THE ROLE OF IMMUNOHISTOCHEMISTRY IN THE DIFFERENTIAL DIAGNOSIS OF SOFT-TISSUE TUMORS

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Introduction

Soft-tissue tumors are rare neoplasms. Benign tumors occur with an annual incidence of 300 per 100,000 population and outnumber malignant tumors by a margin of approximately 100:1.1 Malignant soft-tissue tumors, therefore, constitute less than 1% of all cancers. Due to their rarity as well as the wide variation and frequent overlap in their histopathological features, accurate diagnosis of soft-tissue tumors is a constant challenge to pathologists. For these reasons, close communication among pathologists, radiologists, and surgeons is as essential in the evaluation of soft-tissue tumors as it is in the evaluation of tumors of the bone. In many cases, a diagnosis can be reached with confidence by histopathology alone, but in certain cases, even the application of the full armamentarium of available diagnostic methods leaves the pathologist uncertain about the exact nature of the neoplasm. In many situations, however, treatment may not differ for tumors of similar histological grade, regardless of the cell of origin, and the clinician is usually satisfied knowing the histological grade and the status of the margins of resection. However, today’s advances in diagnostic imaging and therapeutic strategies require more than ever an accurate classification of soft-tissue tumors, both for statistical purposes and for the correct application of newly developed therapeutic protocols.

Classification of soft-tissue tumors can be approached from a scientific point of view (based on their presumed histogenesis or cellular differentiation) or from a clinical management point of view (based on identification of subgroups of therapeutic importance) (Table 1 – please see hard copy of journal). In any case, it is important to acquire as much information as possible regarding the following factors: (1) general clinical information (age, sex, previous medical history, etc), (2) specific information about the tumor itself (location, size, relationship to surrounding tissues, rate of growth, etc), (3) histopathological features (cellularity, growth pattern, matrix production, cell size and shape, atypia and anaplasia, mitoses, necrosis, etc), (4) antigenic profile, and, (5) whenever necessary, electron microscopic features and molecular data. Ancillary studies are not needed in all cases, however, and common sense and knowledge about advantages and limitations of each procedure should reduce the unnecessary use of limited resources. Furthermore, the use of ancillary studies generally plays a supportive role and should always be subordinate to the evaluation of the overall data regarding the case.

Over the years, the role of one of these ancillary procedures, immunohistochemistry (IHC), has greatly enhanced our capabilities to properly classify certain entities. Interpretation of IHC results, however, is dependent on proper technique, strict use and interpretation of well-characterized positive and negative controls, and detailed knowledge about the performance of reagents.

Pathologists must proceed cautiously and consider IHC results in the context of all available data in a given case. This is due in part to our limited understanding of the ontogenesis of soft-tissue tumors and to the demonstrated tendency to aberrant antigen expression in neoplasias in general and soft-tissue tumors in particular. Therefore, pathologists must be aware not only of the typical profile and reported antigenic infidelities of a particular entity, but also of the pitfalls that can be introduced by technical factors, such as tissue processing and fixation as well as the IHC procedures themselves. It is estimated that IHC is confirmatory of a single diagnosis in 30% to 40% of cases, it is helpful in guiding the differential diagnosis in 50% to 60% of cases, and it is not contributory in 1% to 2% of cases. IHC in fact adds confusion to the diagnostic process in 5% to 10% of cases.3

Over the years, the number of commercially available high-quality reagents has steadily increased. Markers initially thought to be specific for a particular cell type, however, have proven to be nonspecific. Examples are the expression of muscle markers in myofibroblastic and fibrohistiocytic tumors, desmin in bladder carcinomas, neuron-specific enolase and S-100 in rhabdomyosarcomas, and cytokeratins in melanomas and sarcomas. Approximately 30 markers are used regularly in the differential diagnosis of soft-tissue tumors (Table 2).

