Research (5G10, NH3), Axcel, Biodesign (MIG-N3, 5E2), Biogenesis (BG10), Biogenex (MIG-N3), Biotec (XNE12), Cymbus Bioscience (5E2), Dako (BBS-NC, VI-H14), Immunotech, Novocastra (VI-H14, SE2), Research Diagnostics (5E2, 5G10, 5A4), Sanbio (MIG-N3), Seralab (MIG-N3), Serotec (MIG-N3), Shandon (BBS-NC, VI-H14) and Zymed.

Fixation/Preparation

Both polyclonal and monoclonal antibodies are immunoreactive in routinely prepared tissue sections. Immunostaining is enhanced by HIER.

Background

Neuron-specific enolase (NSE) is the glycolytic isoenzyme of the enolase γ-γ dimer specifically detected in neurons and neuroendocrine cells, and their corresponding tumors (Wick et al, 1983; Venores et al, 1984). In addition, NSE has been demonstrated in the non-neoplastic cells of the pituitary, peptide-secreting tissues, pinealocytes, neuroendocrine cells of the lung, thyroid, parafollicular cells, adrenal medulla, islets of Langerhans, Merkel cells of the skin (Leong et al, 1986) and melanocytes. NSE immunostaining is also positive in normal striated muscle, hepatocytes and, to a lesser extent, smooth muscle (Cooper, 1994).

While highly sensitive, NSE has low specificity. Antibodies to NSE enjoyed great popularity in the earlier days of diagnostic immunohistochemistry as markers of neural and neuroendocrine differentiation. However, it was soon realized that most anti-NSE preparations showed a high rate of unwanted crossreactivities even among the monoclonal antibodies and a high level of background staining often made interpretation difficult. NSE has since become known facetiously as "non-specific enolase" (Bjerkehagen et al, 1994). While its application in immunohistochemistry is limited, assays for NSE are increasingly being performed as diagnostic and prognostic markers in the serum, pleural effusions and cerebrospinal fluid in cases of head injury, status epilepticus, small cell carcinoma of the lung, neuroblastoma, various neuroendocrine tumors, germ cell tumors and malignant melanoma.

Applications

NSE is still a useful marker to identify peripheral nerves (Leonard et al, 1995). In the context of desmoplastic melanomas, which are often negative for the melanoma-specific markers HMB45 and NKIC3, NSE and S100 are sensitive markers (Anstey et al, 1994). However, when used for the identification of neuroendocrine differentiation, it is necessary that it be employed in a panel with more specific markers such as chromogranin, PHE 5 and synaptophysin. NSE positivity has been demonstrated in as many as 83% of testicular carcinoma in situ cases as well as in overt testicular germ cell tumors including seminomas, non-seminomas and mixed germ cell tumors (Kang et al, 1996).

Comments

Monoclonal antibodies to NSE produce less background staining, but specificity is only slightly increased. PGP 9.5 stains a very similar spectrum of cells and tumors. As PGP 9.5 shows greater sensitivity it would serve as a suitable substitute for NSE; however, neither marker should be used in isolation, especially when

employed for the identification of neuroendocrine differentiation (Leong et al, 1989).

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Neutrophil Elastase

Sources/Clones

Axcel/Accurate (MP57), Biogenesis (AHN-10), Calbiochem (polyclonal), Chemicon (AHN10), Dako (NP57).

Fixation/Preparation

NP57 may be used on both formalin-fixed, paraffin-embedded sections and frozen sections. If other fixatives are used, e.g. acetone or methanol, there is a tendency for the antigen to diffuse from the myeloid cell cytoplasm and to localize in the cell nucleus.

Background

Neutrophil elastase is a neutral protease which plays a major role in the killing of microorganisms and the initiation of tissue injury during inflammatory reactions. The enzyme is present in the primary (azurophilic) granules of myeloid cells (Baggiolini et al, 1978). Neutrophil elastase consists of three isoenzymes with similar molecular masses (approximately 30 kD) (Ohlsson & Olsson, 1974). Monoclonal antineutrophil elastase (NP57) was raised against human neutrophil granule proteins (Pulford et al, 1988). This antibody labels neutrophils in routinely processed histological specimens and also reacts (although more weakly) with a minor population of normal blood monocytes. Other cell types, including epithelial cells, are NP57 negative (Pulford et al, 1988).

Applications

Neoplastic cells in 27/37 (73%) bone marrow specimens of acute myeloid leukemia were NP57 + (Ralfkiaer et al, 1989). The number of positive cells varied from few (5-10%) to virtually all of the cells. In routinely processed biopsy specimens from lymphoid organs with extramedullary hematopoiesis or infiltrates of chronic myeloid leukemia, NP57 was confined to neutrophils and their precursors (Ralfkiaer et al, 1989). Other studies (Van Der Schoot et al, 1990; Traweek et al, 1993) have demonstrated NP57 positivity in 53% of acute myeloid leukemia and 54% of extramedullary myeloid cell tumors respectively. These percentages appear to be slightly lower than that obtained when staining for myeloperoxidase. This probably indicates that elastase is synthesized later during myeloid maturation than myeloperoxidase. Leukemias of lymphoid origin are not stained.

Comments

The above results indicate that detection of elastase with monoclonal NP57 forms a useful supplement to other immunohistochemical markers for myeloid disorders e.g. myeloperoxidase, lysozyme, CD43 and CD15.

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nm23/NME1

Sources/Clones

Accurate (NM301), Accurate/Novocastra (37.6), Dako (polyclonal), Novocastra (nm23-301, polyclonal), Oncogene (NM301, polyclonal) and Pharmingen (NM301).

Fixation/Preparation

Some of the available antibodies are immunoreactive in fixed, paraffin-embedded sections. HIER is required.

Background

The nm23 gene family was originally identified in a murine melanoma cell line and nm23 H1 was found to be transcribed at a ten-fold higher rate in cells of lower metastatic potential. Two highly homologous human genes have subsequently been identified nmE1 and nmE2 located on chromosomes 17q and coding for the 18.5 and 17 kD proteins nm23 H1 and nm23 H2 respectively. nm23 is mainly cytoplasmic, but nuclear and membrane localization has also been seen (Urano et al, 1993).

Applications

The nm23 gene product was initially believed to play a role in suppressing tumor metastasis. This may be too simplistic a view, with both metastasis suppression and disease progression being linked to elevated gene expression in different tumors. Until its precise roles are elucidated, its value as a prognostic indicator is limited.

Isotype-specific studies on breast neoplasms have indicated that it is nm23 H1 and not nm23 H2 that correlates with metastases (Royds et al, 1993). Somatic allelic deletions of nm23 H1 have been reported in some human neoplasms such as breast, kidney, colon and lung cancer, in some cases associated with an increased incidence of metastases (Leone et al, 1991). The loss of nm23 function appears to correlate with phenotypic markers of metastatic potential in some human tumors.

However, there is no strong evidence of direct involvement of the nm23 in metastasis and a bystander effect rather than a causative role for nm23 cannot be ruled out, the reduced nm23 level being a reflection of a more dedifferentiated state of the tumor. nm23 expression correlates inversely with metastatic potential in in vitro and experimental animal systems, with transfection of the nm23 gene into melanoma K1735 cells resulting in a reduction of tumor metastases.

Preliminary studies in esophageal carcinoma (Patel et al 1997) indicate that failure to express p53 and nm23 may be related to an unfavorable prognosis in patients with advanced esophageal carcinoma. Similarly, there is reduced staining of nm23 H1 in laryngeal squamous cell carcinoma compared with laryngeal polyps (Lee et al, 1996b). In contrast, progression of ovarian carcinoma is accompanied by overexpression of nm23 protein (Harlozinska et al, 1996; Srivatsa et al, 1996). While some studies suggest that overexpression of nm23 H1 is an early event in the development of prostatic adenocarcinoma (Igawa et al, 1994; Myers et al, 1996), others show elevated levels of nm23 H1 and H2 in benign prostatic hyperplasia and postulate a role in the suppression of malignancy (Konishi et al, 1993).

In pituitary adenoma, strong expression of nm23 H2 is associated with non-invasive adenomas and may restrain tumor aggression (Takino et al, 1995). Expression in uveal melanoma appears to be inversely proportional to the depth of scleral invasion (Greco et al, 1997) but in melanoma of the skin, there are conflicting studies. Lee et al (1996a) found reduced nm23 H1

immunohistological expression to be associated with melanomas that have high metastatic potential and poorer prognosis. Kanitakis et al (1997) found nm23 to not have a direct correlation with metastatic potential.

In transitional cell carcinoma of the bladder (Shiina et al, 1996) and FIGO stage IB cervical carcinoma (Kristensen et al, 1996), nm23 protein immunoreactivity is not an independent prognostic factor. Staining for nm23 has little value in testicular seminoma, where expression of both the nm23 H1 and nm23 H2 proteins was found not to be associated with metastatic or invasive status of the tumor (Hori et al, 1997).

Comments

Polyclonal antiserum to nm23 produces strong cytoplasmic staining after HIER.

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P27^{kip1}

Sources/Clones

Lab Vision Corp (DCS70), Pharmingen (G173-524) and Transduction Laboratory.

Fixation/Preparation

The anti-p27 antibody is immunoreactive in fixed paraffin-embedded sections but only following HIER in citrate buffer at neutral pH.

Background

The p27^{kip1} (p27) gene encodes an inhibitor of cyclin-dependent kinase (CDK) activity. Two families of proteins that generally inhibit cell cycle progression regulate the activity of cyclin-dependent kinase complexes. These are the INK4 group of p16, p15, p18 and p19, which may have suppressor functions and whose activities are dependent on a normal retinoblastoma protein and show maximal expression during S-phase, and the group of CDK inhibitors which include p21/WAF1/CIP1, p27kip1 and p57/kip2. Overexpression of the latter group inhibits kinase activities of several cyclins and causes cell cycle arrest. The role of kip protein in regulating cell cycle progression in normal and neoplastic cells has not been elucidated although p27-deficient mice develop multiple organ hyperplasia, suggesting that this CDK inhibitor has anti-proliferative activity in vivo (Toyoshima & Hunter, 1994; Hengst & Reed, 1996).

Applications

Several studies have revealed a marked decrease in the percentage of cells expressing p27 in benign and malignant neoplasms compared to normal tissues, with an inverse relationship to Ki-67 antigen, a marker of cell proliferation. Studies with transgenic knockout mice deficient in p27 have shown that p27 protein inhibits proliferation in tissues such as the thymus, pituitary and spleen, leading to hyperplasias of these organs. The exact role of p27 abnormalities in tumor development remains uncertain. Mutations are relatively uncommon in the p27 gene and other mechanisms such as translational control with decreased p27 or downregulation of p27 by specific mitogens may occur during tumor development. The observations that p27 levels are markedly decreased in highly malignant tumors such as anaplastic thyroid carcinomas compared with normal thyroid and benign adenomas suggests that loss of p27 expression may be associated with tumor progression. Evaluation of p27 protein has the potential of predicting the biological behavior of various neoplasms and can be employed to study cell cycle regulation during tumor progression.

Comments

The antibody from Transduction Laboratory, Lexington, KY, is immunoreactive in routine-fixed, paraffin-embedded tissues (Lloyd et al, 1997). The antigen is located in the nucleus. Current applications are primarily in research.

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p53

Sources/Clones

Antibodies to both wild-type and mutant p53 are available from Accurate, Biodesign (Pab1801, 53-12), BioSource, Bioprobe (BP53-12), Chemicon, Cymbus Bioscience, Medac (CM-1), Dako (DO-7), Gibco BRL, Immunotech, Novocastra, Oncogene (Pab1801, Pab421, Pab122), Oncor, Pharmingen (G59-12), Serotec (Pab1801, BP53-12) and Signet.

Antibodies to mutant p53

Biogenesis, Chemicon and Oncogene (Pab240)

Antibodies to Wild-Type p53

Biodesign (Pab246), Oncogene (Pab1620) and Serotec (Pab246)

Fixation/Preparation

Fresh or frozen tissues can be used. Clones Pab1801 and DO7 are effective in formalin-fixed tissue with best results following MW epitope retrieval.

Background

In the current constellation of oncogenes and recessive tumor suppressor genes, the p53 molecule represents one of the most common genetic changes associated with human cancer, being implicated in a wide range of malignancies. The p53 gene displays several unusual features, the most important of which is the ability to act as either a dominant oncogene or a recessive tumor suppressor gene. A combination of genetic events that affect both alleles of the gene results in the loss of expression of wild-type (WT) p53. This may occur as a complete loss of one allele of the gene as a result of a large chromosomal deletion combined with a point missense mutation on the other allele. Mutation leads to the loss of DNA binding and transcriptional regulatory activities of the p53 phosphoprotein with a corresponding loss of its growth suppressive activity and its role as "the guardian of the genome". The mutated protein has abnormal conformation, impaired DNA binding and a prolonged or stabilized half-life, the latter resulting in immunohistochemically stainable levels within nuclei in nearly all tumors showing p53 gene mutation. While a loss of transformation suppression activity and a gain of transforming potential often accompany mutation of p53, not all p53 mutants are equal in terms of their biological activity. Mutations at different hotspots manifest different and distinct phenotypes and there is geographic variation in the sites of mutations thought to reflect the effects of different environmental and regional carcinogens and cofactors.

The p53 gene is located on the short arm of human chromosome 17 and the majority of mutations in the gene are clustered in the most highly conserved domains spanned by 4-9 axons. An important relationship exists between DNA damage hotspots and the capacity to repair the DNA as mutation abolishes the arrest or delay seen in the normal cellular response to DNA damage. Although the WT p53 gene product is not essential for progress of cells through the cell cycle, it does negatively regulate cell growth or division. By binding to specific DNA sequences, the p53 WT product is able to inhibit adjacent gene transcription and serves to prevent uncontrolled cellular proliferation. Thus, loss of WT p53 activity induces a release from G1-S cell cycle checkpoint control following DNA damage, increasing genomic instability and promoting gene amplification.

Binding of WT p53 to a variety of viral proteins such as protein E6, a product of the human papilloma virus, simian virus 40 T-antigen and the Epstein-Barr nuclear antigen, as well as to cellular proteins such as heat shock protein 70 and MDM-2 replication protein, may result in an inactivated complex and a loss of transformation suppression activity (Chang et al, 1993; Batsakis & El-Naggar, 1995).

Applications

Immunohistochemical detection of nuclear p53 protein is based on the increase in concentration of the protein to detectable levels, secondary to an increased synthesis and a lower degradation with longer half-life. In general, there is good agreement between the frequency of positive immunostaining and the frequency of tumors with mutations detected by direct DNA sequencing. However, there are discrepancies between these findings and analysis at the protein level. There is also a danger in assuming positive staining to be an indication of an underlying mutation as p53 protein can be stabilized by other means such as sequestration of normal nuclear protein in the cytoplasm with inactivation of its tumor suppressor function or by binding with the cellular proteins previously mentioned (Hall & Lane, 1994). Also, the use of anti-p53 antibodies that do not react with all mutant forms and other events may lead to failure to detect p53 in neoplasms. The analysis of p53 in neoplastic and preneoplastic states is a powerful tool which provides molecular information on the oncogenic process and the ability to stain for abnormal forms of the protein in tissue sections, particularly those fixed in formalin, allows an important avenue of investigation. Furthermore, there is evidence to suggest that the expression of abnormal p53 may be a prognostic parameter in some neoplasms (Batsakis & El-Naggar, 1995).

Comments

Immunostaining of p53 can be affected by degradation of antigen during tissue processing and it is important to recognize the fixation conditions and the nature of the antibody employed (Fisher et al, 1994). Monoclonal antibody PabI801 (Biogenesis, Gibco BRL, Medac) recognizes most of the mutant and wild types of p53 but 1801 is not suitable for paraffin-embedded tissues. Our own experience is largely with DO7 (Medac, Biogenex, Dako) which identifies both wild-type and mutant protein in formalin-fixed, paraffin-embedded sections and best results are obtained after MW epitope retrieval. Phenol formol saline has been suggested to be a useful fixative for this antigen.

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Pancreatic Hormones (Insulin, Somatostatin, Vasoactive Intestinal Polypeptide, Gastrin, Glucagon, Pancreatic Polypeptide

Pancreatic endocrine tumors have been associated with several distinct clinical syndromes, such as hypoglycemia, glucagonoma syndrome, Zollinger-Ellison syndrome and WDHA (watery diarrhea, hypokalemia and achlorhydria) syndrome (Mukai et al, 1982). Routine histological examination usually fails to predict the behavior and endocrine manifestations of these neoplasms (Creutzfeldt, 1980). Immunohistochemistry permits the specific demonstration of various pancreatic hormones in tissue sections.

Sources/Clones

Insulin

Accurate (K36AC10), Axcel/Accurate (polyclonal), Biodesign (MAb 1, E2-E3, polyclonal), Biogenesis (E6E5, D4B8, IN05, C7C9, polyclonal), Biogenex (AE9D6, polyclonal), Caltag Laboratories (polyclonal), Cymbus Bioscience (MAB1), Dako (polyclonal), EY Labs, Fitzgerald (M91284, M91285, M322212, M322213, polyclonal), Immunotech Inc/Immunotech SA (E2E3), Novocastra (polyclonal), Research Diagnostics (MAB 1), Sanbio/Monosan (N-05, polyclonal), Sigma (K36AC10) and Zymed (Z005, Z006, polyclonal).

Somatostatin

Accurate (YC7, BM17), Axcel/Accurate (polyclonal), Biogenesis (170.3, polyclonal), Biogenex (polyclonal), Caltag Laboratories (polyclonal), Dako (polyclonal), Fitzgerald (polyclonal), Novocastra (polyclonal), Pharmingen (YC7), Sanbio/Monosan (polyclonal) and Zymed (polyclonal).

Vasoactive Intestinal Polypeptide

(VIP): Accurate, Biodesign (polyclonal), Biogenesis (VIP-001), Biogenex (polyclonal), Immunotech (103.10), Serotec and Zymed (polyclonal).

Gastrin

Axcel/Accurate (polyclonal), Biodesign (polyclonal), Biogenesis (polyclonal), Biogenex (polyclonal), Caltag Laboratories (polyclonal), Dako (polyclonal), Fitzgerald (M28046, M28047, polyclonal), Immunotech (4C7A1), Novocastra, Sanbio/Monosan (polyclonal) and Zymed (polyclonal).

Glucagon

Accurate/Sigma (K79bB10), Axcel/Accurate (polyclonal), Biodesign (polyclonal), Biogenesis (polyclonal), Biogenex (polyclonal), Caltag Laboratories (polyclonal), Dako (polyclonal), Fitzgerald (polyclonal), Immunotech (polyclonal), Sanbio/Monosan (polyclonal) and Zymed (polyclonal).

Pancreatic Polypeptide (PP):

Axcel/Accurate (polyclonal), Becton Dickinson, Biodesign (polyclonal), Biogenesis (polyclonal), Biogenex (polyclonal), Dako (polyclonal), Eli Lilly (polyclonal) and Zymed (polyclonal).

Fixation/Preparation

These antibodies are applicable to formalin-fixed, paraffin-embedded tissue as well as frozen sections. No pretreatment or antigen unmasking is necessary for any of the antibodies.

Background

The antigens used as immunogens to raise rabbit antibodies against the pancreatic hormones were as follows: insulin - porcine pancreatic insulin; somatostatin - synthetic peptide somatostatin - 14;VIP - natural porcine VIP, conjugated to glutaraldehyde as carrier protein; gastrin - synthetic human gastrin-17 non-sulfated form(I) conjugated to bovine

serum albumin; glucagon - porcine glucagon.

