Application of markers in the diagnosis of soft tissue tumours

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In this review we describe the application of markers which are useful for the diagnosis of soft tissue tumours in paraffin sections. Detection of intermediate filament proteins appears to be most useful for first screening of these neoplasms because all, except neuroblastomas, express vimentin; cytokeratin is expressed in synovial sarcomas, epithelioid sarcomas and mesotheliomas; desmin in myogenic tumours and glial fibrillary acidic protein in astrocytomas and gliomas. Tissue-specific markers are: factor VIII—related antigen—endothelial cells; myoglobin and skeletal muscle myosin—skeletal muscle cells; neuron specific enolase—neurons and cells of the APUD systems; and leukocyte-associated antigen—leukocytes. Markers which are present in a variety of cell types and therefore do not serve as tissue-specific markers are: S-100 proteins, alpha-1-antichymotrypsin, creatine kinase M and actin. The S-100 antigens have been detected in melanomas, granular cell tumours, chondrosarcomas and in some schwannomas and liposarcomas. Alpha-1-antichymotrypsin has been found in fibrohistiocytic and 'true' histiocytic tumours and creatine kinase M and actin in myogenic tumours. No specific markers have, as yet, been described for fibrosarcomas, Ewing's sarcomas and hemangiopericytomas.

Keywords: soft tissue tumour, immunohistochemical markers

Introduction

Soft tissue tumours constitute a heterogeneous group of widely variable histological appearance (Enterline 1981, Enzinger & Weiss 1983). Many of these neoplasms

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present diagnostic problems and difficulties are often encountered in differential diagnosis. Immunohistochemistry is a technique that is being applied in an increasing number of cases in tumour pathology (Brooks 1982a, Erlandson 1984, Ramaekers et al. 1983a). During the last few years several general and specific markers for soft tissue tumours have been described and antibodies prepared against them. In this review the usefulness of markers for different tumour types is evaluated, subdivided according to their origin. A scheme for a stepwise use of these markers is incorporated and techniques necessary to obtain satisfactory immunohistochemical results are described.

**Tissue preparation for immunohistochemistry**

Sections of formaldehyde-fixed paraffin-embedded tissue are used most frequently for the immunohistochemical detection of antigens in normal tissues and in tumours. This offers the advantage of good preservation of morphology together with convenient handling and storage and it allows retrospective studies. However, many antigenic sites are destroyed by fixation and embedding procedures. Unfixed cryostat sections offer the best preservation of immunoreactivity and are required for the demonstration of antigens, such as plasma membrane-associated antigens, that lose their immunoreactivity after fixation with formaldehyde. However, the morphology of frozen sections is poor and this can introduce difficulties in the distinction between tumour and non-tumour cells.

Fixation may produce chemical changes which lead to partial loss, destruction or inaccessibility of the tissue antigenic sites. Pretreatment of the deparaffinized sections with protease and the development of sensitive immunohistochemical staining techniques have improved detectability of some antigens in paraffin sections and allow us to obtain results comparable to those in cryostat sections.

The application of protease pretreatment to paraffin sections depends on the antigen being looked for. For example, no enzymatic pretreatment is needed for the detection of desmin, vimentin or glial fibrillary acidic protein, at least in our experience, and may result in a decreased staining intensity. On the other hand, the detection of factor VIII related antigen (F VIII RAg) or cytokeratin is greatly enhanced after enzyme preincubation (Table 1). For a detailed review of tissue preparation methods for immunohistochemistry, we refer to the review article of Brandtzaeg (1982).