<table>
<thead>
<tr>
<th>Histogenesis</th>
<th>Markers</th>
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<tr>
<td>Mesenchymal (general)</td>
<td>Vimentin</td>
</tr>
<tr>
<td>Epithelial</td>
<td>Cytokeratins, epithelial membrane antigen</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>Desmin, HHF35, smooth muscle actin</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>Myoglobin</td>
</tr>
<tr>
<td>Fibrohistiocytic</td>
<td>CD68, factor XIIIa</td>
</tr>
<tr>
<td>Nerve sheath</td>
<td>Leu7, glial fibrillary acidic protein</td>
</tr>
<tr>
<td>Melanocytic</td>
<td>HMB45</td>
</tr>
<tr>
<td>Neuronal</td>
<td>Neurofilament</td>
</tr>
<tr>
<td>Endothelial, perivascular</td>
<td>Factor VIII, CD34, CD31, Ulex europaeus</td>
</tr>
<tr>
<td>Hematopoietic</td>
<td>Leukocyte common antigen, CD3, CD20, Ki-1</td>
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<tr>
<td>Lipomatous</td>
<td>Immunohistochemistry generally not used</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>Neuron-specific enolase, chromogranin, synaptophysin</td>
</tr>
</tbody>
</table>

Ewing's sarcoma/PNET  MIC-2 (O-13)

PNET = primitive neuroectodermal tumor
Immunohistochemical Markers

**Mesenchymal Marker**

- **Vimentin** -- Although of limited value in diagnosis, vimentin, a mesenchymal intermediate filament, can be demonstrated in most properly fixed tissues and therefore is used to identify antigen loss during processing. Thus, if vimentin is not identified in tissue that should express it, the test sample should be interpreted cautiously or entirely avoided.

**Neuronal, Nerve Sheath, and Melanocytic Markers**

- **S-100 Protein (S-100)** -- Widely distributed in peripheral and central nervous systems, the S-100 protein may play a role in ion regulation. It localizes to both the nucleus and the cytoplasm and, given the appropriate histology and a specific differential diagnosis, S-100 is one of the most useful markers. It is expressed in astrocytes, oligodendrocytes, Schwann cells, adenohypophysis, adrenal medulla, and a variety of other cells including chondrocytes, adipocytes, hystiocites, and interdigitating reticulum cells of the lymph nodes. Neurofibromas and neurilemmomas express S-100 diffusely, and 50% to 70% of malignant peripheral nerve sheath tumors express S-100 focally. S-100 is also expressed in 90% of clear-cell sarcomas or melanoma of soft parts, occasionally expressed in leiomyomas and leiomyosarcoma, liposacomas, osifying fibromyxoid tumors, and rarely expressed in synovial sarcomas and chondrosarcomas. Melanomas express S-100, a feature that helps in the differential diagnosis of sarcoma-like melanomas. Chordomas coexpress both S-100 and cytokeratins.

- **HMB45** -- The antigen recognized by this antibody is located in premelanosomal vesicles. HMB45 is very helpful for melanocytic lesions and related entities since it is expressed in 89% of melanomas, almost all clear-cell sarcomas, and tumors related to tuberous sclerosis (rhabdomyoma, angiomylipoma, and lymphangiomatosis). However, it is expressed in only 22% of desmoplastic, neurotropic spindle-cell melanomas. HMB45 is not expressed by alveolar soft-part sarcoma, chondroid lipoma, leiomyoma, leiomyosarcoma, malignant peripheral nerve sheath tumors, or osifying fibromyxoid tumors.

- **Neurofilament Protein** -- Useful in the differential diagnosis of small round-cell tumors, neurofilament protein is expressed by many neuroblastomas, medulloblastomas, retinoblastomas, and peripheral neuroepitheliomas, and it is expressed focally in rhabdomyosarcoma and occasionally in malignant fibrous histiocytoma. It has also been demonstrated in Merkel cell tumors and tumors of endocrine origin.

- **Leu-7 (CD57)** -- An antigenic marker for natural killer cells, Leu-7 can be expressed by a variety of neuroendocrine and non-neuroendocrine tumors. Although expressed in nerve sheath tumors and small round-cell tumors of childhood such as neuroblastoma, prominent expression in rhabdomyosarcoma limits its use in the differential of small round-cell tumors.

- **Synaptophysin** -- Present in the presynaptic vesicles of nerve cells, synaptophysin is expressed by tumors of neuronal origin including neuroblastoma, ganglioneuroblastoma, oolfactory neuroblastoma, melanotic neuroectodermal tumor of infancy, peripheral neuroepitheliomas, and rare rhabdomyosarcomas.

- **Neuron-Specific Enolase** -- The use of neuron-specific enolase is limited due to frequent, nonspecific background staining, particularly when polyclonal antibodies are used. It is expressed in over 50% of neuroblastomas, paragangliomas, and various endocrine tumors, in one third of malignant melanomas, and in 2% of nonneural tumors.

