α-Fetoprotein (AFP)

Sources/clones

Accurate, Biodesign (polyclonal), Biogenesis (219–2, BIOAFP003, polyclonal), Biogenex (A-013–01), Bioprobe (F2, C3), Cymbus Bioscience (946.11), Dako (polyclonal), Immunotech (IC5, C3), Pierce (ZGAFP1), Sigma (C3), 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. Other antibodies employed in a panel may be useful in this context. They include anti-albumin (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 is of low specificity, being expressed in various carcinomas), sialoglycoproteins such as B72.3 and Leu-M1 (found in some metastatic adenocarcinomas). Other mimics of HCC are hepatoid tumors that have immunophenotypic characteristics similar to that of HCC including staining for AFP, canalicular staining for CEA and α-1-antitrypsin. Such tumors have been seen in ovary, testis, urinary bladder, breast, lung, thymus, stomach, colon, gallbladder, and pancreas, and focally in germ cell tumors, and represent areas of true hepatocellular differentiation.

Comments

A-013–01 is used routinely following HIER.

Selected references

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NOTES

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 (asml), RDI (asm-1), Sigma (1A4) and Zymed (Z060).

Fixation/preparation

Several of the antibody clones to α-smooth muscle actin (α-SMA) are immunoreactive in fixed paraffin-embedded sections. HIER at 120 °C enhances immunolabeling.

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 kDa. α-Actins are found in muscle cells, beta- and gamma-actins may be present in muscle cells as well as most other cell types in the body including non-muscle cells. 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 muscle-specific 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 single α-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, salivary, and sweat glands, allowing their distinction from neoplastic proliferations. Myoepithelial cells also line benign ductules of the breast, compared to their absence in neoplastic tubules. α-SMA is also a useful marker to identify myofibroblastic differentiation and has been used in studies of idiopathic pulmonary fibrosis and of the fibrogenic Ito cells in the liver. In diagnostic pathology, α-SMA is used mostly as a discriminator of smooth muscle tumors in the identification of spindled and pleomorphic tumors. It is important to emphasize that this marker should not be used in isolation. 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 include α-SMA, desmin, and muscle-specific actin (Appendix 1.23, 1.24). In the diagnostic context of the morphologically indeterminate spindle cell tumor, it should also be remembered that myofibroblasts might 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. 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. Myofibroblastic differentiation is not uncommon in malignant fibrous histiocytoma, so "pleomorphic myofibrosarcoma" has been suggested as an alternative name for this tumor. α-SMA is a useful marker to identify myoepithelial cells and has been used to show the presence of myoepitheliomas in several extrasalivary sites such as the breast, larynx, retroperitoneum, and, more recently, the skin.

Some melanomas may exhibit an aberrant immunophenotype that includes contractile proteins α-SMA and desmin. SMA-positive cells have been suggested to play a

role in the pathogenesis of tubulointerstitial damage in the kidney. The significance of muscle actin expression observed in mesotheliomas is presently unknown. α-SMA expression and staining has recently been demonstrated in articular chondrocytes.

Comments

Clone 1A4 produces the best results in our hands. Immunoreactivity appears not to be enhanced by boiling or proteolytic digestion and is best demonstrated following retrieval at 120 °C in citrate buffer at pH 6.0.

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AMACR

Sources/clones

Clone 13H4: Biocare Medical, Walnut Creek, CA; Corixa Corporation, Seattle, WA; Zeta, DAKO, Carpinteria, CA, USA

Clone P504S: Abgenix Biopharma Inc., Vancouver, BC, Canada; Zeta Corp, Sierra Madre, CA, USA

Fixation/preparation

Standardized for formalin-fixed paraffin-embedded tissue. Cytoplasmic immunohistochemical stain.

Background

α-Methylacyl CoA racemase (AMACR), also called P504S, is a mitochondrial and peroxisomal enzyme involved in the metabolism of branched chain fatty acid and bile acid intermediates. It catalyzes the racemization of α-methyl branched carboxylic coenzyme A thioesters. Deficiency of AMACR is associated with certain adult-onset sensory motor neuropathies. The National Cancer Institute Cancer Genome Anatomy Project Expressed Sequence Tags (ESTs) and Serial Analysis of Gene Expression (SAGE) databases found variable levels of AMACR protein overexpression in a wide range of tissues and cancers including colorectal, prostate, ovarian, breast, bladder, lung, and renal cell carcinomas. Additionally AMACR is expressed in lymphoma and melanoma. AMACR is thus considered a common abnormality in human cancers and is thought to participate in the early stages of development. Prostate and colorectal carcinomas show the highest expression, at 92% and 83% respectively. In prostate carcinoma, AMACR is overexpressed in high-grade prostatic intraepithelial neoplasia (high-grade PIN), invasive adenocarcinoma, and metastatic androgen-independent prostate cancer. AMACR is extensively used as an adjunct immunohistochemical marker for diagnosis of challenging prostate biopsies.