Although there are at least eight different cell types identified in the pancreatic islets (Dayal and O'Brian, 1981), only the resident four major cell types (A, B, D and PP cells) and G and VIP cells (in neoplastic conditions) will be considered here. In the normal adult islet, insulin-containing B cells account for 60-80 % of endocrine cells (Erlandsen et al, 1976) and occupy the central portion of the islets. Glucagon-containing A cells constitute 20-30% and somatostatin-containing D cells, 5-11%. A and D cells are mostly present in the periphery of the islets and are also scattered within the islets along capillaries. Physiologically, glucagon increases hepatic glucose production and opposes hepatic glucose storage; insulin increases peripheral glucose uptake and opposes glucagon-mediated hepatic glucose production. Hence, the delicate balance of these two hormones maintains blood glucose homeotasis. Somatostatin has inhibitory actions on both A and B cells through a "paracrine" effect, thereby regulating the balance of A- and B-cell functions. PP cells are the least numerous and are present both within and outside the islets. The function of pancreatic polypeptide has not been fully understood. PP cells have a variable distribution in the pancreas, with PP cell-rich islets being occasionally present in the posterior lobe of the pancreatic head. Hence, caution should be exercised when evaluating hyperplastic changes of PP cells (Mukai, 1983).

Although the presence of gastrin in D-cells has been disputed, recent studies indicate that gastrin is not present in normal adult islets (Dayal and O'Brian, 1981). VIP has been localized in human islets but the exact cellular origin has not been fully understood (Said, 1980).

In the gastroduodenal segment gastrin has been immunolocalized to the G cells of the gastric antrum, whilst somatostatin has been found in endocrine cells and nerves of the intestinal wall digestive mucosa.

Applications

Endocrine tumors of the gastrointestinal tract and pancreas may demonstrate a wide variety of histomorphological patterns:

solid (nodular solid nests with peripheral invading cords)

solid and glandular (with focal glandular formation)

gyriform (trabecular or ribbon-like structures forming an anastomosing pattern)

glandular (tubular or acinar structures) (Mukai et al, 1982).

With the availability of antibodies to the secretory products, specific designation of these neoplasms has led to terms such as insulinoma, glucagonoma, gastrinoma, somatostatinoma and VIPoma. However, small tumors found incidentally at autopsy may be clinically silent and do not necessarily cause clinical symptoms. Further, many pancreatic endocrine tumors are multihormonal (Mukai et al, 1982). Hence, Rosai prefers the designation `pancreatic endocrine tumor' followed by the description of the hormone(s) demonstrated in situ (e.g. insulin-producing) whenever this can be ascertained. Pancreatic endocrine tumors, which do not cause clinically apparent endocrine syndromes, are usually labeled as non-functioning tumors. However, with the acceptance of hormone production as a sign of function, the number of non-functioning tumors decrease with application of immunohistochemical staining procedures using antibodies to specific hormones.

In general, most insulin-producing tumors associated with hypoglycemia are benign. Conversely, endocrinologically active gastrin-producing tumors, glucagon producing tumors, VIP-producing tumors and somatostatin-producing tumors are often malignant. However, there are no definite morphologic criteria to predict hormonal activity or behavior. Metastases are the only sign of malignancy.

Therefore, all pancreatic endocrine tumors should be regarded as potentially malignant, even though metastases may not be apparent at the time of initial surgery (Mukai, 1983).

The common clinical syndromes and their causative hormones are as follows:

hypoglycemia (insulin)

Zollinger-Ellison syndrome (gastrin).

glucagonoma syndrome (glucagon).

WDHA syndrome (Verner-Morrison syndrome) (vasoactive intestinal pancreatic polypeptide)

somatostatinoma syndrome (somatostatin).

The first two syndromes are relatively frequent but the remaining three are either infrequent or rare (Larsson, 1978). Occasionally, pancreatic endocrine tumors fail to demonstrate

immunoreaction in the presence of clinical syndromes. Explanations for this aberrant phenomenon include abnormal peptides (although biologically active) which may not react with specific antihormone antibodies, fixation artifact or alternatively rapid turnover in tumor cells resulting in only minute amounts being stored (Mukai et al, 1982).

Tumors from some patients with WDHA syndrome have been found to secrete PP (Lundqvist et al, 1978). PP also appears to be the most commonly found in hormone silent/non-functioning tumors (Mukai et al, 1982). Whilst the physiologic function of PP is not yet fully understood, PP cells are nevertheless a component often demonstrated in multihormonal tumors (Larsson, 1978). The frequency of multihormone production by islet cells tumors has been stated as being as high as 50% (Owyang and Go, 1980). These tumors usually cause only one clinical syndrome and a combination of syndromes is extremely rare. In fact, the predominant cell type in a tumor does not necessarily cause the corresponding syndrome (Larsson et al, 1975). Any combination of cell types is possible in pancreatic endocrine tumors, the most striking example being the high frequency of PP cells in tumors secreting VIP and causing the WDHA syndrome (Schwartz, 1979). The most likely explanation for the common presence of several cell types in pancreatic endocrine tumors is that they derive from a multipotential stem cell which may differentiate in various directions (Mukai et al, 1982).

Antibodies to pancreatic hormones may also be applied to the diagnosis of islet cell hyperplasia seen in the non-neoplastic pancreas of patients with islet cell tumors (Larsson, 1977) and primary G-cell hyperplasia (gastrin producing) in the antrum of the stomach. The latter is clinically indistinguishable from Zollinger-Ellison syndrome due to gastrinoma (Lewin et al, 1984). The demonstration of an increase in number and size of the B cell mass in the ductuloinsular complexes in neonatal hyperinsulinemic hypoglycemia is another application of pancreatic hormone immunohistochemistry (Jaffe et al, 1980).

Duodenal (periampullary) somatostatin-rich carcinoid tumors (psammomatous somatostatinoma) need to be distinguished from adenocarcinoma, because the prognosis is better in the former even though lymph node metastases may occur with carcinoids (Chetty et al, 1993). Other neuroendocrine tumors of the duodenum that require immunohistochemistry for their recognition include gastrinomas (most common), gangliocytic paraganglioma (Hamid et al, 1986),

serotonin/calcitonin/pancreatic polypeptide- producing tumors and poorly differentiated neuroendocrine carcinomas. A characteristic feature of MEN-associated gastrinoma is their frequent multicentricity (Pipeleers-Marichal et al, 1990).

Gastrointestinal carcinoid tumors have also benefited from the development of immunohistochemical technology: gastrin, VIP, PP and glucagon have been demonstrated (apart from serotonin in cases of carcinoid syndrome). In children, WDHA syndromes have been reported in association with VIP-secreting ganglioneuromas and ganglioneuroblastomas (Long et al, 1981).

Comments

Immunohistochemistry has contributed extensively to the understanding of the morphofunctional relationship of pancreatic (and related) endocrine tumors. Apart from the cellular localization of secretory products in these tumors, prediction of biological behavior has also been possible. Positive control tissues for this panel of pancreatic hormones include: normal pancreas (insulin, glucagon, somatostatin and PP), gastric antrum (gastrin) and colon (VIP).

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Parathyroid Hormone-Related Protein (PTHrP)

Sources/Clones

Calbiochem (212-10.7).

Polyclonal antibodies raised in rabbits and sheep: PTHRP (1-34), PTHRP (50-69) and PTHRP (106-141) (Danks et al, 1990); PTHRP (1-130) (Druker and Campos, Toronto, Canada).

Fixation/Preparation

Applicable to both frozen sections and formalin-fixed, paraffin-embedded tissue sections.

Background

Humoral hypercalcemia of malignancy (HHM) is a syndrome characterized by low levels of PTH, few/absent bone metastases and hypophosphatemia. Parathyroid hormone-related protein (PTHrP) has been isolated from tumors with HHM and shown to be responsible for the PTH-like effects and disruption of calcium homeostasis (Ralston et al, 1991; Roskams & Desmet, 1997) The amino acid sequence of PTHrP bears homology to PTH from amino acid 1-13, but is unique thereafter (Burtis et al, 1990). Although functioning via PTH receptor, PTHrP is the product of a separate gene located on the short arm of chromosome 12 (Suva et al, 1987). Antibody to PTHrP (Ab-1) reacts with amino acid residues 38-64 of human PTHrP and shows no crossreactivity with human parathyroid hormone.

In addition to being produced by malignant tumors, PTHrP is found in normal keratinocytes, lactating mammary tissue, placenta, parathyroid glands, the central nervous system and a number of other sites, suggesting that it may have a widespread physiologic role (Burtis et al, 1990; Kramer et al, 1991). PTHrP is thought to act in an autocrine and paracrine manner in various tissues to modulate other functions in addition to regulating calcium mobilization.

Immunostaining for PTHrP suggests that production of the peptide by stromal cells and giant cells may be involved in the formation of osteoclast-like cells in giant cell tumor of tendon sheath by acting in an autocrine/paracrine fashion (Nakashima et al, 1996).

Applications

Most squamous cell carcinomas from a variety of sites synthesize PTHrP irrespective of the calcium status of the patient (Lloyd, 1994). Using a polyclonal antibody to PTHrP (1-130), 93% of 40 invasive squamous cell carcinomas were found to be immunopositive (Liapis et al, 1993). Interestingly, the strongest immunoreactivity for PTHrP in the squamous carcinomas was in areas of invasion and with desmoplasia. Adenocarcinomas (smaller percentage than squamous cancers) of breast, lung and kidney, hepatocellular carcinoma, mesothelioma, neuroendocrine tumors and T-cell leukemias are other neoplasms that may express PTHrP (Lloyd, 1994). A recent study demonstrated the presence of PTHrP and its receptor in normal breast epithelium and breast carcinomas, demonstrating that most breast tumors are able to respond to PTHrP (Downey et al, 1997).

A recent study has shown cholangiocarcinomas to be immunopositive for PTHrP (and chromogranin A), whilst all hepatocellular carcinomas were negative (Roskams et al, 1993). Mixed primary liver tumors contained PTHrP immunoreactivity only in areas of cholangiocellular differentiation. Moreover, all metastatic adenocarcinomas (especially from GIT) were negative except for 2/5 metastatic breast carcinomas.

Using polyclonal antibodies

against synthetic PTHrP peptides, immunopositivity was demonstrated in primary parathyroid adenomata and hyperplastic glands from patients with chronic renal failure, whilst primary hyperplastic glands were negative (Danks et al, 1990).

Comments

The frequency of expression of PTHrP is so great and widespread that it may be useful as a tumor marker in the histological diagnosis of certain cancers, e.g. squamous cell carcinoma of the lung. Furthermore, the role of PTHrP in distinguishing between primary hepatocellular carcinoma and cholangiocarcinoma in the liver appears to be fairly reliable. Reactive bile ductules or squamous epithelium of epidermis are recommended control tissues.

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Suva LJ, Winslow GA, Wettenhall REH et al 1987. A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression. Science 237: 893-896.

Parathyroid Hormone

Sources/Clones

Biodesign (polyclonal), Biogenesis (Bo, polyclonal), Biogenex (polyclonal), Dako (3B3) and Fitzgerald (polyclonal).

Fixation/Preparation

This antibody is applicable to formalin-fixed, paraffin-embedded tissue, frozen sections and cytologic preparations. Although not always required, enzyme pretreatment before immunodetection may improve results on paraffin-embedded tissue.

Background

The parathormone gene, closely linked to that ofβ-globin, is located on the short arm of chromosome 11 in humans (as are the genes for calcitonin and insulin). The initial form in which parathormone is synthesized within the cell is a single-chain polypeptide of 115 amino acid residues, preproparathyroid hormone. This is cleaved within the cell to form a proparathyroid hormone, from which a further six amino acids are split, leaving the 84-amino acid chain of parathormone (Habener et al, 1984). The rate of parathormone secretion is directly responsive to the level of calcium in the serum, and indeed the cytoplasm, of parathyroid cells, as has been shown by studies both in vivo and in vitro. (Brown 1982).

Applications

Surgical pathologists are familiar with the ability of parathyroid proliferations to assume a variety of histological guises increasing the difficulty of categorizing any given lesion as hyperplastic, adenomatous or carcinomatous in nature (Wick et al, 1997). This is usually resolved with the macroscopic appearance of the remaining parathyroid glands as assessed by the surgeon. The role of the surgical pathologist is to identify the lesion as parathyroidal in nature and to assess whether it is normocellular or hypercellular. Although easily accomplished in the majority of instances, rare examples of parathyroid hyperplasia/adenoma showing a follicular/trabecular arrangement may cause concern over the alternative diagnosis of a thyroid adenoma. This becomes more pertinent when the parathyroid lesion protrudes into the thyroid gland or lies within the thyroid capsule. Immunodetection for thyroglobulin and parathyroid hormone (PTH) is especially useful to resolve the problem (Permanetter et al, 1983). Nevertheless, caution should be exercised since parathyroid cells often discharge their hormonal product almost as soon as it is packaged in the cytoplasm, resulting in false-negative PTH immunostaining, although the cells are biologically synthetic (Wick et al, 1997).

PTH antibody is also useful to distinguish cell parathyroid hyperplasia/neoplasms from thyroid and metastatic neoplasms (Wick et al, 1997) although the pathologist is typically aware of the preoperative hypercalcemic status. Occasionally when this information is not supplied by the surgeon, PTH immunohistochemistry is then essential. Even more problematic are situations in which clear cell parathyroid carcinomas are non-secretory, without an abnormality in mineral metabolism (Aldinger et al, 1982). In such situations, metastatic renal cell carcinoma or metastatic clear cell carcinoma of the lung is evident, warranting PTH immunohistochemistry to arrive at the correct diagnosis (Wick et al, 1997). The other instance in which PTH antibodies are useful is in the consideration of parathyroid carcinomas located

primarily in the anterior mediastinum (intrathymically). In this situation distinction from primary thymic metastatic carcinomas, non-Hodgkin's lymphoma and germ cell tumors is necessary (Murphy et al, 1986).

Comments

The diagnosis of the majority of parathyroid proliferation may be accomplished with an adequate history, biochemistry profile and histomorphological assessment. However, rare instances in which the tumors have an abnormal location or clear cell morphology or are non-secretory may result in erroneous diagnoses, warranting PTH immunohistochemistry. Normal parathyroid glands are adequate for positive control tissue.

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P-Glycoprotein (P-170), Multidrug Resistance (MDR)

Sources/Clones

Accurate (MRPr1), Biodesign (JSB-1), Coulter (UIC1), Dako (C494, 4E3, C219), Immunotech (MRK-16, UIC2), Monosan (JSB-1, MRPm6, LRP-56), Novocastra, Oncogene, Sanbio (MRPr1), Seralab (JSB-1), Signet (C219, C494, JSB-1) and Zymed (JSB-1).

Fixation/Preparation

Most antibodies available are immunoreactive in frozen sections and some react in fixed paraffin-embedded sections, enhanced by HIER treatment.

Background

P-glycoprotein (P-170) is a transmembrane protein of 170 kD molecular weight. It has been associated with both intrinsic and acquired resistance to certain chemotherapeutic agents, particularly anthracyclines and vinca alkaloids. It is an energy-dependent pump which functions in drug efflux, reducing intracellular accumulation of chemotherapeutic agents, thus conferring the so-called multi-drug resistance (MDR) phenomenon on cells expressing increased levels of this protein (Kartner & Ling, 1989; Gottesman et al, 1991). One of the most perplexing problems encountered in chemotherapy is the resistance of certain tumors to all chemotherapeutic regimens, while other tumors which are initially chemosensitive to a particular agent show resistance to treatment over time and with disease progression. Furthermore, tumor cells which are resistant to one drug often show crossresistance to a wide variety of other, structurally unrelated drugs. For example, tumor cells resistant to adriamycin can show cross-resistance to diverse drugs to which they have never been exposed, including vinca alkaloids and mitomycin C, but not to other drugs such as alkylating agents. This is known as the MDR phenomenon (Leong & Leong, 1997). A family of so-called MDR genes encodes the P-glycoprotein, apparently with only the protein encoded by the MDR 1 gene inducing the MDR phenotype.

There is extensive evidence from in vitro studies, especially with non-human cell lines, that overexpression of P-glycoprotein results in reduced accumulation of drug within the cell. Recently, mice have been generated with knockout of MDR 1 and these animals show abnormalities of transport at the blood-brain barrier and are more sensitive to drugs.

Applications

Molecular and immunohistochemical studies of P-glycoprotein reveal that it is overexpressed in a number of intrinsically resistant tumors such as carcinomas of the liver, pancreas, colon, adrenal cortex and kidney, and appears to vary according to the differentiation of the cells (Cordon-Cardo et al 1990; Lopes et al 1997). Interestingly, in these cases, high levels of the protein have also been demonstrated in the normal tissues from which the tumors are derived. The physiologic function of P-glycoprotein can be deduced from its normal tissue distribution in that high levels of expression are seen in endothelial cells of the blood-brain barrier and in renal proximal tubules, both cell types having the primary function of moving toxic molecules across cell membranes (Schinkel et al, 1994).

Tumors responsive to chemotherapy generally show low levels of P-glycoprotein expression and solid tumors that are most responsive to systemic chemotherapy, such as seminomas and embryonal carcinomas, rarely display detectable levels of the

protein. Tumors from patients previously treated with chemotherapy show frequent elevation of P-glycoprotein, suggesting that the MDR phenotype is induced by exposure to chemotherapy. The detection of elevated levels of P-glycoprotein expression has the potential to identify tumors likely to be resistant to conventional chemotherapy and may provide a rationale for the use of alternative treatments for such patients. Immunohistological evaluation appears to be the method of choice for the assessment of P-glycoprotein, largely because it allows morphological correlation and discrimination from that in non-tumor cells (Ramani & Dewchand, 1995).

Comments

Only two MDR genes are known to be present in man, namely MDR 1 and MDR 3, but only the MDR 1 gene product confers the MDR phenotype. One of the most widely used antibodies to P-glycoprotein is clone C219 which reacts with both the MDR 1 and MDR 3 gene products. Several other antibodies specific to the MDR 1 gene product have now been described. They include HYB-24, HYB-612, and C494. While earlier studies were conducted on frozen sections, HIER has improved the immunoreactivity in fixed paraffin-embedded sections. Renal proximal tubules are used as the standard positive control because of the high levels of expression of P-glycoprotein in the epithelial cells.

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Pituitary Hormones (ACTH, FSH, HGH, LH, PRL, TSH)

Sources/Clones

Antiadrenocorticotropin (ACTH)

American Qualex, Axcel/Accurate (polyclonal), Biodesign (polyclonal), Biogenesis (polyclonal), Biogenex (polyclonal), Caltag Laboratories (polyclonal), Chemicon (polyclonal), Dako (02A3, polyclonal), Fitzgerald (polyclonal), Milab (polyclonal), Novocastra, Sanbio/Monosan (polyclonal), Seralab, Serotec (A1H5, A5B12), Sigma (polyclonal) and Zymed (polyclonal).

Antifollicle-Stimulating Hormone (FSH)

Axcel/Accurate (polyclonal), Biodesign (301, 1801, 29; 701, 702, 706, 709, S1, polyclonal), Biogenesis (754, 143, BIO-FSHb-00, polyclonal), Biogenex (78/74 1F11, polyclonal), Dako (polyclonal) and Fitzgerald (polyclonal, M27301, M210201, M26092, M94166, M94163, M94164).

Antihuman Growth Hormone (HGH)

Accurate (12), Advanced Immunochemical (GH29), Biodesign (901, 902, polyclonal), Biogenesis (2F10, Rt, polyclonal), Biogenex (54/9 2A2, polyclonal), Dako (polyclonal), Fitzgerald (M94168, M94169, M32222, polyclonal), Novocastra (polyclonal), Seralab (polyclonal) Serotec (B008, E1, G1), Sigma (GHC2) and Zymed (ZMGH2, polyclonal).

Antihuman Luteinizing Hormone (LH)

American Research Products (1561-18), Axcel/Accurate (polyclonal), Biodesign (2004, 6101, 6102, 6103, [6206, 6207, 62], polyclonal), Biogenesis (1C10, 3D7, 4E3, G11, polyclonal), Biogenex (3LH 5B6 YH4, polyclonal), Cymbus Bioscience (6101), Dako (polyclonal), Fitzgerald (polyclonal), Serotec (INNbLH1) and Zymed (ZMLH2, ZSL11).