- **Myelin Basic Protein** -- This protein constitutes approximately one third of the myelin sheath and can be identified in benign and malignant Schwann cell tumors and granular cell tumors. It may be useful to distinguish malignant schwannoma from malignant melanoma.

- **Chromogranin** -- This protein is a member of a family of acidic glycoproteins (the most abundant is chromogranin A) located in the soluble fraction of neurosecretory granules. It is used as a panendocrine marker since it is expressed by a majority of neuroendocrine tumors.

**Endothelial/Vascular Markers**

- **CD31** -- The antigen, GPIIa, the cellular adhesion molecule PECAM-1 (platelet endothelial cell adhesion molecule), belongs to the immunoglobulin superfamily and is expressed by some hematopoietic and endothelial cells. It has been shown to have a sensitivity and specificity of 100% for endothelial lesions. It is expressed by 80% to 100% of angiosarcomas and hemangioendotheliomas. However, it is also weakly expressed by rare carcinomas and mesotheliomas and in rheumatoid arthritis.

- **Factor VIII Antigen (FVIII)** -- This is a complex of factor VIII-C (anti-hemophilic factor) and factor VIII-associated antigen (von Willebrand factor). Restricted to endothelial cells and megakaryocytes, it is less specific for endothelial neoplasms than CD31 and CD34. However, it is useful as a confirmatory marker, particularly in well-differentiated tumors.

- **Blood Group Antigens (ABO)** -- *Ulex* lectin, derived from *Ulex europaeus*, binds to the H substance of the ABO system. It seems to be more sensitive than factor VIII in the recognition of endothelium and angiosarcomas. However, it is less specific since it also recognizes a variety of normal cells and some sarcomas.

- **CD34** -- The antigen CD34, a transmembrane glycoprotein present on human progenitor cells and endothelial cells, is a very sensitive marker for endothelial differentiation, staining neoplastic endothelium more strongly than normal endothelium. It is expressed by 70% of angiosarcomas, 90% of Kaposi’s sarcomas, and 100% of epithelioid hemangioendotheliomas. However, CD34 has a much broader reactivity. It is expressed by certain cells around skin adnexal structures and by nerve sheath lesions, benign and malignant solitary fibrous tumors, gastrointestinal tumors, and 50% of epithelioid sarcomas. The coexpression of CD34 and cytokeratin is observed in epithelioid sarcomas, epithelioid angiosarcoma, and glandular schwannoma. Also, 88% of dermatofibrosarcoma protuberans expressed CD34 compared with only rare cases of benign fibrous histiocytoma and dermatofibroma. CD34 in conjunction with F13a is used in the differential diagnosis of superficial spindle-cell lesions. Both markers are expressed by Kaposi’s sarcoma and are absent in keloids. In dermatofibrosarcoma protuberans, CD34 is expressed while F13a is not. The opposite is true for benign fibrous histiocytoma and dermatofibroma.

**Muscle Markers**

- **Desmin (Des)** -- This intermediate filament of skeletal (Z zone), cardiac, and smooth muscle (dense bodies) is expressed in 95% of rhabdomyosarcomas
Actins -- These contractile proteins are classified as alpha (skeletal, cardiac, and smooth muscle), beta (cytoplasmic), and gamma (smooth muscle and cytoplasmic). Myosin-specific actin recognizes all alpha actins (skeletal, smooth, and cardiac) and gamma smooth muscle actin. It does not react with non-muscle actins. The pattern of reactivity is usually at the periphery of the cytoplasm. Fibromatosis, fibrohistiocytic lesions, malignant fibrous histiocytoma, and myoepithelial lesions may express muscle-specific actin. The specificity of smooth muscle actin is more restricted than muscle-specific actin. It does not detect skeletal and cardiac (alpha actins) or gamma smooth muscle actins. It is expressed in smooth muscle neoplasms and in non-smooth muscle lesions with myoid differentiation such as nodular fasciitis and myofibroblastic lesions, which are characterized by expression of smooth muscle actin and muscle-specific actin but lack expression of desmin.

Myoglobin -- This marker is expressed only in skeletal muscle and in approximately half of rhabdomyosarcomas. Careful interpretation is required because it can be released from adjacent damaged muscle and phagocytosed by neoplastic and non-neoplastic cells.