AMACR has also emerged as a putative therapeutic target in cancer treatment. While overexpression of AMACR is seen in a high percentage of the cancers named above, it is either negative or minimally expressed in the adjacent normal tissue. This property makes it a potential candidate for targeted therapy, either as an antibody or as an enzyme inhibitor.

Applications

A. **Use of AMACR in the diagnosis of prostate carcinoma**

AMACR is a sensitive (82–100%) and relatively specific (70–100%) marker for prostate cancer. It is most specific if circumferential luminal to subluminal and diffuse cytoplasmic staining is noted. It is a commonly used tool in the diagnosis of morphologically difficult prostatic adenocarcinomas, in combination with basal cell markers including p63 and 34BE12 (CK903).

1. **AMACR for evaluation of minimal prostatic adenocarcinoma and atypical proliferations**

Immunohistochemical assays for AMACR have become part of routine surgical pathology practice during the past few years. A cocktail stain containing racemase along with p63 and CK903 is becoming increasingly common in the workup of atypical small acinar proliferations (ASAPs) and to support the diagnosis of small foci of prostatic adenocarcinomas. Approximately 10% of cases thought to be atypical can be diagnosed as carcinoma after addition of AMACR to the basal cell marker cocktail. Approximately 45% of atypical diagnoses, rendered by experts, converted to a definitive diagnosis of carcinoma after a positive AMACR.

AMACR staining is not uniform in prostate carcinoma. Approximately 23% of carcinomas are weakly positive, 41% moderately positive, and 35% strongly positive. Histologically benign prostate tissue can sometimes be positive (~8% of cases).

Additionally, premalignant and benign entities that are known to lose basal cell markers may also be positive for AMACR, such as atypical adenomatous hyperplasia (14% of cases), atrophy, partial atrophy, and crowded benign glands.

Conversely, approximately 18% of cases considered to be carcinoma by H&E may be negative for AMACR and basal cell markers. While initial studies suggested that AMACR was uniformly and strongly positive in 97–100% of prostate cancers, more recently only about 82% cancers on needle biopsies were found to be positive.

It is also important to note that unusual morphologic variants including foamy gland, pseudohyperplastic, and atrophic prostate cancers are less frequently positive for AMACR expression, with only 69–80% of cases staining positive. The diagnostic implications of these findings are that while AMACR is a great addition to the armamentarium of stains for the workup of ASAP, it has its limitations. Interpretations based on a positive or negative AMACR should be made with caution, keeping in mind the impression made on H&E.

A cancer diagnosis based on H&E-stained section should not be downgraded to "atypical" on the basis of a negative AMACR.

Use of triple stain (PIN4 cocktail, p63, and 34beta12) or in combination with CK5/6 and p63 is found to increase specificity for detecting prostatic adenocarcinoma in limited needle biopsy material.

2. **AMACR expression in prostatic "pseudoneoplasms"**

Widely recognized mimics of prostatic adenocarcinoma include atypical adenomatous hyperplasia (AAH), adenosis, atrophy, post-atrophic hyperplasia, basal cell metaplasia, and seminal vesicle or ejaculatory duct epithelium. A small number of AAH cases (4/40) reveal focal staining, hence AMACR staining is helpful in distinguishing most, but not all, cases of AAH from adenocarcinoma. Foci of atrophic glands as well as post-atrophic hyperplasia have been shown to be positive for AMACR in 0–36% of cases.

3. **AMACR staining in post-treatment prostate carcinoma**

Radiation therapy has no effect on the staining of prostatic adenocarcinoma in needle biopsies, TURP (transurethral resection of prostate) chips, or salvage radical prostatectomies. On the other hand, benign glands with radiation atypia are found to be negative. A significant decrease in the intensity of AMACR staining is noted in hormone-refractory prostatic adenocarcinoma when compared to clinically localized cancer. To assess residual post-treatment prostatic adenocarcinoma, a panel of stains containing AMACR is useful.