Antiprolactin (PRL)

Axcel/Accurate (polyclonal), Biodesign (164.22.12, [6201-6204,62], [ME.121, ME.1], S2, [2605, 2606]), Biogenesis (1D5, 626/02, 633/1, polyclonal), Dako (polyclonal), Fitzgerald (M94192, M94193, M94194, M31031, M31032, M31033, M310110, M310111, M310112, polyclonal), Immunotech (164.22.16) and Zymed (ZMPL1).

Antihuman Thyroid-Stimulating Hormone (TSH)

American Research Products (25TH7G12), Axcel/Accurate (polyclonal), Biodesign (9001-90010), Biogenesis (TSH-03, polyclonal), Biogenex (5404, polyclonal), Dako (polyclonal), Fitzgerald (polyclonal), Novocastra (QB2.6, polyclonal), Seralab (JOS2.2, polyclonal) and Zymed (ZMTS2, ZMTS4).

Fixation/Preparation

All the antibodies against the pituitary hormones are applicable to formalin-fixed, paraffin-embedded sections. Although not essential, enzyme antigen retrieval pretreatment with Target Unmasking Fluid (TUF, Signet) or trypsin may improve immunoreaction on paraffin-embedded and frozen sections.

Background

In all instances antibodies against the pituitary hormones were raised using purified extract from human pituitary glands as immunogen. The adenohypophysis comprises approximately 75% of the normal

pituitary gland. It consists of the pars distalis, pars intermedia and pars tuberalis. The

pars distalis is roughly divided into a midline zone (PAS-positive mucosubstance containing ACTH [15-20%], FSH/LH [10%] and TSH [5%] cells) and two lateral portions that stain positively with acidic dyes (PRL 15-20% and GH 50%). It should be noted that cells are not strictly limited in their geographic distribution. Trichromic stains such as the PAS-orange G method serve to highlight the PAS-positive basophils and the orange G-positive acidophils. Since this reactivity correlates only crudely with hormonal function, it is therefore necessary to resort to immunohistochemical characterization for proper identification. The cells are arranged in cords and are encircled by well-formed basement membrane. These cells lie in immediate proximity to a capillary to facilitate the secretory process. The general, histochemical and immunohistochemical characteristics of normal adenohypophyseal cells are summarized in Table 1 (for review, see Scheithauer, 1984).

Applications

The major role of immunohistochemistry utilizing antibodies to pituitary hormones is that it serves as the primary basis of adenoma classification. A study comprising a surgical series (Robert, 1979) showed 80% of pituitary adenomas to be functional whilst a combined surgical/autopsy series found only 50% to be hormonally functional (Earle and Dillard, 1973). In adults, adenomas may present with hyperfunction (amenorrhea-galactorrhea, Cushing's disease, Nelson's syndrome, and acromegaly or gigantism), hypofunction (insufficiency of gonadal, thyroidal or adrenal function) or with compressive signs (visual disturbance, headache, or raised intracranial pressure) (Scheithauer, 1984). Aggression of pituitary adenomas is based on the radiological assessment: grade I, microadenomas (<10 mm); grade 2, intrasellar adenoma; grade 3, diffuse adenomas with erosion of sellar floor, and grade 4, invasive adenomas with widespread sellar erosion and destruction (Hardy and Vezina, 1976).

The conventional tinctorial classification of adenomas, based on affinity of tumor cells for acid or basic dyes correlated crudely with the functional characteristics. Acidophil adenomas were presumed to produce growth hormone, whilst basophilic adenomas were considered synonymous with ACTH secretion and Cushing's disease. Chromophobe adenomas, in contrast, were considered non-functioning, with symptoms being attributed to local destructive or compressive effects (Scheithauer, 1984). Hence, with the advent of commercially available specific antisera to pituitary hormones, a functional classification has emerged (Table 2).

Comments

Histopathology laboratories servicing neurosurgical units need to provide a comprehensive functional characterization of pituitary adenomas. The use of the normal pituitary gland will suffice as a positive control for these six hormones.

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Table 1 Normal cellular composition of the pituitary gland: morphological, functional and immunohistochemical characteristics (modified from Scheithauer, 1984)

Cell	Product	Location/percentage	Immunohistochemistry

Somatotroph	GH 21 kD polypeptide	Lateral 50%	GH
Lactotroph	PRL 23.5 kD polypeptide	Posterolateral 15-20%	PRL
Corticotroph	ACTH 4.5 kD polypeptide	Midline 15-20%	ACTH
Gonadotroph	FSH 35 kD and LH 28.2 kD glycoproteins	Generalized 10%	FSH and LH
Thyrotroph	TSH 28 kD glycoprotein	Anterior midline 5%	TSH

Table 2 Functional classification of pituitary hormones (modified from Scheithauer, 1984)

Adenoma type	**H & E**	**PAS**	**Immunohistochemistry**
Prolactin cell	C, A	–	PRL
Growth hormone cell	A, C	–	GH
Mixed GH cell and PRL cell	A, C	–	GH and PRL
Mammosomatotroph	A	–	GH, strong + PRL, weak +
Acidophil stem cell	C	–	PRL +, GH (variable)
Corticotroph cell	B, C	+	ACTH
Gonadotroph cell	B, C	+	Both or either FSH, LH
Thyrotroph cell	B, C	+	TSH
Null cell	C	–	None
Null cell, oncocytic	A	–	None

Placental Alkaline Phosphatase (PLAP)

Sources/Clones

Accurate (8B6, polyclonal), American Research (polyclonal), Biogenesis (PLAP001, polyclonal), Biogenex (polyclonal), Dako (8B6, 8A9, polyclonal), Novocastra (polyclonal), Sanbio (MIG-P), Sigma (8B6) and Zymed (polyclonal).

Fixation/Preparation

The antigen is resistant to fixation and both polyclonal and monoclonal antibodies are immunoreactive in fixed paraffin-embedded sections. HIER enhances staining.

Background

The alkaline phosphatases (AP) are a heterogenous group of glycoproteins, which are usually confined, to the cell surface (Stolbach et al, 1969). The isoenzymes differ in terms of their biochemical properties, anatomical sites of production and reactivity with different antibodies. APs probably have a role in cellular transport, regulation of metabolism, gene transcription and cellular differentiation. At least three genes encode the human AP isoenzymes, one for tissue-nonspecific AP present in the liver, bone and kidney, one for the synthesis of intestinal AP and one or more genes for the placental isoenzyme (PLAP). The different isoenzymes differ in molecular weight and amino acid composition and have different properties. The tissue-nonspecific and intestinal variants are heat sensitive whereas the PLAP isoenzymes are heat resistant. PLAP occurs only in higher primates and displays a high degree of genetic polymorphism. It is a dimer of 65 kD subunits and is synthesized during the G1 phase of the cell cycle. The enzyme is produced by trophoblasts and is responsible for the hyperphosphatemia observed during pregnancy. Biochemically, immunologically and electrophoretically, PLAP can be separated into three distinct subtypes (Fishman, 1995). The phase 1 isoenzyme corresponds to that produced by 6-8 week trophoblasts, the second is a mixture of the early phase and term placental isoenzymes and the phase 3 corresponds to the 13 weeks-term gestation AP isoenzymes. PLAP-like reactivity has been reported in the serum of about 5% of patients with tumors that included carcinoma of the lung, ovary, breast, colon and endometrium, as well as malignant lymphoma and multiple myeloma. Raised levels of serum PLAP were found in 25% of patients with seminoma. Several isoenzymes of AP have been specifically named. The Regan isoenzyme was named after a patient with lung cancer whose serum had the phase 3-type isoenzyme. It was also found in 4-14% of patients with a variety of neoplasms including testicular germ cell tumors and carcinomas of the breast, ovary, lung, stomach and pancreas as well as in the serum of patients with ulcerative colitis, familial polyposis and cirrhosis of the liver. The Nagao isoenzyme was named after a patient with pleural carcinomatosis and bears some similarities to the phase 3 PLAP. The Nagao AP has been found in the serum and tumor cells of patients with adenocarcinoma of the bile ducts and pancreas. The Kashahara variant was detected in tumor extracts of hepatocellular carcinoma and possesses some of the properties of the placental isoenzyme. Other non-Regan isoenzymes have been described in patients with gastrointestinal cancer, benign gynecological disease and female genital cancer, testicular teratomas and lung tumors.

Applications

Antibodies to PLAP are primarily used as a diagnostic discriminator

of germ-cell tumors in the context of separation anaplastic tumors (Appendices 1.5, 1.31). Membrane-based PLAP has been documented immunohistochemically in seminoma, embryonal carcinoma, gonadoblastoma, endodermal sinus tumor and choriocarcinoma (Manivel et al, 1987) and metastatic deposits of seminoma (Koshida et al, 1996).

Spermatocytic seminoma and immature teratomas were negative. Epithelial neoplasms of the ovary (Nakopoulou et al, 1995) and intratubular neoplastic germ cells also labeled for PLAP. It has been suggested that PLAP immunostaining may help separate partial and complete hydatidiform moles and choriocarcinoma. Partial moles show weak hCG and strong PLAP, complete moles show strong expression of hCG and weak PLAP, whereas choriocarcinoma displays strong expression of hCG and weak PLAP and human placental lactogen (hPL) (Losch & Kainz, 1996). PLAP has also been observed in cell lines from human bladder cancer and in somatic tumors such as tumors of the female genital tract, intestine, lung and less frequently in breast and renal carcinomas (Wick et al, 1987). Epithelial membrane antigen (EMA) is said to help the distinction of germ cell tumors from these somatic tumors which also express EMA, whereas the former do not.

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Pneumocystis Carinii

Sources/Clones

Accurate (3F6), Axcel/Accurate (3F6), Biodesign (092, 093), Biogenesis (0G1/1), Biogenex (3F6), Chemicon and Dako (3F6).

Fixation/Preparation

The Dako antibody reacts with an antigenic epitope of human *Pneumocystis carinii*, which is resistant to fixation in formalin and picric acid, paraffin embedding and extraction with ethanol and xylene. This antibody may also be used to detect *P. carinii* in smears prepared from bronchoalveolar lavage fluid and sputum samples (Elvin et al, 1988). However, enzymatic digestion of smears (e.g. trypsin) must be performed before staining.

Background

The Dako antibody (IgM, k) reacts with an 82 kD parasite-specific component of human *Pneumocystis carinii* (Linder et al, 1987). No crossreactivity was found with a number of parasites and fungi (Elvin et al, 1988).

Applications

The explosion in the AIDS epidemic brought about an increased need for specific markers that recognize *P. carinii*. Newly developed antibodies mark cyst wall and/or trophozoites (Linder and Radio, 1989). While the sensitivity of the immunocytochemical method appears to be greater than the Giemsa stain, it is only slightly better than the GMS stain, warranting the use of immunostaining in sputum, where identification of the pathogen is more difficult than in bronchoalveolar lavage (Linder and Radio, 1989). The other advantage of immunostaining is that only the cyst wall is detected with the silver stain, whilst immunohistochemistry stains both cyst wall and trophozoites (Amin et al, 1992). However, the latter staining pattern may appear amorphous or focally granular, which may be confused with non-specific staining of mucin or intracellular/free particulate material (Amin et al, 1992). The 3F6 monoclonal antibody has been found to be consistently more sensitive at detecting cysts of *Pneumocystis* in both sputum and bronchoalveolar lavage specimens (Elvin et al, 1988; Wazir et al, 1994b).

Comments

Immunohistochemistry for *P. carinii* is a useful adjunct to traditional Giemsa and silver stains, particularly in cytopathology laboratories examining a large number of respiratory specimens from HIV-positive patients.

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Pregnancy-Specific β-1-Glycoprotein (SP1)

Sources/Clones

Axcel/Accurate, Biodesign (BD4D8), Biogenesis (polyclonal), Biogenex (4E4, polyclonal), Chemicon (polyclonal), Dako (polyclonal), Fitzgerald (M32236), Research Diagnostics (BB4E4) and Zymed (polyclonal).

Fixation/Preparation

The antigen is fixation resistant and immunoreactivity may be improved with proteolytic digestion or HIER.

Background

Pregnancy specific β-1-glycoprotein (SP1), together with human chorionic gonadotropin (hCG) and placental alkaline phosphatase (PLAP), are three major proteins produced by the trophoblast of the human placenta. Immunohistochemical studies suggest that SP1 and hCG are also present in the human amnion. Recent molecular cloning studies indicate that the human SP1s form a group of closely related placental proteins that, together with the carcinoembryonic antigen family members, comprise a subfamily within the immunoglobulin superfamily. The main source of SP1 is the syncytiotrophoblast but it has been demonstrated that amniotic as well as chorionic membranes express low levels of SP1 genes, although only certain subpopulations of SP1 transcripts were expressed, with differences in species expression between amnion, chorion and trophoblasts (Plouzek et al, 1993).

Applications

The immunohistochemical applications of SP1 have been mainly in the study of placental elements and their corresponding tumors. Differing levels of expression of hCG, human placental lactogen (hPL) and SP1 were observed in the fetomaternal tissues throughout pregnancy. hCG was strongly localized in the cytoplasm of the syncytiotrophoblast in the 12-day blastocysts, remaining strong until 8-10 weeks before decreasing and becoming almost negative at term. hCG showed variable staining in the implantation site. hPL and SP1 appeared later than hCG in the syncytiotrophoblast, increasingly rapidly by week 8 and remaining strong until term (Sabet et al, 1989). Immunolocalization studies of SP1 in syncytiotrophoblasts suggest a secretory pathway including synthesis in the endoplasmic reticulum, processing by the Golgi and exocytic release into maternal blood in the intervillous space (Schlafke et al, 1992).

The presence of SP1, vimentin, cytokeratin and PLAP, particularly the first three antigens, has been used to identify intermediate trophoblasts in the placental site nodule (Shibata & Rutgers, 1994).

Comments

SP1 is not specific to placental cells. It is expressed in a variety of non-placental tumors. In an immunoelectron microscopic study of colonic carcinomas, SP1 and carcinoembryonic antigen were found in all seven tumors studied, none of the tumors showing morphologic evidence of choriocarcinoma. PLAP and hCG were found in two tumors (Haynes et al, 1985). In urothelial tumors, immunoreactive SP1 were observed in five of 47 high-grade tumors. HCG and hPL were found in nine and seven cases respectively. These findings suggested that morphologic and functional trophoblastic differentiation evolved from transitional cell carcinoma (Campo et al, 1989). Earlier studies suggested that SP1 expression was a poor prognostic

factor in breast carcinoma but this has not been substantiated (Wright et al, 1987). SP1 has been employed in the panel for the distinction of mesothelioma from adenocarcinoma, being positive in almost 60% of adenocarcinomas. However, SP1 is also expressed in mesotheliomas, albeit in lower frequency (6%) (Pfaltz et al, 1987).

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Progesterone Receptor (PR)

Sources/Clones

Abbott (PgR-ICA), Becton Dickinson (PR33, PR4-12), Biogenesis (1A6), Biogenex (PgR-1A), Dako (1A6, polyclonal), Immunotech (PR10A9), Labvision Corp (HPRA2-7), Novocastra (1A6), Oncor (4.12) and Zymed (1A6).

Fixation/Preparation

Most antibody clones currently available are immunoreactive in routinely fixed, paraffin-embedded tissues and enhanced after HIER. Enzymatic predigestion is not required (Leong & Milios, 1993).

Background

In selected target tissues, estrogens have been found to stimulate not only mitogenesis but also the synthesis of specific proteins. One of these estrogen-induced proteins is the progesterone receptor (PR). Progesterone and synthetic progestins activate the receptor, provoke its phosphorylation and DNA-binding ability and induce its regulatory activities. Since the PR is an estrogen-inducible protein, its expression is indicative of an intact estrogen receptor pathway and may identify tumors that are hormonally responsive to estrogen, thereby improving the overall predictive value of steroid receptor assays in selected tumors such as breast carcinoma (Guichon-Mantel et al, 1996).

The PR displays the typical three-domain structure of the steroid-thyroid receptor family. The central domain contains two "zinc finger" structures responsible for the specific recognition of the cognate DNA sequences. The carboxyl-terminal domain contains the hormone and antihormone binding sites. The complete organization of the human PR gene has been determined. It spans over 90 kb and contains eight exons. The first exon encodes the N-terminal part of the receptor, the DNA-binding domain is encoded by two exons, each corresponding to one zinc finger, and the steroid-binding domain is encoded by five exons.

The signal responsible for the nuclear localization of the PR is a complex one. The receptor continuously shuttles between the nucleus and cytoplasm. The receptor diffuses into the cytoplasm and is constantly and actively transported back into the nucleus, similar to the phenomenon for estradiol and glucocorticosteroid receptors. Immunolocalization of PR is confined to the nucleus.

Applications

The value of estrogen and progesterone receptor assays in predicting response to hormonal treatment in advanced breast cancer patients has been well supported by both studies employing cytosol-based ligandbinding methods and immunohistochemical assays, the prognostic utility being strongest in premenopausal women. Approximately 50% of breast cancers are ER+ PR+, 20% ER+ PR-, 5% ER- PR+, and 25% ER- PR-.

Those women whose cancers express both ER and PR show the greatest likelihood of responding to endocrine treatment. Using conventional biochemical assays, the response rate is about 77% for ER+ PR+ tumors, 46% for ER- PR+, 27% for ER+ PR- and 11% for ER- PR- tumors. However, it is clinically recognized that a small proportion of women with tumors which are receptor negative will show a positive response to hormonal therapy and as many as one third of those with receptor-positive tumors may fail to respond to such treatment. The significance of breast carcinomas biochemically negative for estrogen receptor (ER), but positive for PR, is poorly

understood. It has been proposed that these tumors, more common in younger women, contain ER whose presence is masked in a biochemical binding assay by endogenous estrogen. Such tumors should be positive for ER by immunocytochemical assay but this was not proven in one study, which found that ER-PR+ tended to have larger tumor size and higher histologic grade and Sphase fractions compared to ER + PR + tumors. It was concluded that biochemically ER- PR + breast carcinomas are biologically different from ER + PR + tumors (Keshgegian, 1994).

There has been some suggestion that PR may be a more important predictor as there are more responders among patients with ER-PR+ compared to ER+PR- tumors. In some series, although this remains to be proven, the prognostic advantage of steroid receptor positivity was lost after 4-5 years of follow up (Lipponen et al, 1992). As with the estrogen receptor, there is increasing evidence that immunohistological assays provide more accurate prognostication than cytosol-based methods (Mohammed et al, 1986; Pertschuk et al, 1996).

Comments

We employ PgR-ICA which is enhanced following HIER. Immunostaining is further enhanced following HIER in TRS (Dako) as compared to citrate buffer (Leong et al, 1996; MacGrogan et al, 1996). Consistent immunostaining is obtained in cytological preparations that have been fixed in 10% formalin following complete air drying. HIER should be used (Suthipintawong et al, 1997).

Problems associated with reporting of results and the relevance of objective assessment of immunostains with image analysis equipment are discussed under "estrogen receptor".

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Cytopathology 17: 127-133.

Proliferating Cell Nuclear Antigen (PCNA)

Sources/Clones

Biodesign (PC10), Biogenesis (PC10), Biogenex (19A2), Boehringer Mannheim (PC10), Camon (19A2), Chemicon, Coulter (19A2), Dako (PC10), Diagnostic Biosystems (PC10), Medac (PC10), Oncogene (PC10, 19F4) 19A2), Serotec (19.A2), Signet (PC10) and Zymed (ZO49).

Fixation/Preparation

Both the main clones, PC10 and 19A2, to proliferating cell nuclear antigen (PCNA) are immunoreactive in fixed embedded tissues. However, the antigen is fixation dependent and HIER produces significant enhancement of immunostaining. HIER in 1% zinc sulfate is reported to produce the best staining (Shin et al, 1994), although we have found citrate buffer at pH 6.0 to be sufficiently effective.