**Markers in tumour diagnosis**

Figure 1 sets out a scheme for a rational approach to the use of markers described under individual tumours in the ensuing account.
Differential Diagnosis of Carcinoma Versus Sarcoma and Lymphoma

The expression of intermediate filament proteins is specific for a certain tissue type (Osborn & Weber 1983, Ramaekers et al. 1983a) and the presence of cytokeratins in a tumour is a strong indication of its epithelial origin. Squamous cell carcinomas, adenocarcinomas and mesotheliomas can be stained with polyclonal and monoclonal antibodies to cytokeratin in frozen sections (Ramaekers et al. 1983b, c) and, to a lesser extent, with polyclonal antibodies in paraffin sections after predigestion with pro-
Figure 1. Flow diagram for use of markers in soft tissue tumours. AAT=alpha-1-antitrypsin; AACT=alpha-1-antichymotrypsin; F VIII RAg=Factor VIII related antigen; GFAP=glial fibrillary acidic protein; ker=cytokeratin; lam=laminin; LC=leukocyte common antigen; lys=lysozyme; MBP=myelin basic protein; NF=neurofilament proteins; NSE=neuron specific enolase; PNA=peanut agglutinin; SBA=soy bean agglutinin; UEA-I=Ulex europaeus agglutinin I; vim=vimentin; + =positive staining; − =negative staining; −/+=variable result.

 tease. Since the latter may give variable staining results when using the peroxidase-antiperoxidase or avidin–biotinyl–peroxidase complex technique it is best to use these in parallel with the other intermediate filament antibodies, e.g. vimentin. Most sarcomas examined so far have been positive for vimentin in paraffin sections.
Lymphomas can be distinguished from other mesenchymal tumours on the basis of the presence of common leukocyte antigen both in frozen and in paraffin sections (Borowitz, Stevanovic & Gottfried 1984, Dalchau, Kirkely & Fabre 1980, Warnke et al. 1983).

When interpreting staining results of tumours of unknown origin the coexpression of cytokeratin and vimentin in certain tumours should be kept in mind. This has been found in metastatic carcinoma cells in pleural and ascitic fluids and in cultured epithelial cells (Franke et al. 1979, Ramaekers et al. 1983d), as well as in malignant mesotheliomas (Lazarides 1980, Miettinen et al. 1982a, Ramaekers et al. 1983a, Rocca & La Rheinwald 1984, Said, Nash & Tepper 1983), epithelioid sarcomas (Chase et al. 1984), synovial sarcomas (Corson et al. 1984, Miettinen, Lehto & Virtanen 1983), pleomorphic adenomas, renal cell carcinomas (Herman et al. 1983) and nephroblastomas (Ramaekers et al. 1985), and in adenoid cystic carcinoma of the lung.

FIBROSARCOMAS AND FIBROHISTIOCYTIC TUMOURS

This group of tumours demonstrates a broad histological spectrum (Enzinger & Weiss 1983). Positive immunohistochemical reactions have been reported for alpha-1-antitrypsin (AAT) and alpha-1-antichymotrypsin (AACT) (Du Boulay 1982a, Kindblom, Jacobson & Jacobson 1982, Nathrath & Meister 1982, Roholl et al. 1985a) and the expression of these histiocytic markers has been found to correspond with the gradual transition from fibroblast-like cells to histiocytic-like cells (Roholl et al. 1985a). AAT and AACT, however, are also detectable in osteosarcomas and pleomorphic rhabdomyosarcomas (Roholl et al. 1985a). Their presence, therefore, has limited diagnostic value. In addition, albumin (Brooks 1982a, b) and immunglobulin (Inoué et al. 1984) staining has also been noted so that uptake of serum proteins is highly probable.

The enzyme lysozyme can be stained in paraffin sections without protease predigestion (Pinkus & Said 1977), but is of limited value for diagnosis (Burgdorf, Duray & Rosai 1981a, Roholl et al. 1985a), and may only be found in cases of 'true' histiocytic lymphoma (malignant histiocytomas) (Roholl et al. 1985b).

Some lectin receptors have been alleged to be specific for normal (Howard & Batsakis 1982, Roholl et al. 1985b, Strauchen 1983) and malignant histiocytes (Roholl et al. 1985b). We have shown the presence of peanut and soy bean agglutinin binding sites on tumour cells in malignant fibrous histiocytomas, rhabdomyosarcomas and osteosarcomas, whereas fibrosarcomas, leiomysarcomas and liposarcomas were negative (Roholl et al. 1985a).