B. **AMACR in renal tumors**

Contemporary classification of renal tumors is based on clinical, morphological, and molecular genetic characteristics. With the emergence of numerous distinct genetic subtypes, often with overlapping histological features, immunohistochemical stains have come to play an increasingly important role in their accurate classification. AMACR has been found to be strongly expressed in papillary renal cell carcinomas, mucinous tubular and spindle cell carcinoma, and acquired cystic disease-associated renal cell carcinoma (RCC).

In a poorly differentiated RCC, or in cases with overlapping histological appearances, AMACR forms a useful adjunct.

Renal tumors with clear and papillary features are often encountered in our daily practice. In this setting the differential diagnoses will include CCRCC (clear cell renal cell carcinoma), PRCC (papillary renal cell carcinoma) with clear cell changes, CCPRCC (clear cell papillary renal cell carcinoma), and Xp11 translocation RCC. While the majority of CCRCCs present with sheet-like growth patterns, rarely, true papillae can be seen. PRCCs can have clear cells, and Xp11.2 translocation RCCs can have clear cells growing as solid sheets and/or lining true papillae. Given the overlapping histological features of these genetically and prognostically distinct entities, immunohistochemical stains for accurate classification become essential.

A panel of stains that includes CAIX, CK7, AMACR, TFE3, and cathepsin K can be used as follows:

- CCRCC is positive for CAIX, while being negative for CK7 and AMACR. Rare cases may show some focal weak staining with AMACR.
- PRCC with clear cell features is strongly positive for CK7 and AMACR. Focal CAIX staining may be seen.
- CCPRCC is positive for CK7 and CAIX, while being negative for AMACR.
- Xp11 translocation RCC is positive for AMACR, TFE3, and cathepsin K. The majority are negative for CK7, but focal positivity for CK7 may be seen. CAIX may be focally positive as well.

C. **Positive AMACR staining in other sites**

1. In the gastrointestinal tract:
   a. Colon adenomas with high-grade dysplasia stain positive for AMACR.
   b. Barrett-related dysplasia is found to be positive: 93% of cases with high-grade dysplasia and 44% of

cases with low-grade dysplasia have been reported positive.

c. Stomach adenocarcinoma may also show positivity for AMACR in approximately 25% of cases.

2. Extramammary Paget's disease: 71% of cases are reportedly positive.

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NOTES

Amyloid

Sources/clones

Amyloid-A (AA)

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

Transthyretin (ATTR/pre-albumin)

Axcel/Accurate (polyclonal), Biodesign (polyclonal), Biogenesis (polyclonal), Dako (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), Zymed (Z022).

Amyloid-β precursor protein (βAPP)

Boehringer Mannheim (polyclonal), Dako (6F/3D), 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. The pattern of distribution, progress of disease, and complications are dependent on the fibril protein. Amyloid is characterized by the following: (1) a typical green birefringence with polarized light after Congo red staining, (2) non-branching linear fibrils with a diameter of 10–12 nm, and (3) an x-ray diffraction pattern which is consistent with Pauling's model of a cross-β fibril. 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. 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. AA-amyloidosis is commonly associated with chronic inflammatory disorders. AL-amyloidosis (either λ- or κ- light chain origin) is linked mainly to plasma cell dyscrasias or is interpreted as being idiopathic. ATTR-amyloidosis is found in cases with familial amyloidosis. AβM-amyloidosis is associated with long-term hemodialysis.

However, a critical issue in the clinicopathological typing of amyloidosis is the interpretation of the immunostaining. 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, which may themselves "contaminate" amyloid deposits. The most critical of these are the immunoglobulin light chains. Based on these aberrant staining patterns, it has been proposed that the identification of a fibril protein with a single antibody demonstrates an even and homogeneous immunostaining for the entire amyloid deposit, while 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. It is strongly recommended to test 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 immune- and Congo red-staining methods. 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 these should contain submucosa. Other recommended sites include subcutaneous fat, sural nerve, heart, kidney, and bone marrow. While AA-amyloidosis is commonly detected in rectal biopsies, any involved organ or tissue is suitable for identification/classification of AL-amyloidosis. Interestingly, it has been shown that long-term hemodialysis-associated β2-microglobulin amyloid may also involve the gastrointestinal and reproductive systems in addition to the usual osteoarticular involvement.

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), while AA-amyloidosis responds better to colchicine and dimethylsulphoxide.

The role of antibodies against amyloid-β precursor protein has assisted in the diagnosis of Alzheimer's disease and early detection of axonal injury in the brain. Antibodies to transthyretin amyloid protein are useful in the diagnosis of cardiac amyloidosis and familial amyloidotic polyneuropathy.

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