Background

PCNA, formerly called cyclin (now recognized to be a much wider class of proteins ssociated with cell proliferation), represents a component of DNA polymeraseβ (Bravo & Macdonald-Bravo, 1987) and is a 36 kD intranuclear proliferation-associated antigen. An antibody to this antigen was first described in the blood of selected patients with systemic lupus erythematosus. This polypeptide has since been found in both normal and transformed cells and is tightly associated to the sites of DNA replication. Its expression is highest during S-phase of the cell cycle and there is generally a good correlation between expression of PCNA and the S-phase fraction determined by flow cytometry in a given tumor cell population.

However, certain caveats apply to the use of anti-PCNA antibodies as markers of cell proliferation. In malignant cell lines such as HeLa, PCNA levels increase during S-phase but are not zero during the other phases of the cell cycle. Indeed, in this cell line the levels of PCNA increase only by a factor of 2-3 during S-phase (Morris & Matthews, 1989). There are also at least two forms of PCNA, one associated with the "replicon" structure and the other more loosely associated in the cell nucleus. Both proteins are retained by formalin fixation but only the former is retained by alcoholic fixatives such as methacarn (Bravo & MacDonald Bravo 1987). Furthermore, the antigen persists in cells that are no longer in the cycling phase and are in G_0. Generally PCNA counts obtained with clone PC10 have been higher than those obtained with Ki-67 (Leong et al, 1995) or S-phase fraction measured by flow cytometry, despite PCNA being considered to be primarily an S-phase-associated protein. PCNA has a relatively long half-life of 20 h and may be immunohistochemically detected in cells that have recently left the cell cycle and may be in G_0. Discrepancies have also been demonstrated between PCNA counts obtained with PC10 and that of S-phase fraction by thymidine and bromodeoxyuridine uptake in a variety of tumors and in the experimental situation (Yu et al, 1991; Scott et al 1991). The PCNA index was found to be 2-3 times that of values obtained with DNA polymerase-α (Kawakita et al, 1992). There is ample evidence that the antigen is very fixation dependent and different antibody clones show vastly different sensitivities for the antigen (Leong et al, 1993; Coltrera et al, 1993).

Applications

PCNA immunostaining offers an alternative to the well-established but cumbersome methods of

assessing tumor growth fractions, namely tritiated thymidine or bromodeoxyuridine incorporation, or flow cytometry and has been enthusiastically employed in numerous publications, despite the limitations discussed above.

Comments

PC10 is the most sensitive of the antibody clones available but bearing in mind the fixation dependency of PCNA, comparisons between laboratories are invalid unless fixation protocols are standardized. Furthermore, because of the long half-life of the antigen, only strongly stained cells should be counted and weakly stained cells show poor correlation with the S-phase fraction (Yu et al, 1995). Methanol is the fixative of choice (Burford-Mason et al, 1994).

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Prostate-Specific Antigen (PSA)

Sources/Clones

Accurate (ER-PR8), Biodesign (8), Biogenesis (PSA-001, 07), Biogenex (8), Dako (ER-PR8, polyclonal), Enzo, Hybritech, Immunotech, Oncogene (OS94.3), Oxoid (PSB535), Sanbio (8), Serotec (SC.5) and Zymed (2009).

Fixation/Preparation

The antigen is resistant to formalin fixation and immunostaining is enhanced by heat-induced epitope retrieval.

Background

Prostate-specific antigen (PSA) is a chymotrypsin-like, 33 kD singlechain glycoprotein with selective serine protease activity for cleaving specific peptides. The PSA gene is a member of the human kallikrein gene family and is located on the 13q region of chromosome 19. PSA is selectively produced by the epithelial cells of the acini and ducts of the prostatic gland and is secreted into the semen where it is directly involved in the liquefaction of the seminal coagulum that is formed at ejaculation. The sequence of PSA shows extensive homology withγ-nerve growth factor (56%), epidermal growth factor-binding protein (53%) andα-nerve growth factor (51%). This feature, together with its ability to digest insulin growth factor-binding protein-III (IGFBP-3) to release biologically active IGF-I, makes PSA a candidate growth factor or a cytokine or modulator of cell growth. PSA has also recently been suggested to be capable of being produced by cells bearing steroid hormone receptors under conditions of steroid hormone stimulation (Diamandis & Yu, 1995).

Applications

PSA is a useful biochemical marker as any disruption of the normal architecture of the prostate allows diffusion of PSA into the stroma where it gains access to the peripheral blood through the microvasculature. Elevated serum PSA levels are thus seen with prostatitis, infarcts, benign hyperplasia and transiently after manipulation and biopsy. Most importantly, significant elevations are seen with prostatic adenocarcinoma, making it an important tool for diagnosis as well as monitoring response to treatment. Although cancers produce less PSA per cell than normal prostatic epithelium, the greater number of malignant cells and the disruption of stroma in the malignant gland accounts for the elevated serum PSA levels.

Immunostaining for PSA has proven to be an effective method of identifying cells of prostatic origin, but the presence of PSA cannot be used to differentiate between benign and malignant. Antibodies to PSA show high sensitivity although very occasionally carcinomas have been reported to be negative for PSA. Correlations of PSA tissue reactivity with Gleason's grade of prostatic cancer have shown that high-grade tumors may be entirely negative by immunolabeling. There was an initial suggestion that the presence of PSA-negative cells in a prostatic carcinoma correlates with a more aggressive clinical course but this has not been confirmed and most tumors display very heterogeneous staining (Bostwick, 1994).

Comments

As the occasional case of prostatic carcinoma and metastatic deposit may show only weak or no staining for PSA, it is best to use this marker in conjunction with other markers of prostatic tissue such as prostatic acid phosphatase and CD 57 (Leu7). A combination

of these three markers gives the highest diagnostic yield (Appendix 1.14). Furthermore, immunoreactivity to PSA has been shown in a variety of extraprostatic tissues including the epithelium of the urethra, periurethral glands of both males and females, urachal remnants, endometrium (Clements & Mukhtar, 1994), transitional epithelium of the bladder and in cystitis cystica and glandularis, anal mucosa and anal glands (Stein et al, 1982), ductal cells of the normal pancreas and normal salivary glands. PSA immunoreactivity is also seen in urethral and periurethral gland adenocarcinoma, extramammary Paget's disease of the penis and pleomorphic adenoma and carcinoma of the salivary gland (Elgamal et al, 1996). Neutrophils and some neuroendocrine tumors also stain for PSA.

Specificity is improved by using the monoclonal antibodies. We have had consistency of results with clone ER-PR8 from Dako when used with MW-induced retrieval.

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Prostatic Acid Phosphatase (PAP)

Sources/Clones

Accurate (P-29, 4LJ, SB19), AMS Biotech, Biodesign, Biogenesis (501, 503, 504), Biogenex (045), Biomeda, Caltag (SB19), Camon, Chemicon, Dako (PASE/4LJ, polyclonal), Diagnostic, Immunotech (PAP29), Milab/Med, Novocastra, Oxoid (PAY376), Sanbio (4LJ), Saxon, Seralab (8), Serotec, Sigma (PAP12, PAP29) and Zymed (ZMPAP4).

Fixation/Preparation

Both poly- and monoclonal antibodies are immunoreactive in fixed, paraffin-embedded tissues and staining is enhanced by heat-induced epitope retrieval.

Background

Acid phosphatases hydrolyze phosphoric acid esters at acid pH. They are found in a variety of tissues and differences in electrophoretic patterns or sensitivity to isoenzyme inhibitors allowed the distinction of isoforms of the enzyme to specific tissues. Normal prostatic tissue contains several isoforms but only two are secreted in the seminal fluid. Acid phosphatase activity is mainly localized to the lysosomes of prostatic epithelial cells and ultrastructurally is identified within microvilli of the apical cell membranes and in the secretory granules at the supranuclear or apical regions of benign cells. Although synthesized in rough endoplasmic reticulum, PAP is not demonstrable in this site and because it is only recognized in lysosomes it is assumed that antibodies recognize PAP only when packaged into granules. Basal cells are negative for PAP. Serum levels of the enzyme reflect the amount of enzyme released into the circulation and are dependent on the tumor mass and also the rate of synthesis and access to the intravascular space. Low levels of the enzyme have been suggested to represent low rates of synthesis by poorly differentiated tumors.

Applications

PAP immunostaining is a useful discriminator for prostatic tissue and its diagnostic specificity and sensitivity are increased when used in a panel in conjunction with PSA and CD57 (Leu 7). Like PSA, immunoreactivity for PAP is more intense and homogeneous in benign prostatic tissue than in prostatic carcinoma. PAP is localized within prostatic acini and ducts, although the latter tend to show weaker and more heterogeneous staining (Leong & Gown, 1993).

Rare cases of squamous metaplasia of the prostatic epithelium show staining for PAP. There is weak positivity in seminal vesicle epithelium and like PSA, periurethral glands in both men and women are positive for the enzyme. Other non-prostatic tissues which may show PAP immunostaining are anal glands in men, neuroendocrine cells of the rectum, transitional epithelium and Von Brun's nests of the bladder, renal tubular epithelium, pancreatic islet cells, hepatocytes, gastric parietal cells and mammary ductal epithelium. Neutrophils show the strongest concentration of PAP among non-prostatic tissues. Neoplasms that show crossreactivity are mainly those derived from the cloaca, such as urinary bladder, periurethral glands and colon and neuroendocrine tumors (Wahol & Longtime, 1985; Epstein, 1993).

Comments

In general, PAP is relatively specific for prostatic neoplasms. However, because of the crossreactivity of both PAP and prostate-specific antigen (PSA)

with the tissues listed above, it is still best to use PAP in conjunction with PSA, particularly in the context of a tumor in the perineum whose differential diagnosis includes prostatic carcinoma, transitional carcinoma and adenocarcinoma of the bladder and rectal carcinoma (Leong et al, 1996). Besides PAP and PSA, the panel should include an antibody to high molecular weight cytokeratin, CK 20 and CK 7 (Appendix 1.14).

Acid phosphatase consists of several isoenzymes and polyclonal antibodies to PAP crossreact with isoenzyme 4, which is present in small amounts in most human tissues. Furthermore, polyclonal antibodies recognize several antigenic sites and may produce weak background staining but this is not seen with monoclonal antibodies that recognize only one antigenic site. Clone PASE/4LJ from Dako produces satisfactory results.

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Protein Gene Product 9.5 (PGP 9.5)

Sources/Clones

Accurate (31A3, 13C4) and Biogenesis (31A3, 13C4).

Fixation/Preparation

Immunostaining in paraffin-embedded sections is enhanced by HIER in citrate buffer at pH 6.0.

Background

Protein gene product 9.5 (PGP 9.5) is a ubiquitin carboxyl-terminal hydrolase whose gene is mapped to chromosome 4p14, spans 10 kb and contains nine exons (Edwards et al, 1991). It displays 5' features, some common to many genes and others common to neurofilament neuron-specific enolase and Thy-1-antigen gene 5' regions (Wilkinson et al, 1989). PGP 9.5 is a 27 kD soluble protein which has been shown by immunostaining in all levels of the central and peripheral nervous system, many neuroendocrine cells, in part of the renal tubule, spermatogonia and non-pregnant corpus luteum (Wilson et al, 1988). Benign and neoplastic follicular center lymphoid cells also stain for the antigen (Langlois et al, 1995). The function of PGP9.5 is currently unknown. There is some evidence from studies in glioma cell lines that the protein is maximally expressed during the growth phase and that it may play a role in glial cells during brain development, in reactive gliosis or in tumorigenesis of the glial lineage (Giambanco et al, 1991). PGP 9.5 has been demonstrated in pituitary adenoma, medullary carcinoma of thyroid, pancreatic islet cell tumor, paraganglioma, neuroblastoma, carcinoid tumors from a variety of sites and Merkel cell carcinoma (Rode et al, 1985; Gosney et al, 1995).

Applications

PGP 9.5 is distinct from neuron-specific enolase (NSE) and is largely employed as a marker of nervous and neuroendocrine differentiation. However, it is of low specificity as shown in a study of bronchial carcinomas where, like NSE, PGP 9.5 actually labeled more cases of non-small cell tumors than small cell lesions. PGP 9.5 has the advantage of producing a more intense stain with less background compared to NSE but if used as a marker of neural and neuroendocrine differentiation, it must be employed in conjunction with chromogranin and synaptophysin which are more specific markers for this purpose. Other applications of PGP 9.5 include the study of unmyelinated nerve fibers in the skin and colonic mucosa, atrial myxomas and inclusion bodies in the central nervous system (Wilson et al, 1988; Gosney et al, 1995).

Comments

Before the advent of HIER, it was recommended that fresh tissues be fixed in a solution of 95% alcohol-5% acetic acid for 2-3 h to obtain optimal results. This is no longer necessary as HIER produces marked enhancement of immunoreactivity compared to other methods of antigen unmasking, with both increase in number of positive-staining cells and increased intensity of reaction within individual cells and their processes (McQuaid et al, 1995).

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pS2

Sources/Clones

Biogenex (PS2.1), Dako (BC04) and Labvision Corp (PS2.1, R47-94).

Fixation/Preparation

The antigen survives formalin fixation and is enhanced by HIER.

Background

pS2 is a 6660 Dalton, 60 amino acid secretory polypeptide protein that was isolated from the breast carcinoma cell line MCF-7. It belongs to a recently described family of trefoil-shaped growth factors which includes human intestinal trefoil factor (hITF) and human spasmolytic polypeptide (hSP). Although its exact function is unknown, it is believed to be part of a steroid-dependent stimulatory pathway. An estrogen-regulated protein, it has been studied as a marker of an intact estrogen pathway and hence marker hormone sensitivity and favorable prognosis in breast carcinoma. There is growing evidence that members of the trefoil peptide family are involved in active maintenance of the integrity of gastrointestinal mucosa and facilitate its repair (Poulsom, 1996; May & Westley, 1997).

Applications

pS2 positivity is preferentially expressed in hormone-dependent cells in breast cancer. Low concentrations of the protein have been associated with a poor prognosis (Foekens et al, 1990; Predine et al, 1992) while strong expression predicted responsiveness to endocrine treatment (Racca et al, 1995). The five year recurrence-free survival and overall survival were 85% and 95% respectively for estrogen receptor (ER)+/progesterone receptor (PR)+/pS2+ tumors, but only 50% and 54% for patients with ER + /PR + /pS2- tumors (Foekens et al, 1990).

In another study of 72 advanced breast cancer cases, 76% of pS2+ cases had stable disease, complete remission or partial remission as compared with 37% of the pS2- cases. The authors proposed that pS2 may help differentiate the 35-50% of ER+ breast cancer patients who do not clinically respond to hormone therapy and the rare ER - patients who do (Schwartz et al, 1991). However, further studies have found that while pS2 immunostaining correlates with age, estrogen receptor and progesterone receptor status, it is not an independent prognostic factor or an indicator of increased survival in breast cancer (Speider et al, 1994; Wysocki et al, 1994).

pS2 is widely distributed throughout the gastrointestinal tract, particularly adjacent to damaged mucosa (Collier et al, 1995; Poulsom, 1996). It is consistently expressed in superficial and foveolar epithelium of non-neoplastic gastric mucosa and in 66% of gastric carcinomas, but has little value as a prognostic indicator (Machado et al, 1996). Colorectal carcinoma stains with pS2 to a lesser extent but this too lacks statistical significance (Shousha et al, 1993).

Expression in normal pancreas is usually absent but it can be seen focally within occasional ducts in chronic pancreatitis and it is prominent in pancreatic adenocarcinoma and ampullary tumors (Collier et al, 1995).

Comments

We employ clone PS2.1.

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Rabies

Sources/Clones

Accurate (HYB-3R7), Biodesign, Biogenesis (RAB50), Chemicon International (C4-62-15-2) and Research Diagnostics (RV7C5).

Fixation/Preparation

Antirabies monoclonal antibody may be applied to acetone-fixed samples. It is also potentially applicable to formalin-fixed, paraffin-embedded tissue sections, although optimization will be necessary with some form of antigen retrieval.

Background

Rabies is a rod- or bullet-shaped virus with a single-stranded RNA genome and belongs to the family Rhabdoviridae. It is a highly fatal disease of humans and warm-blooded vertebrates, usually transmitted via infected saliva following the bite of a diseased animal, most commonly dogs. Virus introduced into the bite wound enters the peripheral nerves and following an incubation of weeks to months, spreads to the spinal cord and brain. It produces a neurological derangement, lasting a few days to weeks and resulting in death.

Antibody C4-62-15-2 to rabies virus is specific to the N-nucleoprotein. It enjoys a wide range of species reactivity and includes mouse, raccoon, skunk, dog/coyote and bats (Smith, 1989).

Applications

During prolonged incubation periods, the sensory neurons of the dorsal root ganglia may be the site of viral sequestration. Efferent spread of virus in the nervous system may extend terminally to the eye and nerve fibers surrounding hair follicles. Hence, demonstration of antigen in corneal impression smears or skin biopsies may be used for confirmation of diagnosis in a live patient. Unless the diagnosis is confirmed during life, an autopsy must be performed with 10-20 mm blocks of cerebrum, cerebellum, hippocampus, medulla, thalamus and brain stem being taken in duplicate: 50% glycerol-saline for virological examination and 10% buffered formalin for immunohistological examination.

Comments

Antibody to rabies is useful in locating the Negri bodies in sections of brain. In one study of naturally infected domestic and wild animals, rabies antigen was detected in 62% of the brain areas in which inclusion bodies were not found (Palmer et al, 1985). The antigen is not limited to the Negri bodies but also traceable in the cytoplasm (Feiden et al, 1985; Sinchaisri et al, 1992).

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Retinoblastoma Gene Protein ($P110^{RB}$, Rb protein)

Sources/Clones

Accurate (84B3-1), Biodesign (RB1, 1F8), Biogenesis (RB), Biomol Research (MAB245), Dako (Rb1), Labvision Corp (1F8), Novocastra (Rb1), Oncogene (AF11, LM95.1), Pharmingen (245), QED (3C8) and Santa Cruz (C-15)

Fixation/Preparation

The antibodies are mostly immunoreactive only in fresh-frozen sections although some antibodies stain fixed paraffin-embedded sections but only after HIER.

Background

The Rb gene is located on chromosome 13q14 and spans a region of more than 200 kb, including 27 exons. The Rb gene is the only tumor suppressor that has been shown to directly suppress tumor formation. The Rb protein has a molecular mass of 105 kD and a number of antibodies which recognize specific parts of this protein have been developed. Besides loss of function due to chromosomal abnormalities including chromosomal deletion, translocation and point mutation, as with p53, phosphorylation may inactivate the Rb protein. In addition, a variety of viral oncoproteins including simian virus 40 T antigen, E1A from adenovirus and E6 from human papilloma virus may bind and inactivate the Rb protein.

Immunostaining may be a valid way to assess the presence of normal Rb protein but several factors affecting staining should be considered before accepting the relevance of the technique. Firstly, it has been observed that the level of expression of Rb protein is not the same in all cells in any individual tissue, e.g. in the epithelium of the cervix, there are low or undetectable levels of staining in the basal layers and staining increases with cell maturation. In contrast, low or absent anti-Rb protein staining was observed in the well-differentiated epithelial cells of the gastric mucosa such as the foveolar and mucus cells compared to the cells in the crypts and neck of the glands. Astrocytes and microglia do not show detectable Rb protein by immunostaining and other subsets of normal cells such as some stromal cells do not display demonstrable Rb protein. The reasons for failure to demonstrate the protein at an equivalent level in all cells may relate to variations in expression as a function of cell cycling activity, cell differentiation and protein phosphorylation. More importantly, there is a large subset of cells, which include endothelial cells, lymphocytes and stromal cells, in which the ability to demonstrate $p110^{RB}$ expression is critically dependent on the method of staining used (Cordon-Cardo & Richon, 1994).