To aid differential diagnosis, it is useful to use antibodies to desmin—positive in myosarcoma, or S-100 antigen—positive in malignant schwannoma (Weiss, Langloss & Enzinger 1983) and liposarcoma (Hashimoto, Daimaru & Enjoji 1984). The demonstration of desmin in leiomysarcomas is variable. Fibronectin has been documented in some cases of malignant fibrous histiocytoma but not in fibrosarcomas (Du Boulay 1982b).
Antibodies to laminin may be an aid in the differential diagnosis between fibrosarcoma and malignant fibrous histiocytoma which are both negative, and malignant schwannomas which are positive (Miettinen, Foidart & Ekblom 1983b). Granular cell tumours seem to be positive for laminin (Miettinen et al. 1983b, 1984a).

**Rhabdomyosarcoma**

The diagnosis of rhabdomyosarcomas is often difficult, especially when the small cell component of poorly differentiated form has to be distinguished from other types of small round cell tumours such as neuroblastoma, lymphoma and Ewing's sarcoma. To overcome these diagnostic difficulties, several immunohistochemical markers have been introduced. These include desmin (Altmannsberger et al. 1985, Kahn et al. 1983a), the M (muscle) subunit of creatine kinase (De Jong et al. 1985a, Kahn et al. 1983a, Tsokos, Howard & Costa 1983, Wold, Li & Homburger 1981), myoglobin (Brooks 1982b, Corson & Pinkus 1981, Kahn et al. 1983a, Kawaga et al. 1983, Mukai, Rosai & Hallaway 1979, Tsokos et al. 1983), actin (Bussolati et al. 1980, De Jong et al. 1985b, Mukai, Schollmeyer & Rosai 1981), and skeletal muscle myosin (De Jong et al. 1984, Koh & Johnson 1980, Tsokos et al. 1983). These can all be applied to paraffin sections. Only myoglobin, skeletal muscle myosin, and skeletal muscle actin have been found to be specific markers for the detection of cross-striated muscle cell differentiation in tumours. Of the myosin isozymes, only fast myosin (fast twitch type II fibres myosin) has been found to be of value (De Jong et al. 1984). Antibodies against fast myosin and skeletal muscle actin are preferred to antibody against myoglobin. The latter is in general only present in cytoplasm-rich well-differentiated tumour cells and, as a consequence, is of little value for the diagnosis of poorly differentiated rhabdomyosarcoma.

Although creatine kinase M and desmin are not specific markers for the detection of cross-striated muscle differentiation in tumours, they are useful in distinguishing poorly differentiated rhabdomyosarcomas from other types of small round cell tumours in childhood (Altmannsberger et al. 1985). With antibodies against these proteins a higher percentage of poorly differentiated rhabdomyosarcomas is stained than with antibodies against fast myosin, skeletal muscle actin and myoglobin (A.S.H. De Jong, unpublished results).

**Leiomyosarcoma**

So far, no specific markers have been described for leiomyosarcoma, since smooth muscle actin, desmin and myosin can also be detected in other muscle tumour types. Nevertheless, antibodies against desmin are a useful tool in the differential diagnosis of soft tissue tumours (Bures, Barnes & Mercer 1981, Kawai et al. 1983). They can be used to distinguish leiomyosarcoma from malignant fibrous histiocytoma and nerve sheath tumours. The latter may also contain S-100 antigen and myelin basic protein, which are not present in smooth muscle cells. It should be kept in mind that some leiomyosarcomas may give false negative results, in paraffin sections with antibodies.
Markers for soft tissue tumours

The presence of smooth muscle myosin has also been reported in fibrosarcomas, malignant fibrous histiocytoma and in malignant schwannomas (Bures et al. 1981, Donner, De Lanerolle & Costa 1983).