Applications

The p53 and retinoblastoma (Rb) gene products must be the two most-studied tumor suppressor genes. While alterations in the p53 tumor suppressor gene have been recognized as the most frequent genetic alterations in human neoplasia, the extent of Rb gene alterations is less well known. p53 alterations are mostly detected as overexpression of the protein and can easily be done with immunostaining, whereas most normal cells do not contain stainable wild-type p53 protein. In contrast, the Rb protein is detectable immunohistochemically in normal non-transformed cells, although whether this is so for all normal cells and tissues is currently unknown. As abnormality is based on the absence of stainable Rb protein, it is critical that techniques of

maximal sensitivity must be employed and internal positive controls must be present in the sections (Skelly et al, 1996).

Alterations in the RB gene have been described in a number of human tumors including retinoblastoma, osteosarcoma, other sarcomas, leukemias, lymphomas and certain carcinomas including those from the breast, prostate, lung, bladder, kidney and testis (Geradis et al, 1994). Rb gene alterations have been associated with increasing tumor grade and stage in a variety of tumors and there is increasing evidence that alterations of this gene are associated with increased risk for metastasis (Xu et al, 1991, 1993). In breast carcinoma there is some evidence of association with other signs of progression and loss of hormonal receptor expression (Drobnak et al, 1993).

Comments

It was recently demonstrated that HIER in citrate buffer at pH 6.0 with overnight antibody incubation produced maximal sensitivity when staining fixed paraffin-embedded sections. Fixation in methacarn also requires HIER treatment and the use of DNAse produced variable results. The use of low pH buffers can produce false-positive results. Thus, in the assessment of Rb protein, as with other fixation-sensitive antigens, it is clear that the findings of individual laboratories cannot be generalized owing to differences in fixation and immunolabeling techniques. However, these factors do not preclude the assessment of the Rb protein in laboratories where fixation and other variables are strictly controlled.

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S100

Sources/Clones

Accurate (polyclonal), Biodesign (polyclonal), Biogenesis (15E2E2, polyclonal), Biogenex (15E2E2, polyclonal), Chemicon (monoclonal, polyclonal), Cymbus Bioscience (MIG5), Dako (polyclonal), ICN (polyclonal), Immunotech (polyclonal), Medac (S1/61/69), Novocastra (polyclonal), Oncogene (OS94.5), RDI (MIG-5), Seralab (polyclonal), Serotec (polyclonal), Sigma (polyclonal) and Zymed (polyclonal).

Fixation/Preparation

Formalin-fixed tissues are ideally suited for S100 immunostaining and the antigen is resistant to long durations of fixation in formalin. Its reactivity can still be enhanced by heat-induced antigen retrieval but not by proteolytic digestion.

Background

S100 protein, so named because of its solubility in a saturated ammonium sulfate solution, occurs as three biochemically distinct forms. Each is a protein dimer of two subunits, designated α and β. The three dimers are S100A, (α-β), S100A (α-β), and S100B (β-β). The α and β subunits each have a molecular weight of approximately 10.5 kD with extensive amino acid sequence homology between the two subunits. They both have amino acid sequences known to code for the calcium-binding sites of the calmodulin family of proteins. S100 is highly acidic and water soluble with varying affinities for calcium, zinc and manganese. These properties are related to many basic cell functions such as cation diffusion across lipid membranes, microtubule assembly and stability, calcium and cyclic nucleotide regulation and increased activity of RNA polymerase, drug-protein interactions, the plasma membrane function of neurons and interaction with chromosomes and synaptosomes. S100 protein is conserved in nature and is present within the cells of all three germ layers in humans, a reflection of its important role in basic cell function.

Applications

S100 has been demonstrated in a wide variety of normal and abnormal tissues. Formalin fixation and paraffin embedding may alter antigenic sites and aldehyde fixation may prevent diffusion of the highly soluble antigen that can produce artefactual immunolocalization patterns. Indeed, one study has reported granular staining of virtually every cell type when fresh-frozen tissue was stained with a monoclonal S100 antibody.

Normal and neoplastic cartilaginous tissue, including benign and malignant chondroid tumors, express S100 protein and this is useful for the distinction of non-cartilaginous bone tumors which are mostly negative for the antigen. Cartilaginous tumors can be distinguished from chordomas by the presence of cytokeratin and EMA in the latter and their absence in the former. S100 is also useful for the labeling of myoepithelial cells in mammary ducts, particularly when distinguishing sclerosing adenosis from tubular carcinomas, the former displaying a distinct layer of myoepithelial cells. Sustentacular or satellite cells of the adrenal medulla and paraganglia and their corresponding tumors are labeled by S100 antibodies, as are the folliculostellate cells of the anterior pituitary (Nakajima et al, 1982; Takahashi et al, 1984; Loeffel et al, 1985).

The S100 antigen is a useful marker of peripheral nerve cells. The protein is present in the

nuclei and cytoplasm of Schwann cells and satellite cells in parasympathetic and sympathetic ganglia (Daimaru et al, 1985). The β-subunit has been reported in these cells but not in neurons, the latter contain the α-subunit that is not expressed in Schwann cells or satellite cells. Pacinian corpuscles also contain S100 protein. While S100 protein is expressed in the majority of benign nerve sheath tumors, as many as 40-50% of malignant Schwann cells do not stain. A population of S100+ Schwann cells can be demonstrated in neurofibromas but variable numbers of perineural and intermediate cells within these tumors do not stain for S100 protein. Correspondingly, neurogenic sarcomas arising in patients with neurofibromatosis show a spectrum of expression of S100 protein. Both benign and malignant granular cell tumors contain S100 protein expressed as the β-subunit, a feature used to support an origin from Schwann cells.

The other group of cells which are labeled by S100 antibodies are the histiocytes. The interdigitating reticulum cells of the paracortical areas in the lymph node are stained by S100 protein antibodies, as are dendritic reticulum cells of the lymphoid follicles. Langerhans' cells of the skin, mucous membranes and other sites are also positive for S100 protein, expressing S100B activity (β-β). As such, S100 protein is a useful marker for the identification of Langerhans' cell histiocytosis.

One of the most useful applications of the S100 protein is as a marker of nevus cells and melanomas. Virtually all benign melanocytic lesions contain S100 protein which is also observed in over 95% of malignant melanomas. When used in conjunction with a panel comprising cytokeratin, vimentin and LCA, it allows the distinction of malignant melanoma from its common mimics, namely, anaplastic carcinoma and large cell lymphoma. Similarly, the inclusion of anti-CEA forms a useful panel to distinguish Bowens' disease, Pagets' disease of the skin and superficial spreading malignant melanoma. Because a small number of melanomas may fail to express S100 protein, antibodies to HMB-45 and the melanoma-associated antigen NKI/C3 are useful additional markers for melanoma.

S100 protein is expressed by adipocytes and a proportion of liposarcomas also stain positive. Tumors of the cutaneous adnexae and salivary glands also express S100 protein.

Comments

Although S100 protein is a useful marker for the identification of melanoma, Langerhans' cell histiocytosis and peripheral nerve tumor, the antibodies should be used in the context of the differential diagnosis derived from morphologic and clinical appearances. A wide variety of carcinomas, including those from the lung, pancreas and female genitourinary tract, as well as *Mycobacteria ulcerans* organisms have been reported to show positivity so that S100 antibodies should not be used or interpreted in isolation. We have also observed the staining of be ign skeletal and smooth muscle cells with some anti-S100 antibodies.

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Takahashi K, Isobe T, Ohtsuki Y et al 1984. Immunohistochemical study on the distribution of α and β subunits of S100 protein in human neoplasm and normal tissues. Virchow's Archives Cellular Pathology 45: 385.

Serotonin

Sources/Clones

Accurate (5HTH209, YC5/45, polyclonal), Axcel/Accurate (5HTH209), American Qualex (polyclonal), Biodesign (polyclonal), Biogenesis (polyclonal), Biogenex (polyclonal), Caltag Laboratories (polyclonal), Chemicon (polyclonal), Dako (5HT-H209), Fitzgerald (M09203), Immunotech (polyclonal), Pharmingen (YC5-45), Sanbio/Monosan (polyclonal), Serotec (polyclonal), Seralab (polyclonal) and Zymed (polyclonal).

Fixation/Preparation

The antibodies to serotonin are immunoreactive in formalin-fixed, paraffin-embedded tissue sections. HIER enhances immunoreactivity.

Background

Serotonin (5-hydroxytryptamine) is a neurotransmitter substance which is found in a broad range of normal, hyperplastic and neoplastic tissues, including the gastrointestinal tract, central nervous system, adrenergic nerve fibers, platelets and basophils. The major use of this marker has been to identify serotonin-secreting carcinoid tumors, which mostly arise from the midgut (Westberg et al, 1997).

Applications

Immunostaining for serotonin has been employed as a marker of neuroendocrine differentiation. However, like other specific neuropeptides such as bombesin, ACTH, calcitonin and VIP, it is of low sensitivity and specificity and should only be employed in a panel of several antibodies with more specific markers such as chromogranin and synaptophysin. The major application of serotonin lies in the detection of carcinoid tumors (Zavala-Pompa et al, 1993; Zea-Iriarte et al, 1994; Burke et al, 1997), particularly as such tumors may respond to specific therapy with the somatostatin analog octreotide and α-interferons (Wilander et al, 1989; Westberg et al, 1997). Serotonin may also be detected in scattered cells within other neuroendocrine tumors from a variety of sites (Le Bodie et al, 1996; Gilks et al, 1997; LaGuette et al, 1997; Linberg et al, 1997). Whereas all tumors of the lung with dense core granules contained neuron-specific enolase, fewer contained serotonin (Wilson et al, 1985). In another study of 53 carcinoid tumors, 34 were argentaffin positive, 50 were argyrophil positive and 43 contained immunologically detectable serotonin (Shaw, 1988).

Comments

Serotonin has limited application as a marker of neuroendocrine differentiation. If used for this purpose, it should be employed with a panel of more specific and sensitive antibodies such as chromogranin and synaptophysin. Its main application today would be in a secondary panel to identify the specific neuropeptides produced in an established neuroendocrine tumor.

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Zea-Iriarte WL, Ito M, Naito S et al 1994. Goblet cell carcinoid of the appendix. Internal Medicine 33: 422-426.

Simian Virus 40 (SV40 T antigen)

Sources/Clones

Biogenesis (0H9, 0G5), Chemicon, Oncogene (PAb416, PAb419, PAb280), Pharmingen (Pab101, Pab122) and Santa Cruz (PAb101, Pab108).

Fixation/Preparation

The use of this antibody was confined to the staining of fixed tissue culture cells (Montano and Lane, 1984) until the recent application of antigen retrieval (Baker-Cairns et al, 1996).

Background

SV40 T antigen (Ab-3) is a mouse monoclonal antibody with specificity for antigenic determinants unique to the SV40 small t antigen and non-reactive with SV40 large T antigen (Montano & Lane, 1984). Both antigens are encoded by the early region of the SV40 genome (Tooze, 1973).

SV40 large T antigen is an 81 kD multifunctional viral phosphoprotein. Some of its functions are essential to the viral replication in monkey cells. Others contribute to its neoplastic transforming activity (Livingston & Bradley, 1987).

The large T antigen binds DNA and complexes with p53 protein (Lane and Crawford, 1979). It also forms a specific complex with the P105 product of the retinoblastoma susceptibility gene (De Caprio et al, 1988).

Applications

The use of this antibody has been confined to the research laboratory to define the cellular location of small t antigen in subcellular extracts of SV40-infected cells (Montano and Lane, 1989). Pab280 reacted strongly with a cytoplasmic form of small t antigen that appears to be associated with the cytoskeleton. Small t was found to accumulate late in the SV40 lytic cycle and was localized in both the cytoplasm and the nucleus of cells infected with wild-type SV40 (Montano and Lane, 1984). Research applications have centered around the use of SV40 as an effective gene transfer vector in vitro (Strayer, 1996), the immortalization of cell lines and the stimulation of developmental abnormalities and tumorigenesis in transgenic mice (Kelly et al, 1991; Rutland et al, 1991; Kivela et al, 1991; Kon et al, 1997; Webber et al, 1997).

Comments

The recent demonstration that 60% of human mesotheliomas contain and express SV40 sequences stimulated a great deal of interest. It has also been shown that SV40 large T antigen interferes with the normal expression of the tumor suppressor gene p53 in human mesotheliomas which raises the possibility that SV40 may contribute to the development of human mesotheliomas (Carbone et al, 1997). SV40 has been demonstrated in fixed tissue with the novel application of a DNA thermal cycler for antigen retrieval (Baker-Cairns et al, 1996).

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Spectrin/Fodrin

Sources/Clones

Accurate (SB-SP1, SB-SP2), American Qualex (polyclonal), Biodesign (polyclonal), Biogenesis (B12G3, D4D7, 2C5, polyclonal), Calbiochem (polyclonal), Chemicon (polyclonal), ICN Immunologicals (AA6), Locus Genex, Helsinki (101AA6), Finland, Novocastra (RBC1.5B1, RPC2.3D5), Serotec (D7A3, D4D7), Sigma (polyclonal) and Zymed (Z068).

Fixation/Preparation

The antibody is immunoreactive in fresh-frozen tissue sections and in fixed paraffin-embedded sections following HIER.

Background

Spectrin is a flexible rod-shaped molecule of 200 nm length found in mammalian and avian erythrocytes. It is composed of two non-identical subunits,α and β, and linked to the plasma membrane by the protein ankyrin. Along with actin, ankyrin and band 4.1, spectrin forms a network or membrane skeleton that lies immediately beneath the plasma membrane. The main function of the spectrin cytoskeleton is that of structural support for the bilipid layer of the cell membrane and the spectrin-based membrane skeleton also controls lateral mobility of the erythrocyte membrane proteins (Bennett, 1989, 1990a/b). Thermal denaturation of spectrin leads to disintegration of the erythrocytes into vesicles and deficiencies or structural abnormalities of the membrane skeleton proteins lead to loss of shape or tensile strength of the erythrocytes, resulting in fragmentation and destruction as they pass through the spleen. Defects of spectrin are associated with fragile erythrocytes in hemolytic anemias such as hereditary elliptocytosis, pyropoikilocytosis and spherocytosis (Bennett & Gilligan, 1993).

Non-erythroid cells also show a membrane skeleton which contains spectrin, although the molecular organization in such cells is less understood. Non-erythroid spectrin, known as fodrin with a molecular weight of 240 kD, exhibits many similarities to spectrin, including immunochemical crossreactivity, and is found in virtually all nonerythroid cells. Besides the function of maintaining some specialized membrane domains, fodrin appears to be redistributed in a variety of cell surface events, suggesting that it acts as a dynamic mediator between the cell membrane, membrane skeleton and cytoskeleton. For example, there is significant reorganization of the spectrin network in cells treated with growth factors. In chromaffin cells, stimulation with a calcium ionophore results in secretion and a relocation of spectrin as cytoplasmic aggregates, antibody-induced capping of B lymphocyte surface immunoglobulin leads to redistribution of spectrin similar to the surface proteins and in A-431, an epidermoid carcinoma cell line, EGF induces cell surface remodeling and the accumulation of spectrin in membrane ruffles coincident with its phosphorylation. It is thought that calcium ions influence membrane skeleton assembly and maintenance by binding to spectrin, by calcium-regulated, calmodulin-mediated influence of the interactions between spectrin and other proteins, or by calcium-dependent protease cleavage of spectrin (Harris & Morrow, 1990; Wallis et al 1992; Davis & Bennett, 1994).

Applications

Until recently, the antibody to spectrin/fodrin was employed

only on fresh-frozen tissue sections; however, with the use of microwave antigen retrieval, we were able to demonstrate immunoreactivity in fixed paraffin-embedded sections (Sormunen et al, 1997). The interest in fodrin lies in its role in cell adhesion during embryogenesis and in neoplasms. In comparison to their non-neoplastic counterparts, neoplastic epithelial cells show elevated levels of fodrin immunostaining regardless of tumor type. There was strong fragmented and circumferential staining for fodrin which often became accentuated with increasing grades of anaplasia and loss of membrane staining corresponded with loss of tumor cell cohesiveness (Sormunen et al, 1997). More recent work suggests that fodrin is linked to E-cadherin and β-catenin, together having a role in cell-to-cell adhesion. The breakage of this complex is heralded by detachment of β-catenin and associated with change in cell shape and cell adhesion in breast carcinoma (Sormunen et al, 1998).

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Synaptophysin

Sources/Clones

Accurate (SVP-38, S5768), American Research Products, Biodesign (SY-38), Biogenesis (SY-38), Biogenex (SY-38), Boehringer Mannheim (SY-38), Calbiochem, Cymbus Bioscience (SY-38), Dako (SY 38, polyclonal), Sanbio/Monosan (SY-38), Seralab (SY-38) and Sigma (SVP-38).

Fixation/Preparation

Applicable to formalin-fixed, paraffin-embedded sections. Microwave antigen retrieval in citrate buffer improves the immunostaining of this antibody. Enzyme pretreatment is not recommended for the monoclonal antibody. Also applicable to frozen sections and cell smears. The polyclonal antibody requires enzyme pretreatment before immunostaining.

Background

Synaptophysin is an integral-membrane glycoprotein (38 kD) of presynaptic vesicles (Jahn et al, 1985). The protein is a component of the classic, locally recycled small synaptic vesicle present in almost all neurons (Navone et al, 1986). Synaptophysin is present in empty vesicles and is both chemically and topographically different from chromogranin (68 kD), a membrane protein of dense-core neuroendocrine granules (Lloyd & Wilson, 1983).

Antibody (SY38) to synaptophysin has been raised against presynaptic vesicles from bovine brain. Hence, the antibody shows reactivity with neuronal presynaptic vesicles of brain, spinal cord, retina, neuromuscular junctions and small vesicles of adrenal medulla and pancreatic islets of human, bovine, rat and mouse origin (Navone et al, 1986). In normal tissues, neuroendocrine cells of the human adrenal medulla, carotid body, skin, pituitary, thyroid, lung, pancreas and gastrointestinal mucosa are labeled with this antibody (Wiedenmann et al, 1986).

The polyclonal antibody (Dako) was raised against the synthetic human synaptophysin peptide coupled to keyhole limpet hemocyanin.

Applications

Antibody to synaptophysin allows specific staining of neuronal, adrenal and neuroepithelial tumors: these include pheochromocytoma, paraganglioma, pancreatic islet cell tumors, medullary thyroid carcinoma, pulmonary/gastrointestinal/medias tinal carcinoid tumors and pituitary/parathyroid adenomas. Other neural tumors like neuroblastomas, ganglioneuroblastomas, ganglioneuromas, central neurocytoma and ganglioglioma also demonstrate immunoreactions with this antibody (Chejfec et al, 1987; Gould et al, 1986; Stefaneanu et al, 1988). The DAKO-rabbit antihuman synaptophysin is also useful for the identification of normal and neoplastic neuroendocrine cells.

Comments

Synaptophysin is a specific and fairly sensitive marker for neural/neuroendocrine tumors of low and high grades of malignancy. The recommended positive control tissue is pancreas (islets).

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TAG-72 (B72.3)

Sources/Clones

Biogenesis, Biogenex, Labvision Corp, Medac and Signet.

Fixation/Preparation

This antibody is applicable to formalin-fixed, paraffin-embedded tissue sections and cell blocks prepared from pleural and ascitic fluids.