LIPOSARCOMAS

Although the histology of this group of tumours varies greatly, they have in common the synthesis and storage of lipids. The presence of vimentin and S-100 antigen has been reported in liposarcomas. It is not clear at present whether all liposarcomas can be typed by the S-100 antigen. Cocchia et al. (1983) and Hashimoto et al. (1984) reported the presence of S-100, whereas Kahn et al. (1983b) and Nakajima et al. (1982) could not detect this antigen in liposarcomas. A differential diagnosis between malignant schwannomas and the pleomorphic form of liposarcomas is therefore not possible on the basis of this marker. Malignant schwannomas also vary in S-100 positivity (Weiss et al. 1983). Pleomorphic forms of malignant fibrous histiocytomas and liposarcomas may be distinguished on the basis of S-100 (Hashimoto et al. 1984).

NEUROBLASTOMAS

Neurofilaments have been shown to be of little value in the diagnosis of neuroblastoma. In general they can only be detected in ganglion-like cells in ganglioneuroblastoma, whereas neuroblastomas themselves are negative for all of the other types of intermediate filament proteins (Carlei et al. 1984, Osborn et al. 1982, our own observation). Ewing's sarcoma, rhabdomyosarcoma and lymphoma do, however, stain in all cases with an antibody to vimentin, when frozen sections are used. Therefore a negative reaction with all intermediate filament antibodies, at least in frozen sections is, in our opinion, a strong indication of neuroblastoma.

Neuron-specific enolase (NSE) (Taylor et al. 1983) has been reported to be a useful marker in distinguishing neuroblastoma from other types of small round cell tumours in childhood (Dhillon, Rode & Leathem 1982). However, the studies of Tsokos et al. (1984) and Vinores et al. (1984) show that NSE in neoplasia is not restricted to neuronal neoplasms, APUD-derived neoplasms (Schmeckel, Maraugos & Brightman 1979, Sheppard et al. 1984) and melanomas (Dhillon et al. 1982). Neuron-specific enolase was found for instance in many types of tumours of the central nervous system, ductal carcinomas and fibroadenomas of the breast, schwannomas, rhabdomyosarcomas (Vinores et al. 1984), metastatic carcinomas in the brain (Staal et al. 1985) and in neuroendocrine tumours of the gastrointestinal tract and the pancreas (Simpson et al. 1984). Therefore, caution should be exercised in relying on the immunohistochemical demonstration of NSE in tumour diagnosis. Thus we would conclude that so far, no (specific) markers have been described for neuroblastomas.

PERIPHERAL NERVE TUMOURS

In contrast to neuronal cells, the supporting cells of the central and peripheral nervous system and their tumours do not contain neurofilament proteins or NSE (Trojanowski
& Lee 1983). They do contain however the S-100 antigen (Stefansson, Wollman & Jenkovic 1982. Weiss et al. 1983), myelin basic protein (Bonnin & Rubenstein 1984) and vimentin. Not all malignant schwannomas contain S-100 antigens (Weiss et al. 1983). It seems that S-100 proteins are demonstrable only in peripheral nerve neoplasms of Schwann cells (Herrera & Pinto de Moraes 1984). Angervall, Kindblom & Haglid (1984) in contradiction to Enzinger & Weiss (1983), have described the presence of this marker in dermal nerve sheath myxomas. It should be mentioned that the alpha and beta-subunit of S-100 protein is present in malignant melanomas and chondrosarcomas (Takahashi et al. 1984), but only the beta-subunit is present in malignant schwannomas (Isobe et al. 1984). Unfortunately, the heterologous antisera commercially available do not discriminate between these subunits (Takahashi et al. 1984).

Myelin basic protein is a major component of the myelin sheath in the central and peripheral nervous system (Bonnin & Rubenstein 1984) and has been demonstrated in malignant schwannoma (Mogollon et al. 1984) and also in granular cell tumours (Penneys et al. 1983). Its distribution in other soft tissue tumours remains to be studied.

**VASCULAR TUMOURS**

Factor VIII related antigen (F VIII RAg) has been found in endothelial cells lining blood and lymph vessels (Svanholm, Nielsen & Hauge 1984) and in tumour cells derived from them (Burgdorf, Mukai & Rosai 1981b, Nadji et al. 1980, Rucheti, Gerber & Schaffner 1984, Sehested & Hon-Jensen 1981). It is absent in proliferating or newly formed endothelium (Jurco et al. 1982) and in pericytes. This marker is sensitive to formaldehyde fixation and dehydrating procedures (Van Pelt-Verkuil & Emeis 1981). Pretreatment of the paraffin section is therefore necessary and pepsin is preferable to trypsin (Table 1).

The presence of Ulex europaeus agglutinin I (UEA-I) binding sites in endothelium was originally reported by Höltööer et al. (1982). In a comparative study to determine the usefulness of the two endothelial cell markers, Ordóñez & Batsakis (1984) have found UEA-I to be a more sensitive marker than factor VIII related antigens but it may not be more specific. The detection of UEA-I receptors depends on the blood group of the patient. Specimens from patients with blood group O always express these receptors intensely, whereas with other blood groups there is variable expression of them (Watanabe, Shoda & Hanioka 1984). Recently two monoclonal antibodies have been described, recognizing antigenic determinants specifically present on blood vessel endothelium (Cui et al. 1983, Schlingemann et al. 1985) but not on lymph vessel endothelium (Schlingemann et al. 1985). These antibodies do not bind to pericytes, and do not cross react with F VIII RAg or the UEA-I receptors. They can be used on frozen sections only. Cardiac myxomas are positive for F VIII RAg (Morales et al. 1981) and also bind UEA I (Elbers, personal communication). Not all tumours, diagnosed as myxomas, however, are positive for F VIII RAg (McComb 1984) and/or for UEA-I receptors (Elbers, personal communication).
CHONDRO- AND OSTEOSARCOMAS

Both tumours can express the histiocytic markers AT and AACT (Roholl et al. 1985a) and vimentin, whereas chondrosarcomas also express immunoreactivity for S-100 (Kahn et al. 1983b, Weiss et al. 1983) and collagen type II (Horton et al. 1983, Roessner et al. 1982, 1983). Several monoclonal antibodies directed against osteosarcoma cell lines have been produced (Campbell, Price & Baldwin 1984). To our knowledge these antibodies have not yet been used in tumour diagnosis.

SYNOVIAL SARCOMAS

The immunoreactive pattern of spindle- and epithelium cells is different. Cytokeratin is demonstrable in the epithelium-like cells (Corson et al. 1983, Miettinen, Letho & Virtanen 1982b, Mirra, Wang & Bhuta 1984), and these cells also express several enzymes which are lacking in the spindle cells (Pisa et al. 1982). The spindle cell component, however, contains both vimentin and keratin (Corson et al. 1984, Miettinen et al. 1983a). Based on differences in lectin binding properties between normal synovial cells and synovial sarcoma cells, Miettinen & Virtanen (1984) proposed that synovial sarcomas are not the malignant analogues of synovial cells, but are carcinosarcomatous tumours.

EPITHELIOID SARCOMAS

These tumours demonstrate immunoreactivity for both vimentin and cytokeratin, a feature that may be of diagnostic help in distinguishing epithelioid sarcoma from nodular fasciitis, benign and malignant fibrous histiocytoma, malignant melanoma and necrotizing granuloma (Chase et al. 1984). The absence of keratin pearls and dyskeratosis in the epithelium allows differentiation from a squamous cell carcinoma (Enzinger & Weiss 1983).

MESOTHELIOMAS

Malignant mesotheliomas show a positive reaction for cytokeratin (Ramaekers et al. 1983a, Said, Nash & Tepper 1983). The differential diagnosis between sarcomatoid mesotheliomas and fibrosarcomas is difficult so detecting cytokeratin is helpful. The spindle cell component of mesotheliomas may, however, coexpress cytokeratin and vimentin. Furthermore, it has been shown that mesothelial cells produce collagen type I and III in a different ratio from fibroblasts (Harvey & Amlot 1983), a feature that might be used as an additional help in the differential diagnosis.