Background

Clone B72.3 represents the monoclonal antibody to tumor-associated glycoprotein (TAG-72) (isotype: IgT1). The immunogen is a membrane-enriched fraction of a breast carcinoma derived from a liver metastases. This antibody recognizes a tumor-associated oncofetal antigen (TAG-72) expressed by a wide variety of human adenocarcinomas (Szpak et al, 1986; Muraro et al, 1988). It reacts with a sialyl-Tn epitope (72 kD) expressed on mucins (Gold & Mattes et al, 1988). TAG-72 expression in fetal tissue is only observed in tissues of the gastrointestinal tract, including the colon, esophagus and stomach. Although weak reaction with some tissues of adults has been observed, no reactivity is seen with tissue from organ systems including lymphoreticular, cardiovascular, hepatic, pulmonary, neural, muscular, skin, endocrine and genitourinary tract.

Applications

Immunoreactivity of TAG-72 has been observed in 19 of 22 (86%) pulmonary adenocarcinomas (Szpak et al, 1986). In contrast, none of the 20 mesotheliomas studied showed reactivity with this antibody. Other studies have confirmed these findings that malignant mesotheliomas generally do not react with this antibody and if they do, the reactivity is weak. Alternatively, adenocarcinomas from a variety of sites show strong, usually focal and predominantly cytoplasmic reactivity with TAG-72 (Sheibani et al, 1992). This antibody has shown immunoreaction with 84% of invasive ductal breast carcinomas and 85-95% of colon, pancreatic, gastric, esophageal, lung, ovarian and endometrial adenocarcinomas. Other workers have found this antibody to be less sensitive, labeling only 30-40% of adenocarcinomas (Sheibani et al, 1992).

Studies investigating TAG-72 staining of serous effusions have found similar high specificity but with variable sensitivity. Metastatic adenocarcinoma has been reported to be positive in 58-95% of cases from effusion specimens (Shield et al, 1994). Although rare cases of TAG-72 staining in reactive mesothelial cells have been reported (Esteban et al, 1990), other studies did not observe staining in benign, reactive or malignant mesothelial cells.

Comments

Strong reactivity for TAG-72 appears to be relatively specific for adenocarcinoma, but the utility of this antibody is somewhat limited by the variable sensitivity. Nevertheless, it is recommended that TAG-72 be included in an immunodiagnostic panel for evaluation of suspected cases of mesothelioma.

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Szpak CA, Johnston WW, Roggli V, et al 1986. The diagnostic distinction between malignant mesothelioma of the pleura and adenocarcinoma of the lung as defined by a monoclonal antibody B72.3. American Journal of Pathology 122: 252-260.

Tau

Sources/Clones

Accurate (TAU2, polyclonal), Accurate/Sigma, Biodesign (TAU2), Biosource (AT8, BT2, HT7), Calbiochem, Chemicon, Dako (polyclonal), Labvision Corp (TAU5), Pharmingen (TAU2.1), Sigma (TAU2, polyclonal) and Zymed (T14, T46).

Fixation/Preparation

Most of the antibodies are immunoreactive in fixed paraffin-embedded sections.

Background

The major components of the neuronal cytoskeleton are α and β tubulin, the microfilament-associated proteins (MAPs), neurofilaments and actin. Tau is a neuronal microtubule-associated protein which is the major antigenic component of neurofibrillary tangles and senile plaques in Alzheimer's disease (Joachim et al, 1987). Comparison of tau-immunoreactive lesions in three relatively uncommon neurodegenerative diseases, namely supranuclear palsy, Pick's disease and corticobasal degeneration, demonstrated unexpected pathological similarities, but also fundamental differences between these disorders (Feany & Dickson, 1996).

Tau2 was produced using bovine MAP as immunogen. It reacts exclusively with the chemically heterogenous tau in both the phosphorylated and non-phosphorylated forms. Tau2 does not react with other MAPs or with tubulin and localizes along microtubules in axons, dendrites, somata and astrocytes and on ribosomes. Tau2 crossreacts with bovine, monkey and chicken tissue. A variety of antibodies to phosphorylated neurofilament proteins have been shown to crossreact with phosphorylated epitopes of tau (Perry et al, 1985; Cork et al, 1986).

Applications

Applications of tau are mainly in the field of neuropathological research in neurodegenerative disorders. In the diagnostic setting, conventional silver impregnation stains such as Bielchowsky or Bodian are used for the demonstration of neurofibrillary tangles. These can now also be detected with antibodies to phosphorylated tau epitopes and ubiquitin.

Comments

None

References

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USA 82: 3916-3920.

Terminal Deoxynucleotidyl Transferase (TdT)

Sources/Clones

Accurate (polyclonal), Biodesign (monoclonal), Biogenex (6A6.09), Chemicon, Dako (HT1, HT3, HT4, polyclonal), Gentrak, Immunotech (HTdT, polyclonal), Seralab (HTdT-1, polyclonal), Sigma (8-1 E4) and Supertechs (polyclonal).

Fixation/Preparation

While both immunofluorescent and immunoenzyme techniques were initially applied to cryostat sections and cell suspensions, immunohistochemical staining of formalin-fixed, paraffin-embedded sections is now possible. Paraffin section immunostaining is greatly enhanced by heat-induced antigen retrieval so that terminal deoxynucleotidyl transferase (TdT) can be demonstrated on routine and archival specimens without the need for DNAse digestion and prolonged incubation previously necessary. Both 4 M urea and citrate buffer pH 6.0 are suitable retrieval solutions (Orazi et al, 1994). Polyclonal antibodies are preferable to monoclonal antibodies for formalin-fixed, paraffin-embedded sections.

Background

Terminal deoxynucleotidyl transferase (TdT) is a 58 kD protein encoded by a 35 kb gene on chromosome 10q23-25. It is a nuclear enzyme that catalyzes the random addition of deoxynucleotidyl residues on the 3'OH termini of single-stranded DNA and of oligo-deoxynucleotide primers and differs from other DNA polymerases by not requiring template instruction for polymerization.

TdT is recognized to exert its DNA polymerase function during the early variation of genes coding for T and B cells, perhaps by resulting in the addition of non-germline-encoded nucleotides (N-regions) although its function is still debated.

TdT is normally present only in hematopoietic tissues such as thymus and bone marrow, where it is restricted to a proportion of multipotent cell precursors and immature T and B lymphocytes. TdT positivity is never observed in normal peripheral blood cells.

Approximately 1-2% (more in young individuals) of bone marrow cells show TdT positivity and these mostly express B-cell precursor phenotype in cell suspension studies. In trephine biopsies, TdT-+ cells do not display preferential localization and are sparsely dispersed in interstitial spaces.

In the thymus, T lymphocytes can be classified into three maturation stages corresponding to their microenvironment. Stage I thymocytes, accounting for 0.5-5% of thymocytes, reside in the subcapsular zone of the thymus and comprise large TdT blast cells which express CD 7, CD 2, CD 5, and cCD 3 (cytoplasmic). Stage II thymocytes, accounting for 60-80% of thymocytes, are TdT and express CD 7, CD 5, cCD 3, CD 2, CD 1, CD 4 and CD 8. Stage II thymocytes, accounting for 15-20% of thymocytes, reside in the medulla, do not express TdT nor CD 1 and show differentiation into either CD 4+ or CD 8+ cells.

Applications

TdT as a marker is mostly used in the diagnosis of lymphomas and leukemias. TdT activity is seen in acute lymphoblastic leukemias (ALL) of both B- and T-cell lineages so that TdT is a useful diagnostic marker for lymphoblastic leukemias (Chilosi & Pizzolo, 1995). In addition, as many as 30% of patients with chronic granulocytic leukemia develop a lymphoid blast crisis which is characterized by a lymphoblastic phenotype including nuclear TdT expression.

These TdT lymphoblastic crisis have a better prognosis than TdT-nonlymphoid blast crisis and respond to ALL-like therapy.

About 20% of cases of acute non-lymphoid leukemias also express TdT in which the proportion of TdT blasts coexpressing various myeloid markers is variable. It has been suggested that the expression of TdT in such cases is a marker of poor prognosis but this is controversial. Such cases often show the phenomenon of phenotypic and genotypic "lineage infidelity" in which there is expression of lymphoid antigens such as CD 7 and rearrangement of Ig and T-cell receptor genes.

The L3 ALL in the FAB classification which represents Burkitt-type leukemia is an exception as the blast cells of this type of leukemia represent a "mature" B-cell phenotype with surface immunoglobulin expression.

TdT is a reliable marker to distinguish lymphoblastic lymphoma (LL) from other lymphomas that are always TdT -. LL are related to T-ALL and their distinction from the latter can be difficult, but, clinical and phenotypic differences have been observed with the latter tending to show a more immature immunophenotype. While LL is frequent in children, forming about one third of all non-Hodgkin's lymphoma cases, it also makes up about 5% of cases in adults and cases of non-T, non-B or pre-B-cell LL have been reported in extranodal sites in both children and adults.

TdT is thus a useful marker for diagnosis as well as for staging as it helps identify tumor cells from reactive lymphocytes. TdT staining can be used for the detection of early involvement and in staging, especially in extranodal sites such as the testes, CNS (through cerebrospinal fluid examination), skin, liver, kidney and other sites of extramedullary involvement and for monitoring minimal residual disease following chemotherapy.

Comments

TdT represents a powerful tool in leukemia and lymphoma diagnosis but it should be used in the context of a complete panel of markers and relevant histochemical enzyme stains. The ability to stain for this DNA polymerase in paraffin-embedded tissues, especially with polyclonal antibodies, following heat-induced antigen retrieval has greatly enhanced its diagnostic utility (Orazi et al, 1994). When immunostaining cryostat sections, it is necessary to employ brief fixation in buffered formalin or Zamboni's fixative and to reduce diffusion of the enzyme the sections must be immersed in fixative immediately after cryosectioning (Chilosi et al, 1983). We employ the rabbit anti-TdT from Dako.

References

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Thrombomodulin

Sources/Clones

Advanced Immunochemical (polyclonal), American Diagnostic (polyclonal), Axcel (24FN, 3E2) and Dako (1009).

Fixation/Preparation

Antibodies to thrombomodulin are applicable to formalin-fixed, paraffin-embedded tissue.

Background

Thrombomodulin (TM) is a transmembrane glycoprotein composed of 575 amino acids (molecular weight 75 kD) with natural anticoagulant properties (Wen et al, 1987). It is normally expressed by a restricted number of cells, such as endothelial and mesothelial cells (Marnyama et al, 1985). In addition, synovial lining and syncytiotrophoblasts of human placenta also express TM. Although TM contains six domains that are structurally similar to epidermal growth factor (EGF), there is no cross-reaction of anti-TM with EGF (Collins et al, 1992). The anticoagulant activity of TM results from the activation of protein C and the subsequent action on factors Va and VIIIa and from the binding of thrombin (Suzuki et al, 1987).

Applications

Several immunohistochemical endothelial markers are currently available (Suthipintawong et al, 1995) and thrombomodulin serves as another marker, staining blood and lymphatic channels and their corresponding tumors consistently. In a recent study, TM antibody stained 95% of benign lymphatic lesions (including lymphangioma and lymphangiectasia) (Appleton et al, 1996). In addition, TM demonstrated positivity in 100% benign vascular tumors (pyogenic granuloma and hemangioma) and 94% of malignant vascular tumors (Kaposi's sarcoma, angiosarcoma and epithelioid hemangioendothelioma). Hence, TM serves as a sensitive marker for lymphatic endothelial cells and their tumors. There has also been recent interest in the use of TM as an immunohistochemical marker for mesothelial cells and malignant mesotheliomas. The results have been rather variable with some studies claiming high specificity whilst others were less specific in distinguishing mesothelioma from adenocarcinoma. Table 1 illustrates the positivity rate for TM:

Based on these data, it appears that TM cannot be totally depended upon for the purpose of distinction between mesothelioma and pulmonary adenocarcinoma.

Comments

Clearly, the major role of TM remains in the confirmation of lymphatic and vascular tumors, although some advocate the use of TM as a mesothelioma-binding antibody in the standard panel of antibodies used for the evaluation of malignant mesothelioma (Ordonez, 1997).

References

Appleton MAC, Attanoos RL, Jasani B 1996. Thrombomodulin as a marker of vascular and lymphatic tumors. Histopathology 29: 153-157.

Table 1

	Mesothelioma %	Pulmonary adenocarcinoma %

Collins et al, 1992	100	8
Brown et al, 1993	59	60
Attanoos et al, 1996	52	6
Doglioni et al, 1996	80	77

Attanoos RL, Goddard H, Gibbs AR 1996. Mesothelioma-binding antibodies: thrombomodulin, OV632 and HBME-1 and their use in the diagnosis of malignant mesothelioma. Histopathology 29: 209-215.

Brown RW, Clark GM, Tandon AK, Allred DC 1993. Multiple marker immunohistochemical phenotypes distinguishing malignant pleura mesothelioma from pulmonary adenocarcinoma. Human Pathology 24: 347-354.

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Wen D, Dittman W, Ye R, Deaven L, Margerus P, Sadler J 1987. Human thrombomodulins: Complete cDNA sequence and chromosome localization of the gene. Biochemistry 26: 4350-4435.

Thyroglobulin

Sources/Clones

Axcel/Accurate (polyclonal, DAK-Tg6), Biodesign (polyclonal, 101, 102, 103, 104), Biogenesis (polyclonal), Biogenex (polyclonal), Caltag Laboratories (14/14), Dako (polyclonal, DAK-Tg6), Chemicon (polyclonal), Fitzgerald (M370108, M310136, M310137, M310138, M310139), Immunotech SA (J7B49, J7C9-3, J7C76-20), Labvision Corp (2H11, 6E1), Novocastra (polyclonal, ID4), Sanbio/Monosan (14/14) and Zymed (polyclonal).

Fixation/Preparation

The antibodies to thyroglobulin are applicable to formalin-fixed paraffin sections, acetone-fixed cryostat sections and fixed-cell smears.

Background

DAK-Tg6 (IgG1, k) and 1D4 (IgG2a) were raised against purified human thyroglobulin. These antibodies react with thyroglobulin (300 kD) in normal, hyperplastic and neoplastic thyroid glands. Circulating iodide, derived from dietary sources and deiodination of thyroid hormones, is selectively trapped by the thyroid gland. Oxidation of iodine to the organic form is then effected by a thyroid peroxidase enzyme (Magnusson et al, 1987), which is sited at the apical border of the follicular cell. This is now recognised as the antigen to thyroid antimicrosomal antibody in autoimmune disease (Portmann et al, 1988). Organic iodide is then incorporated into mono- and diiodotyrosine by binding to tyrosine residues on thyroglobulin stored in colloid. Thyroglobulin contains 140 tyrosine residues but not all of these are iodinated and T4 and T3 synthesis occurs only at specific sites (Dunn et al, 1987). Hormone release is brought about by endocytosis of thyroglobulin at the apical pole of the follicular stem cell, fusion of endocytotic vesicles with lysosomes and release of T3 and T4 by the proteolytic cleavage of thyroglobulin. These hormones are then secreted into the peripheral blood via the basal pole.

Applications

Apart from being immuno-positive in all papillary and follicular carcinomas, thyroglobulin may also be useful in poorly differentiated and anaplastic carcinomas in up to 50% of cases (Wilson et al, 1986; De Micco et al, 1987). Although both latter entities have been shown biochemically to synthesize 19S thyroglobulin, immunohistochemistry often fails to detect thyroglobulin in these tumors (Monaco et al, 1984). H黷thle cell tumors also demonstrate immunopositivity with thyroglobulin. The other major role of antibodies to thyroglobulin is in the identification of metastatic thyroid carcinomas. A note of caution is necessary, since thyroglobulin may be demonstrated in medullary carcinoma of the thyroid gland (Wilson et al, 1986). In such instances, attention to morphology as well as application of calcitonin antibodies would be crucial in avoiding an erroneous diagnosis. Antibodies to thyroglobulin do not react with epithelial cells from GIT, pancreas, kidney, lung and breast nor the malignancies that arise in these organs.

Comments

The main role of thyroglobulin antibody lies in the identification of poorly differentiated/anaplastic carcinomas and metastatic thyroid carcinoma. Normal thyroid tissue may be used as positive controls.

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Portmann L, Fitch FW, Harvan W, et al 1988. Characterisation of the thyroid microsomal antigen and its relationship to thyroid peroxidase, using monoclonal antibodies. Journal of Clinical Investigation 81: 1217-1224.

Wilson NW, Pambakian H, Richardson TC, Stokoe MR, Heyderman E 1986. Epithelial markers in thyroid carcinoma: an immunoperoxidase study. Histopathology 10: 815-829.

Toxoplasma gondii

Sources/Clones

Accurate, American Research Products (1637-18), Biodesign (polyclonal), Biogenesis (polyclonal), Biogenex (GII-9), Chemicon, Dako (polyclonal) and Fitzgerald (M26303).

Fixation/Preparation

Applicable to formalin-fixed, paraffin-embedded tissue sections. Proteolytic enzyme pretreatment is essential before immunostaining. These antibodies are also applicable to cryostat sections and fixed-cell smears.

Background

T.gondii is a protozoan parasite which causes a mild and self-limiting infection in adults. Toxoplasmosis occurs in patients who eat raw or partially cooked meat, reflecting the widespread presence of this protozoan in animals used as food sources. Following gastrointestinal infection, active toxoplasmosis is accompanied by fever with enlargement of lymph nodes and spleen. The immune reactions cause the intracellular *toxoplasma* to adopt a cystoid form in which they can persist for a lifetime. However, infections in immunocompromised patients may be fatal, causing acute toxoplasmosis including toxoplasmosis encephalitis. Activation of a latent infection during pregnancy may lead to intrauterine transmission of the organism to the fetus, resulting in spontaneous abortion, stillbirth or severe central nervous system damage (Kriek & Remington, 1978).

The production of monoclonal antibodies against protozoa has been limited by the complex life cycles of these parasites. Clone GII antibody recognizes a tachyzoite membrane antigen of 30 kD. The polyclonal antibody (Dako) was raised against formalin-fixed tachyzoites of *T.gondii* isolated and purified from infected mice. This latter antibody does not crossreact with the following organisms: *Cryptosporidia, Microsporidia, Histoplasma capsulatum, Candida, Blastomyces, Pneumocystis carinii, Entamoeba histolytica, Aspergillus, Cryptococcus neoformans and Mycobacterium tuberculosis* (Conley et al, 1981).

Applications

Both tachyzoites (or trophozoites) and encysted bradyzoites forms of T.gondii are demonstrated with these antibodies. Infected *Toxoplasma* tissue including brain, lung, spleen and lymph nodes may be positively identified with these antibodies. This is particularly pertinent when examining tissue from immunocompromised patients, e.g. AIDS, where a high index of suspicion along with application of anti-*toxoplasma* antibody may help a definite diagnosis (Luft & Remington, 1988).

Comments

These antibodies are best optimized using *toxoplasma*-infected tissue.

References

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Luft BJ, Remington JS 1988. Toxoplasmic encephalitis. Journal of Infectious Diseases 157: 1-6.

Ubiquitin

Sources/Clones

Accurate/Novocastra (FPM1), Biodesign (polyclonal), Biogenesis (242.9, polyclonal), Dako (polyclonal), Fitzgerald (polyclonal), Serotec (polyclonal) and Zymed (UBI1).

Fixation/Preparation

This antibody is applicable to formalin-fixed, paraffin-embedded tissue sections.

Background

Ubiquitin is an 8.5 kD polypeptide found almost universally in plants and animals. The best documented function for ubiquitin involves its conjugation to proteins as a signal to initiate degradation via the ubiquitin-mediated proteolytic pathway (Jahngen-Hodge et al, 1992). Ubiquitin-mediated proteolysis is involved in the turnover of many short-lived regulatory proteins. This pathway leads to the covalent attachment of one or more multiubiquitin chains to target substrates which are then degraded by the 26S multicatalytic chains proteasome complex (Rolfe et al, 1997). Ubiquitin modification of a variety of protein targets within the cells also plays an important role in many cellular processes: regulation of gene expression, regulation of cell cycle and division, involvement in the cellular stress response, modification of cell surface receptors, DNA repair, import of proteins into mitochondria, uptake of precursors into neurons, and biogenesis of mitochondria, ribosomes and peroxisomes (Ciechanover & Schwartz, 1994).