MISCELLANEOUS

Hemangiopericytomas

The presence of vimentin and smooth muscle myosin (Donner et al. 1983) have been described in these tumours.
Ewing's sarcomas and Kaposi's sarcomas

The presence of F VIII RAg has been reported for Ewing's (Roessner et al. 1982) and also for Kaposi's sarcomas (Guarda et al. 1981) but Navas-Palacios, Aparicio-Duque & Valdé (1984) could not detect this antigen or UEA-I binding sites in tumour cells of Ewing's sarcomas. Endothelial cells should possess predominantly type III collagen and not type I (Prockop et al. 1979). Cultured tumour cells of Ewing's sarcomas produced collagen type III and not type I (Stern et al. 1980) but others (Harvey et al. 1982) reported that these tumour cells synthesized predominantly collagen type I.

Mixed mesodermal tumour

The different components within mixed mesodermal tumours, carcinomatous sarcomatous and rhabdomyoid, can be recognized using antibodies to the appropriate intermediate filament proteins (Ramaekers et al. 1983c).

Granular cell tumours


Discussion

Several tumour characteristic antigens have been described as useful markers in diagnostic tumour pathology. For example, F VIII RAg and UEA-I binding sites specifically recognize endothelial cells and the presence of these antigens justifies the presumption of a tumour of endothelial origin. Their absence or their non-detectability, however, does not rule this out. Absence of staining can be caused by several fixation artefacts, but may also be the result of reduced expression of marker antigens. Reduced or non-expression of marker antigens can be dependent on the degree of differentiation of a tumour. A differentiation-dependent expression has been described for some intermediate filament proteins (Damjanov 1982), S-100 (Herrera & Pinto de Moraes 1984, Weiss et al. 1983), neuron specific enolase (Carlei et al. 1984), and myogenic markers (Altman et al. 1985, De Jong et al. 1984). Reduced expression of marker antigens may also be a phenotypic change due to malignant transformation per se. This phenomenon has been reported for fibronectin (Yamada & Olden 1978).

A consequence of decreased expression is that a well developed sensitive staining method is a prerequisite for detection of such antigens. In this way a minimal number of false-negative results will be obtained. Current developments in this area are promising (Hsu, Raine & Fanger 1981, Holgate et al. 1983). The application of other fixatives and/or other embedding procedures (Brandzaeg 1982, Hancock, Kraft & Atkins 1982, Stein et al. 1984) may affect detection level of antigenic markers and thus
facilitate the introduction of immunohistological techniques in routine histopathology. For an immunodiagnostic evaluation it is not only important to confirm a presumed diagnosis but in addition to exclude other diagnostic possibilities. Knowledge of the distribution pattern of markers is therefore a prerequisite for immunodiagnostic evaluation. The reported distribution patterns of S-100 proteins illustrate this. Originally these were thought to be specific for nerve tissue. Their presence has now been described in a variety of non-nerve sheath cell types, but not all nerve sheath cell tumours are positive for these antigens (Weiss et al. 1983). In addition, the alpha and beta subunit of S-100 each have their own distribution patterns (Isobe et al. 1984, Molin et al. 1984, Takahashi et al. 1984). In our opinion their distribution patterns in soft tissue tumours have not yet been completely investigated. The same applies to other markers, such as myelin basic protein and laminin, which have been selectively used whereas myogenic markers have been widely investigated in different tumour types.

It has to be borne in mind that large numbers of macrophages may be present within tumours (Evans & Cullens 1984). These cells are able to phagocytose degenerate material. In addition they themselves may express certain of the markers we have discussed (Eusebi, Bondi & Rosai 1984). It is essential therefore to exclude these non-malignant cells from the immunodiagnostic evaluation. Finally we would stress that not all the markers so far described are of value in diagnosis. Furthermore, specific markers or a characteristic pattern of markers have not yet been recognized for two large groups of soft tissue tumours: malignant fibrous histiocytomas and liposarcomas. It is obvious that more sophisticated markers are needed in this area, and monoclonal antibodies will perhaps bring us closer to this goal.

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