Applications

Ubiquitin immunostaining has been shown to be a highly sensitive and specific method for the detection of Mallory bodies, thereby making it a valuable tool in the study of alcoholic liver disease, adding objectivity to the diagnosis of alcoholic hepatitis (Vyberg & Leth, 1991). In the human spongiform encephalopathies, ubiquitin immunoreactivity has been demonstrated in a punctate distribution at the periphery of prion protein amyloid plaques and in a finely granular pattern in the neurophil around and within areas of spongiform change (Ironside et al, 1993). Ubiquitin has also been simultaneously present with GFAP in the cytoplasm and cell processes of tumor cells of astrocytomas (Galloway & Likavec, 1989). The demonstration of ubiquitin immunolabeling in both ductus efferentes and ductus epididymidis epithelia has suggested that ubiquitinated proteins are secreted into the epididymal lumen (Fraile et al, 1996). Ubiquitin expression has been demonstrated in the lung and adrenal gland in autopsy cases that died by fire accident (Shoji, 1997), suggesting that the adrenal gland reacts strongly to heat shock. Evidence of ubiquitin-positive myocytic intranuclear or cytoplasmic inclusions or positive-staining rimmed vacuoles in the setting of an inflammatory myopathy may be suggestive of a diagnosis of inclusion body myositis (Prayson & Cohen, 1997). On the therapeutic front, prevention of p53 ubiquitination (and subsequent degradation) in human papilloma virus-positive cervical tumors should lead to programed cell death (Rolfe et al, 1997).

References

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Shoji T 1997. Demonstration of heat shock protein, ubiquitin, in fire death autopsy cases by immunohistochemical study: Nippon Hoigaku Zasshi 51: 70-76

Vyberg M, Leth P 1991. Ubiquitin: an immunohistochemical marker of Mallory bodies and alcoholic liver disease. APMIS Supplement 23: 46-52

Ulex Europaeus Agglutinin 1 Lectin (UEA-1)

Source

Dako (UEA-1).

Fixation/Preparation

Carbohydrates reactive with UEA-1 are generally active in formalin-fixed, paraffin-embedded tissue sections. Background staining may be reduced by the addition of 5% human serum to the anti-UEA-1 dilution buffer.

Background

UEA-1 is a plant lectin isolated from*Ulex europaeus* seeds (gorse seeds) by affinity chromatography. The lectin is homogeneous, containing 4.2% neutral sugar and 1.4% glucosamine (Horejsi, 1979). Its molecular weight is approximately 110 kD, comprising two covalently bound basic subunits. UEA-1 is specific to certain terminalα-L-fucosyl residues of glycoconjugates and also detects blood group H antigen (Pereira et al, 1978).

Applications

UEA-1 has been used successfully as a marker for endothelial cells. It has been shown to be more sensitive for benign vascular tumors than thrombomodulin or factor VIII-related antigen (Yonezawa et al, 1987). In fact, other workers have shown UEA-1 to be a more sensitive marker for endothelial cells of vascular tumors than factor VIII-related antigen (Miettinen et al, 1983; Ordonez & Batsakis, 1984). UEA-1 does not distinguish between the endothelial cells of blood vessels and lymphatics (Suthipintawong et al, 1995). In some tissues Ulex lectin has demonstrated additional binding to epithelial structures (Holthofer et al, 1982). This latter immunoreaction has been exploited with UEA-1 demonstrating specific binding to collecting duct carcinoma of the kidney, enabling distinction from other types of renal cell carcinoma (Amin et al, 1997).

Comments

There are more specific and sensitive markers of endothelial cells available, such as C 34 and CD 31. It would appear that the use of UEA-1 is confined to identifying collecting duct carcinoma of the kidney. Benign vascular tissue makes appropriate positive controls for UEA-1.

References

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marker for vascular tumors. Comparative study with Factor VIII and Ulex europaeus I lectin. American Journal of Clinical Pathology 88: 405-411

Villin

Sources/Clones

Accurate, Biodesign (ID2C3), Biogenesis (20/24), Chemicon (15E2), Immunotech (ID2C3) and Serotec (ID2C3).

Fixation/Preparation

HIER is required for fixed paraffin-embedded sections. Fixation in Carnoy's solution or methacarn preserves immunoreactivity. The antibody is also immunoreactive in fresh cell preparations and frozen sections.

Background

Microvilli increase the absorptive surface of epithelial cells by as much as 20 times. They comprise a highly specialized plasma membrane of a thick extracellular coat of polysaccharide and digestive enzymes and a core comprising a central rigid bundle of 20-30 parallel actin filaments that extend from the tip of the microvillus down to the cell cortex. The actin filaments are all oriented with their plus ends pointing away from the cell body and are held together at regular intervals by actin-bundling proteins. Besides fimbrin, which occurs in microspikes and philopodia, the most important bundling protein is villin, which is found only in microvilli. Like fimbrin, villin crosslinks actin filaments into tight parallel bundles, but in a different actin-binding sequence and is capable of stimulating the formation of long microvilli in cultured fibroblasts which do not normally contain villin and have only a few small microvilli.

Villin, a 95 kD, Ca_{2+} -regulated actin-binding protein, is found in absorptive cells of the small and large intestines, in ductal cells of the pancreas and biliary system and in the cells of the proximal renal tubules. Villin is also found in undifferentiated normal and tumoral cells of intestinal origin in vivo and in cell culture so that its expression is seen in cells that do not necessarily display microvilli-lined brush borders (Robine et al, 1985).

Applications

Villin has been employed as a marker of gastrointestinal tumors, particularly those from the colon, stomach and pancreas, all such tumors staining positive in one study (Bacchi & Gown, 1991). Gallbladder and hepatocellular carcinomas were also shown to express villin (Moll et al, 1987). A subset of non-gastrointestinal tumors, including some adenocarcinomas of the ovary, endometrium and kidney, were also positive (Moll et al, 1987; Bacchi & Gown, 1991). Lung adenocarcinomas were rarely positive and no staining was observed in breast carcinoma or mesothelioma. The presence of villin in renal carcinomas is variable and is frequently seen in clear cell and chromophilic tumors but not in chromophobe cell tumors (Moll et al, 1987). Villin also appears to be expressed in the tubular and glandular areas of better differentiated tumors and is not observed in sarcomatoid renal carcinoma, leading to the suggestion that it may be a potential grading marker (Grone et al, 1986). Its expression in renal carcinomas suggests that they display proximal rather than distal tubular differentiation. It is also observed in the glandular areas of Wilm's tumor (Droz et al, 1990).

Comments

Villin shows apical localization but may also be seen in the basement membrane area surrounding tumor nests (West et al, 1988). Clone ID2C3 shows reactivity with human, porcine and chicken villin.

References

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West AB, Isaac CA, Carboni JM et al 1988 Localization of villin, a cytoskeletal protein specific to microvilli, in human ileum and colon neoplasms. Gastroenterology 94: 343-352.

Vimentin

Sources/Clones

Accurate (V9, J144, Vim-13.2), Amersham, Biodesign (V9), Biogenesis (Vim-01, LN6), Biogenex (LN6, V9), Biotest (VIM 3B4), Boehringer Mannheim (3B4, V9), Chemicon, Cymbus Bioscience (VIM3B4), Dako (VIM3B4, V9), Diagnostic Biosystems (V9), Enzo, Immunotech (V9, V3260), Medac, Milab, Novocastra, Oncogene (V9), Pierce (ZSV5), RDI (VIM3B4), Serotec (J144), Sigma (LN9) and Zymed (ZSV5, ZC64).

Fixation/Preparation

Most antibody clones currently available are immunoreactive in fixed paraffin-embedded tissues and immunostaining is enhanced by HIER.

Background

Vimentin is a 58 kD protein which has been purified from a variety of sources and has been shown to form homophilic filaments with an average diameter of 10 nm. Its name is derived from the Latin word vimentum, which means arrays of flexible rods. Similar to the other intermediate filaments, vimentin is a protein monomer of highly elongated fibrous molecules with an amino-terminal head, a carboxyl-terminal tail and a central rod domain. The latter consists of an extended α-helical region containing long tandem repeats of a distinctive amino acid sequence motif called the hepatad repeat. This central rod domain shows a striking sequence homology between intermediate filaments of different species and an even more marked homology of as high as 30% between cytokeratin, desmin, glial fibrillary acidic protein, neurofilaments and vimentin of the same species. Immunohistochemical staining revealed vimentin filaments as part of a wavy network of filaments in the cytoplasm of fibroblasts, associated with both nuclear and plasma membranes. It has been suggested that vimentin, like other intermediate filaments, serves as a modulator between extracellular influences governing calcium flux into the cell and nuclear function at a transcriptional or translational level and may thus have a role in gene expression. Vimentin filaments can be precipitated as juxtanuclear whorls following treatment of cells with colcemid or vinblastine.

Applications

Vimentin is the most widely distributed intermediate filament and is expressed in virtually all mesenchymal cells and also by most other cell types in culture (Lane et al, 1983). With the widespread application of intermediate filament analysis to human neoplasms, it soon became apparent that although individual cell types and their corresponding tumors generally express a single intermediate filament class, several neoplasms may express more than one intermediate filament class (Azumi & Battifora, 1987). In many instances, this coexpression of one or more intermediate filament classes occurs in a predictable manner and may be employed as a diagnostic discriminator. Vimentin expression, traditionally accepted to be class specific for cells of the mesenchyme, can be coexpressed with cytokeratin in a number of epithelial cell types and their corresponding tumors. These include the endometrium, thyroid, gonadal epithelial cells, renal tubules, adrenal cortex, lung, salivary gland, hepatocytes and bile duct. Furthermore, there is increasing evidence to suggest that a variety of high-grade epithelial tumors may acquire the expression of vimentin intermediate filaments (Leong, 1991). Vimentin expression has been described in carcinomas of the skin (Iyer &

Leong, 1992), urinary bladder, breast (Raymond & Leong, 1989), prostate (Leong et al, 1988), gastric mucosa (Takemura et al, 1994) and uterine cervix. Several reports have indicated a correlation of vimentin expression with high tumor grades in breast carcinoma (Raymond & Leong, 1989; Heatley et al, 1993; Domogala et al, 1994; Koutselini et al, 1995) and ovarian epithelial malignancy (Nakopoulpou et al, 1995). One report suggested that vimentin expression was a poor prognostic marker in node-negative breast carcinoma (Domogala et al, 1990) although this has not been confirmed (Seshadri et al, 1996). Expression of vimentin in epithelial tumors also corresponds to changes in cell shape and forms from epithelioid to fibroblastoid or spindle forms so that vimentin is regularly expressed in spindle cell carcinomas.

Many tissues in embryos and fetuses, including surface ectoderm, neural groove and brain, gut mucosa and musculature, and renal tubular epithelium, display coexpression of vimentin with another intermediate filament during their developmental stages before being replaced by the intermediate filament protein specific for the mature tissue type (Goel et al, 1997). Vimentin is expressed in epithelial cells in vitro, culture preparations, cell suspensions and in exfoliated and metastatic cells in body fluids, suggesting that altered cell-to-cell contact and changes in cell shape may account for this apparent aberrant expression. Studies of cell cultures of mouse parietal endodermal cells led to the hypothesis that the acquisition of vimentin may be related to reduced cell-to-cell contact and the ability of epithelial cells to survive independently.

Immature muscle fibers contain desmin and vimentin and mature fibers lack vimentin. Regenerating muscle fibers react with anti-vimentin antibodies and more intensely for desmin than mature fibers. The detection of vimentin has therefore been applied to identify muscle regeneration, especially in cases of infantile spinal muscular atrophy and the high incidence of reactive fibers in some congenital and early-onset disorders may indicate developmental arrest (Bornemann & Schmalbruch, 1993).

Comments

Due to variability of fixation and HIER, vimentin has been used as an internal control or reporter molecule to assess the quality of antigen preservation and the uniformity of tissue fixation in fixed paraffin-embedded tissue sections (Battifora, 1991).

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VS38

Sources/Clones

Dako (VS38c).

Fixation/Preparation

The antibody is reactive in paraffin-embedded sections and staining is enhanced by heat-induced antigen retrieval.

Background

VS38 was shown to detect a protein similar to the p63 protein. The latter is a non-glycated, reversibly palmitoylated type II transmembrane protein, which is found in rough endoplasmic reticulum. VS38 was originally described as a marker of neoplastic and non-neoplastic plasma cells (Turley et al, 1994).

Applications

It is now recognized that the protein detected by VS38 is not exclusive to plasma cells but serves to distinguish plasma cells from other lymphoid cells because of their high secretory activity (Banham et al, 1997). It has been recommended for inclusion in a panel of antibodies for the immunostaining of bone marrow trephines fixed in common fixatives including Bouin's solution (Gala et al, 1997).

VS38 immunostaining has been reported in neuroendocrine tumors and in melanocytic lesions and caution should be exercised when using this marker to identify plasma cell lineage (Banarjee et al, 1997).

Comments

There is a need for a specific marker of plasma cell differentiation as a variety of neoplastic cells can display plasmacytoid features and the converse, that is, poorly differentiated plasma cells and plasmacytoid cells can be difficult to recognize morphologically. In a recent study of endometritis, it was found that besides labeling plasma cells, VS38 also stained epithelium and stromal cells of the endometrium. In contrast, CD 38 produced strong labeling of plasma cells and not the other endometrial components, suggesting that CD 38 may be a more specific marker of plasma cell differentiation (Leong et al, 1997).

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SECTION 2
APPENDICES

Appendix 1
Selected Antibody Panels for Specific Diagnostic Situations

Appendix 1.1 Bone/Soft tissue-chondroid-like tumors

	CK	VIM	S100	EMA	CEA	GFAP	CD 57
Chordoma	+	+	+	+	–	+	–
Chondroblastoma	+	+	+	+	–	–	–
Chordoid chordoma	+	+	+	+	–	–	–
Myxoid chondrosarcoma (chondroid sarcoma)	–	+	+	–	–	–	–
Mesenchymal chondrosarcoma	–	+	+*	–	–	–	+
Clear cell sarcoma	–	+	+	–	–	–	–

*Chondroid cells

Appendix 1.2 Brain-metastatic carcinoma vs glioblastoma vs meningioma

	CK	EMA	VIM	GFAP
Metastatic carcinoma	+	+	+	–
Glioblastoma	–	–	+/–	+
Meningioma	–	+	+	–

*Secretory meningioma may be focally keratin positive

Appendix 1.3 Childhood-round cell tomurs

	LCA	VIM*	CK	DES	MSA	p30/32	Myog	MYOD1	NSE	SY
Hematolymphoid tumor	+/–	+	–	–	–	+	–	–	–	–
Neuroblastoma	–	+	+	–	–	+	–	–	+	+/–
ES	–	+	+	–	–	+	–	–	+	–
PNET	–	+	+	+	+	+	–	–	+	+
DSRCT	–	+	+	+	+	+	–	–	+	+

Rhabdomyosarcoma	–	+	–	+	+	–	+	+	–	–

*Vimentin labeling shows up the cytoplasm which is often not visible on H&E stains

ES-Ewing's sarcoma (skeletal and extraskeletal)

PNET-peripheral/primitive neuroectodermal tumor

DSRCT-desmoplastic small round cell tumor

Appendix 1.4 Gastrointestinal and aerodigestive tract mucosa-basaloid squamous vs adenoid cystic vs neuroendocrine carcinoma

	HMWtCK	**CEA**	**S100**	**Chgn**	**SYN**
Basaloid squamous carcinoma	+	+	–	–	–
Adenoid cystic carcinoma	+	+*	+	–	–
Neuroendocrine carcinoma	–	–	–	+	+

* Confined to luminal aspect of gland-like spaces

Appendix 1.5 Gonads-germ cell tumors vs somatic adenocarcinoma

	PLAP	αFP	hCG	CD 30	CK	EMA	VIM	hPL
Seminoma	+	–	–*	–	–*	+	–	–
Embryonal carcinoma	+	+/–	–	+	+	–	–	–
Yolk sac tumor	+/–	+	–	–	+	–	–	–
Choriocarcinoma	+/–	–	+	–	+	+	+	+
Somatic carcinoma	+**	–	+	–	+	+	–	–

*Occasional trophoblasts may be positive

**Mullerian tract, breast, gut and pulmonary tumors may occasionally be positive

Appendix 1.6 Granulocytic sarcoma vs lymphoma vs carcinoma

	CD 45	CD 38	CK	NE	CD 15*
Granulocytic sarcoma	+	–	–	+	+
Lymphoma	+	–	–	–	–
Plasmacytoma (poorly differentiated)	+/–	+	–	–	–
Carcinoma	–	–	+	–	+

*Other markers of granulocytic sarcoma include myeloperoxidase, CD 34, CD 68

Appendix 1.7 Intracranial tumors

	GFAP	VIM	CK	NF
Astrocytoma	+	+	+	–
Oligodendroglioma	+	+	–	–
Ependymoma	–	+	+	–
Neuroma/neurocytoma	–	–	–	+

Schwannoma	+	+	–	–
Metastatic carcinoma	–	+	+	–

Appendix 1.8 Liver-hepatocellular carcinoma vs metastatic carcinoma vs cholangiocarcinoma

	CEA	**AFP**	**CK 7**	**CK 19**	**VIM**	**Hep Par 1**	**Albumin**
Hepatocellular carcinoma	+*	+	+	+	+	+	+/–
Cholangiocarcinoma	+	–	+	+	+	+	–
Metastatic carcinoma	+/–	–	+/–	+/–	+	–	–

* Staining of canaliculi in hepatocellular carcinoma

Appendix 1.9 Lung束lear cell tumors

	CK	**VIM**	**Chgn**	**SYN**	**HMB-45**	**S100**
Carcinoma with clear cell change	+	–	–	–	–	–
Clear cell tumor (`sugar' tumor)	–		–	–	+	
Renal carcinoma, metastatic	+	+	–	–	–	–
Carcinoid	+	–	+	+	–	–

*Rare cells may stain positive

Appendix 1.10 Lymph node-round cell tumors in adults

	CK	**VIM**	**S100**	**HMB-45**	**CD 45**
Melanoma	–	+	+	+	–
Carcinoma	+	–	+	–	–
Lymphoma	–	+	–	–	+

Appendix 1.11 Mediastinal tumors

	CD 45	**CK**	**EMA**	**PLAP**	**CD 99**
Thymoma	–	+	+	–	+
Lymphoma	+	–	–	–	–
Germ cell tumor	–	+	+	+	–

Appendix 1.12 Nasal tumors

	VIM	**CK**	**S100**	**HMB-45**	**NF**
Neuroblastoma	+	–	+(SC)	–	+
Melanoma	+	–	+	+	–

Carcinoma	+	+	+	–	–

SC-sustentacular cells stain positive

Appendix 1.13 Pelvis-metastatic colonic adenocarcinoma vs ovarian endometrioid carcinoma

	VIM	**CEA**	**CA 19.9**	**CA 125**	**CK 7**	**CK 20**
Colonic adenocarcinoma	+	+	+	+	–	+
Ovarian endometrioid carcinoma	+/–	–	–	+	+	–

Appendix 1.14 Perineum-prostatic vs bladder vs rectal carcinoma

	CK 7	**CK 20**	**PSA**	**PSAP**	**CEA**
Prostatic carcinoma	–	–	+	+	+
Bladder carcinoma	+	+	–	+	+
Rectal carcinoma	–	+	–	–	+

Appendix 1.15 Peritoneum-myxoid tumors

	SMA	**Des**	**S100**	**Col IV**
Myxoma	–	–	–	–
Myxoid neurofibroma	–	–	+	+L
Myxoid liposarcoma	–	–	+	+C
Myxoid fibrosarcoma	–	–	–	–
Aggressive angiomyxoma	+	+	–	+F
Angiomyofibroblastoma	+	+	–	+F

C-circumferential; F - fragmented, thin; L - linear, continuous

Appendix 1.16 Pluera-mesothelioma vs carcinoma

	LMWCK	**HMWCK**	**VIM**	**EMA**	***a*SMA**	**CEA**	**CR**	**CD 15**[#]	**B**
Mesothelioma	+	+	+	+[*]	+/–	–	+	–	–
Secondary carcinoma	+	–	+	+	–	+	+	+	+

[*]Circumferential, with long microvilli

[#] Can be substituted with other myelomonocytic markers, e.g. LN1 (CD w75), LN2 (CD 74), Mac 387

LMWCK-low molecular weight cytokeratin

HMWCK-high molecular weight cytokeratin

CR-calretinin

Appendix 1.17 Retroperitoneum-renal cell carcinoma vs adrenocortical carcinoma vs phechromocytoma

	EMA	**VIM**	**CK**	**Chgn**	**SYN**	**S100**
Renal carcinoma	+	+/–	+	–	–	+
Adrenocortical carcinoma	–	+	+	–	–	–
Pheochromocytoma	+	–	+	+	+	+*

*Sustentacular cells

Appendix 1.18 Retroperitoneum-vacuolated clear cell tumor

	VIM	**CK**	**EMA**	**S100**	**GFAP**	**CEA**
Chordoma	+	+	+	+	+	–
Colonic adenocarcinoma	+	+	+	–	–	+
Renal cell carcinoma	+	+/–	+	+	–	–
Myxopapillary ependymoma	+	+/–	+	+/–	+	–

Appendix 1.19 Skin-adnexal tumors

	CK 20	**S100**	**EMA**	**CEA**	**GCDFP-15**	**SA**	**CD 15**	**Chgn**	**NF**	**I**
Squamous carcinoma	–	–	–	–	–	–	–	–	–	
Eccrine tumor	–	+	+/–	+/–	+	+/–	+/–	–	–	–
Apocrine tumor	–	–	+/–	+/–	+	+/–	+/–	–	–	+
Sebaceous tumor	–	–	+	–	–	–	+	–	–	–
Pilar tumor	–	–	–	–	–	–	–	–	–	–
Merkel cell carcinoma	+	–	+	–	–	–	–	+	+*	

*Merkel cell carcinoma often shows juxtanuclear whorls of neurofilaments and/or cytokeratin

Apppendix 1.20 Skin-basal cell carcinoma vs squamous carcinoma vs adnexal carcinoma

	EMA	**Ber-EP4**
Basal cell carcinoma	–	+
Squamous carcinoma	+	–
Adnexal carcinoma	+	+

Appendix 1.21 Skin-Pagetoid tumors

	LMWtCK	HMWtCK	S100	CEA	VIM
Melanoma	–	–	+	–	+
Paget's disease	+	–	+	+	–
Bowen's disease	+	+	–	–	–

Appendix 1.22 Skin-spindle cell tumors

	CK	VIM	CD 34	CD 31	αSMA	S100	HMB-45	Leu7	DES
Spindle SCC	+	+	–	–	–	–	–	–	–
Melanoma	–	+	–	–	–	+	+	+	–
AFX	–	+	–	–	–	–	–	–	–
DFSP	–	+	+	–	–	–	–	–	–
PNST	–	+	+	–	–	+/–	–	+/–	–
Smooth muscle	+	+	–	–	+	+	–	–	+/–
Kaposi's sarcoma	–	+	+	+	–	–	–	–	–
Angiosarcoma	+	+	+	+	–	–	–	–	–

AFX - atypical fibroxanthomas

DFSP - dermatofibrosarcoma protuberans

PNST - peripheral nerve sheath tumor

SCC - squamous cell carcinoma

Appendix 1.23 Soft tissue-epithelioid tumors

	CK	VIM	EMA	CD 34	CD 31	DES	αSMA	CD 57	S100
Metastatic carcinoma	+	+	+	–	–	–	–	–	+
Synovial sarcoma	+	+	+	–	–	–	–	–	–
Epithelial sarcoma	+	+	+	–	–	–	–	–	–
Angiosarcoma	+	+	–	+	+	–	–	–	–
PNST	–*	+	+	+	–	–	–	+/–	+/–
Leiomyosarcoma	+	+	–	–	–	+	+	–	–
Melanoma	–	+	–	–	–	–	–	+	+

* Occasional cells stain positive

PNST - epithelioid peripheral nerve sheath tumor

Appendix 1.24 Soft tissue-pleomorphic tumors

	VIM	**DES**	**S100**	**MSA**	**ScA**	**HMB-45**	**CK**	**MYOD1**
Rhabdomyosarcoma	+	+	–	+	+	–	–	+
MFH*	+	–	+	+	–	–	–	–
Melanoma	+	–	+	–	–	+	–	–
Carcinoma	+	–	–	–	–	–	+	–

* MFH - malignant fibrous histiocytoma may express factor XIIIa

Appendix 1.25 Stomach-undifferentiated-spindle cell tumors

	CD 34	SMA	MSA	S100	Chgn	SYN	CK
LyMo/LyMSa	+(weak)	+	+	+	–	–	+
GIST	+	–	–	–	–	–	–
PNST	–	–	–	+	–	–	–
GAN	+/–	–	–	–	+	+	–

GIST - gastrointestinal stromal tumor. This term is used for spindled tumors, which do not show evidence of myogenic or neurogenic differentiation, morphologically and immunophenotypically.

GAN - gastrointestinal autonomic nerve tumors. Amorphous extracellular collagen or skenoid fibers are often present and ultrastructural examination may be necessary to confirm the diagnosis of this aggressive tumor.

LyMo/LyMSa - leiomyoma/leiomyosarcoma

PNST - peripheral nerve sheath tumor

Appendix 1.26 Thyroid carcinomas

	34BE12	VIM	Thy	Chgn	SYN	CEA	Cal
Papillary carcinoma	+	+	+	–	–	–	–
Follicular carcinoma	–	+	+	–	–	–	–
Medullary carcinoma	–	+*	–	+	+	+	+
Metastatic carcinoma	+	+	–	–	–	–	–

* Vimentin expressed in spindle cells of medullary carcinoma

Appendix 1.27 Urinary tract-spindle cell proliferations

	CK	EMA	Des	MSA	SMA	S100
Inflammatory pseudotumor	–	–	–	+	+	–
Leiomyosarcoma	+	–	+/–	+	+	+
Spindle cell carcinoma	+	+	–	–	–	+

Rhabdomyosarcoma	–	–	+	+	–	–
MFH	–	–	–	+**	+**	+
Neurofibrosarcoma	–	–	++*	+*	–	+/–
Malignant melanoma	–	–	–	–		+
Postoperative spindle cell nodule	+	–	–	+	+	–

* Triton tumor

** In reactive myofibroblasts

Appendix 1.28 Uterine cervix-endometrial vs endocervical carcinoma

	VIM	CK 20	CK 7	CEA
Endometrial carcinoma	+	–	+	–
Endocervical carcinoma	–	–	–	–
Colonic carcinoma (metastatic)	+	+	–	+

Appendix 1.29 Uterus-trophoblastic cells

Trophoblastic cell	**1st trimester**		**2nd trimester**		**3rd trimester**	
	hCG	**HPL**	**hCG**	**hPL**	**hCG**	**hPL**
Cytotrophoblast	–	–	–	–	–	–
Intermediate trophoblast	+	++	+	+++	+	+/++
Syncytiotrophoblast	++++	+	++	+++	+	++++

Percentage of cell staining for the respective antigen: + = 1-24%; ++ = 25-49%; +++ = 50-74%; ++++ = > 75%

Appendix 1.30 Uterus-Immunophenotyping of syncytiotrophoblast in trophoblastic proliferations

	hCG	**hPL**	**PLAP**	**SP1**	**CK**	**VIM**
Partial mole[*]	+ Diffuse	+/++ Diffuse[#]	+/+++ Diffuse[#]	NK	+++ Diffuse	–
Complete mole	+++ Diffuse	+/++ Focal[#]	+ Focal	+++ Diffuse	+++ Diffuse	–
ChorioCa	+++ Diffuse	+ Focal	+ Focal	NK	+++ Diffuse	–
Placental site tumor (intermediate trophoblasts)	++ Focal	+ Focal	+++ Diffuse	+++ Diffuse	+++ Diffuse	+++ Diffuse

NK - not known; + - weak; ++ - intermediate; +++ - strong staining; [#] - expression increases with advancing pregnancy

[*] In the 1_{st} trimester, the pattern of expression of hCG in partial and complete moles is very similar. Similarly, the immunophenotypic profile of hydropic abortus and partial moles is very similar to that of normal pregnancy in th trimester.

Appendix 1.31 Tissue-associated antigens in `treatable tumors'

Lymphoma/leukemia	LCA (CD 45)
Germ cell tumor	PLAP
Breast carcinoma	GCDFP-15
Thyroid carcinoma	Thyroglobulin
Prostatic carcinoma	PSA, PSAP
Trophoblastic tumor	hCG, hPL
Rhabdomyosarcoma	Des, MYOD1, Myoglob*
Ewing's sarcoma/PNET	CD 99
Neuroblastoma	NF
Neuroendocrine tumor	Chgn, SYN

Chgn - chromogranin; Des - desmin; hCG - human chrorionic gonadotropin; hPL - human placental lactogen; Myoglob - myoglobin; NF - neurofilaments; PLAP - placental alkaline phosphatase; PSA - prostate-specific antigen; PSAP - prostatic acid phosphatase; SYN - synaptophysin

* Low sensitivity

Appendix 1.32 Epithelial tumors which may coexpress vimentin intermediate filaments*

Thyroid carcinoma

Endometrial carcinoma

Adrenocortical carcinoma

Ovarian epithelial tumors

Gonadal tumors

Salivary gland tumors

Renal cell carcinoma

Choroid plexus tumors

Breast carcinoma

Prostatic carcinoma

Ependymal tumors

Lung carcinoma

Hepatocellular carcinoma

Pheochromocytoma

Adamantinoma

Primitive/peripheral neuroepithelial tumor

* Any carcinoma, when sufficiently dedifferentiated, may coexpress vimentin; these tumors show this property with some regularity.

Appendix 1.33 Mesenchymal tumors which may coexpress cytokeratin

Angiosarcoma

Leiomyosarcoma

Chordoma

Chondroid chordoma

Chondroblastoma

Synovial sarcoma (monophasic and biphasic)

Epithelioid sarcoma

Mesothelioma

Meningioma

Malignant peripheral nerve sheath tumor*

Malignant melanoma**

Anaplastic large cell lymphoma Ki-1 (rare)

Dendritic cell sarcoma

* Glandular component
** Only in cryostat sections

Appendix 1.34 Tumors which may coexpress three or more intermediate filaments

Astrocytoma	GFAP, Vim, CK
DSRCT	Vim, CK, Des, NF
ES/PNET	Vim, CK, Des
Leiomyosarcoma	Vim, Des, CK
Pheochromocytoma	CK, NF, Vim
Rhabdomyosarcoma	Des, Vim, CK
Pleomorphic adenoma	CK, Vim, GFAP
Endothelial cells	Vim, CK, Des
Teratoma	CK, Des, GFAP, NF, Vim
True mixed tumors	CK, Des, Vim

(including Mullerian tumors)

Mesothelioma	CK, Des, Vim

ES/PNET - Ewing's sarcoma/primitive peripheral neuroepithelial tumor; CK - cytokeratin; Des - desmin; GFAP - glial fibrillary acidic protein; NF - neurofilaments; Vim - Vimentin

Appendix 1.35 Abbreviations to antibodies-and their sources

α FP - α - fetoprotein (Dako)

bcl-2 (Dako)

Ber-EP4 (Dako)

CA 125 (Signet)

CA 19.9 (Signet)

CD 15 - LeuM1 (Becton Dickinson)

CD 31 (Dako)

CD 34 (Oxoid)

CD 57 - Leu7 (Becton Dickinson)

CEA - carcinoembryonic antigen (Biogenex)

Chgn - chromogranin (INC)

CK - broad-spectrum cytokeratin (clone MNF116 - Dako; AE1/3 - Boehringer Mannheim; bovine keratin - Dako)

CK 19 - cytokeratin 19 (Novocastra)

CK 20 - cytokeratin 20 (Dako)

CK 7 - cytokeratin 7 (Dako)

Col IV - type IV collagen (Dako)

CR - calretinin (Dako)

DES - desmin (Dako)

EMA - epithelial membrane antigen (Seralab)

GCDFP-15 - gross cystic disease fluid protein-15 (Signet)

GFAP - glial fibrillary acidic protein (Dako)

Hep Par 1 (Dako)

hCG - human chorionic gonadotropin (Biogenex)

hPL - human placental lactogen (Dako)

HMB-45 - melanoma-associated antigen (Dako)

HMWtCK - high molecular weight cytokeratin (34BH11 - Dako)

LCA - CD 45, leukocyte common antigen (Dako)

LMWtCK - low molecular weight cytokeratin (35BE12 - Dako)

MSA - muscle-specific actin (Enzo)

MYOD1 (Novocastra)

Myog - myogenin (clone F5D - Dako)

NE - neutrophil esterase (Dako) p30-32 = p30-32 glycoprotein (clone MIC2 - Dako; 013 - Signet)

PLAP - placental alkaline phosphatase (Dako)

PSA - prostate-specific antigen (Dako)

PSAP - prostatic acid phosphatase (Dako)

S100 - S100 protein (Dako)

SA - salivary/amylase (Biodesign)

ScA - sarcomeric actin (Dako)

SMA - α-smooth muscle actin (Sigma)

SYN - synaptophysin (Boehringer Mannheim)

VIM - vimentin (Dako)

Appendix 2
Heat-Induced Epitope Retrieval and Antigen Retrieval Protocol

Heat-Induced Epitope Retrieval (HIER)

The need to employ heat-induced epitope retrieval (HIER) as a routine procedure before the commencement of any immunostaining protocol cannot be overemphasized. Since the seminal work of Shi et al (1991), it has been recognized that the heating of deparaffinized tissue sections in a variety of retrieval solutions, up to boiling temperature, results in the `unmasking' of a very wide range of tissue antigens (Leong & Milios, 1993a, b; Gown et al, 1993, Catorretti et al, 1993; Cuevas et al, 1994). Tissues fixed in formaldehyde as well as a variety of common fixatives respond to this treatment with enhancement of a wide range of antigens (Byron, 1997), making it an indispensable requirement of immunolabeling. The method is equally applicable to cytological preparations and cell blocks (Suthipintawong et al, 1996, 1997).

Various solutions have been employed for antigen retrieval but a 10 mM solution of citrate buffer at pH 6.0 is the best universal reagent and can be prepared in the laboratory (2.1 g citric acid monohydrate per litre of water, adjusted to pH 6.0 with NaOH) (Leong et al, 1996). Besides citrate buffer and other `home-made' retrieval solutions, several commercial reagents are available, including Antigen Retrieval Solution (Biogenex), Target Retrieval Solution (Dako), Target Unmasking Fluid (Monosan) and Target Unmasking Fluid (Serotec). We found that all the commercial reagents enhanced immunolabeling but no single reagent was consistently best for all diagnostic antibodies. The Dako product had a slight edge over other reagents including citrate buffer and EDTA at pH 8.0 (Leong et al, 1996). An important observation was that all reagents, including commercial retrieval solutions, were reusable for as many as 20 times without appreciable loss of reactivity, allowing considerable cost savings should commercial reagents be employed.

Shi et al (1995) suggested that pH is an important variable in epitope retrieval solutions and grouped antigens into three categories based on their reactivity at different pH. Antigens like CD 20, PCNA, AE1, EMA and NSE showed excellent retrieval throughout the pH range. MIB1 and estrogen receptor were immunoreactive following treatment in solutions of very low pH and at neutral to high pH, but displayed a dramatic drop of immunoreactivity at moderately acidic pH (pH 3-6). The third group, comprising antibodies such as MT1 (CD 43) and HMB-45, showed increasing intensities of staining in solutions of increasing pH and only weak staining at acidic pH.

Other variables such as temperature, duration of heating, composition of the retrieval solution, including molarity, salt content and presence of metallic ions, and the nature of the antigen of interest may influence immunoreactivity and should be considered when testing a new antibody.

Heat is considered to be the major factor responsible for the `unmasking' of the epitopes `hidden' as a result of protein crosslinking induced by formaldehyde. Microwave irradiation was the first form of heating introduced by Shi et al (1991) as it produces instantaneous and uniform heating in small pieces of biological tissues (Leong et al, 1985). Other methods for generating heat have been employed for antigen retrieval including the conventional water bath, wet autoclaving, steaming,

pressure cooking, hot oven heating in a humidified chamber (reviewed by Taylor et al, 1996) and even the sauna bath (A M Gown, personal communication). The most recent method of generating heat for antigen retrieval is the DNA thermal cycler (Baker-Cairns et al, 1996). While the virtues of each method have been argued, we find microwaves to be the most convenient.

Recently, antigen retrieval has been successfully achieved with ultrasound (Portiansky & Gimeno, 1996). As ultrasound generates negligible amounts of heat, this raises doubts about whether heat is a requirement of the procedure or if molecular kinetics is the mechanism of action. Another area of controversy lies in the concept of `unmasking' of antigenic epitopes produced by crosslinking fixatives. Interestingly, the demonstration that the antigen retrieval procedure also enhances immunoreactivity in tissues exposed to fixatives such as Zenker's and Carnoy's solutions (Byron, 1997), which are not considered to act by protein crosslinkage, raises more questions as to the mechanism of antigen retrieval.

Protocol for Heat-Induced Antigen Retrieval Using Microwaves

1. Mount 5 micron formalin-fixed, paraffin-embedded sections on aminoalkylsilane-coated slides.

2. Deparaffinize in xylene and rehydrate through graded alcohols.

3. Rinse in deionized water followed by rinsing in phosphate-buffered saline (PBS).

4. Block endogenous peroxidase with 0.5% hydrogen peroxide/methanol for 30 min.

5. Wash in PBS.

6. Stack slides on edge in a plastic container (Kartell, Milan, Italy). The transparent container allows stacking of up to 20 slides.

7. Fill the container with 10 mM citrate buffer at pH 6.0, taking care to completely immerse all tissue sections (the container takes about 250 ml of buffer).

8. Cover the container (the Kartell box is provided with a fitting lid that prevents boiling over).

9. Place the container with slides in a microwave oven that has a carousel (NEC model 702, 650 watts). If more than one container is used, place them equidistant at the periphery of the carousel.

10. Irradiate at maximum setting until boiling (about 5 min).

11. As soon as boiling is attained, adjust the power setting so that the solution simmers. Simmer for a further 10 min.

12. Turn off power. Allow the tissue sections to remain in the hot buffer for another 25 min.

13. Remove sections. Block in 3% non-immune horse serum for 20 min before incubating with the primary antibody. Immunostain with a standard immunoenzyme technique.

Note

The duration of heating depends on the fixation of the tissue sections. Over fixed tissues will require longer periods of heating, whereas inadequately fixed tissue sections will detach from the slides. Appropriate adjustments will be necessary to optimize this step.

Immunoreactivity of some antigens can be further enhanced when HIER is performed in special commercial retrieval solutions (Leong et al, 1996) or by variation of the pH of the retrieval solution (Shi et al, 1995) and these will require evaluation especially when detecting antigens of low immunoreactivity.

Proteolytic digestion may enhance the immunoreactivity of some tissue antigens. The optimal stage at which to apply proteolytic enzymes will vary according to the antigen of interest; for example, with E-cadherin, proteolytic digestion is best performed before HIER, whereas for collagen type IV, laminin and cytokeratins, proteolytic digestion is optimally performed after HIER and just before incubation with the primary antibody. As with the duration of heating, the type, concentration of enzyme and duration of digestion will require optimization to suit the antigen of interest as well as the fixation of the tissue.

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