Fibrohistiocytic Markers

CD68 -- This 110-kd glycoprotein is found in the lysosomes of monocytes and macrophages and in primary granules of neutrophils found in normal hepatocytes, renal tubules, and melanomas, as well as potentially in any tumor containing lysosomal granules or phagolysosomes. Since it is variably expressed in approximately 50% of malignant fibrous histiocytoma cases, it is considered not specific of this diagnosis. Expression of CD68 should not be used as evidence of histiocytic lineage.

Factor XIIIa (F13a) -- This intracellular form of the fibrin-stabilizing factor is found in serum and may be engulfed by neoplastic cells instead of being actively produced. It is expressed by histiocytic cells such as the dermal dendrocyte, which also expresses CD34. F13a can be used in the differential diagnosis of benign fibrous histiocytoma/dermatofibrosarcoma protuberans (see above) and juvenile xanthogranuloma vs histiocytosis X.

Epithelial Markers

Epithelial Membrane Antigen (EMA) -- This antigen represents a complex of high-molecular-weight cytokeratins isolated from the human milk fat globule (HMFG) membrane. Approximately 75% of the epithelial-like sarcomas (epithelioid and synovial sarcomas) express EMA. EMA is also expressed in perineural tissues, malignant peripheral nerve sheath tumors, leiomyosarcomas, surface of plasma cells, histiocytes, and T-cell lymphomas.

Cytokeratins -- Cytokeratins consist of a group of 19 polypeptides with molecular weights ranging from 40 to 67 K. Cytokeratins are expressed in the vast majority of, if not all, epithelial-like sarcomas such as epithelioid and synovial sarcomas, in many rhabdoid tumors, and in mesotheliomas. Cytokeratins, particularly 8 and 18, are expressed transiently in many mesenchymal cells, a phenomenon more readily apparent in frozen sections and demonstrated at the mRNA level. Whether this represent a regression to the embryonic stage, a result of cell proliferation, or some other reason is unclear. To complicate matters further, many sarcomatoid carcinomas lack diffuse expression of cytokeratins and may aberrantly express other mesenchymal markers.

Miscellaneous

P503/2/MIC-2 Gene Product (CD99) -- Located in the short arm of the sex chromosome, it encodes a surface protein first described in T-cell and null-cell acute lymphoblastic leukemia. Two recent antibodies that detect MIC-2 epitopes, HBA-71 and O13, are useful in the diagnosis of Ewing’s sarcoma and peripheral neuroepitheliomas.

Immunoprofiles of Soft-Tissue Tumors

Interpretation of IHC profiles requires knowledge about the phenotypic range of each marker and the range of marker reactivity in each entity. The use of predefined marker panels is highly recommended, although given current health care cost containment, the inclusion or exclusion of a particular reagent should be considered in the context of the histopathological features of the neoplasm (Table 3).
Fibrous Tumors

The main components of fibroconnective tissue are fibroblasts and myofibroblasts (a modified fibroblast), and the extracellular matrix containing collagen and a gel-like (ground) substance. Fibroblasts and myofibroblasts produce procollagen and collagen and express vimentin, actin and, to a lesser degree, desmin. These markers are also expressed by fibrous tumors, both benign and malignant. In some entities such as fibromatosis, other markers (eg, smooth muscle actin, factor XIIIa, myosin) can also be found.

Fibrohistiocytic Tumors

Fibrohistiocytic tumors are probably derived from fibroblasts. Benign tumors, fibrous histiocytes, are frequently confused with other lesions (eg, nodular fasciitis, neurofibroma, leiomyoma) or other fibrohistiocytic tumors (eg, dermatofibrosarcoma protuberans). Due to the lack of specific markers for fibrohistiocytic lesions, the diagnosis is generally based on the absence of markers for other lineages. Dermatofibrosarcoma protuberans, for instance, strongly express CD34, and S-100 is expressed by neurofibromas. Malignant tumors such as malignant fibrous histiocytomas have been the subject of a long-lasting debate not only about whether they are of histiocytic origin -- a hypothesis that is now seriously disputed -- but also about whether they represent a homogeneous entity or a collection of sarcomas in which differentiation is not readily evident. IHC helps to differentiate them from other pleomorphic tumors such as anaplastic carcinoma, melanoma, or other pleomorphic sarcomas. Focal immunoreactivity can be found for markers such as keratin, desmin, and neurofilament protein. This should not be interpreted as evidence of a particular ontogenesis. In general, the initial diagnosis of malignant fibrous histiocytoma is maintained in only approximately 50% of cases once sophisticated diagnostic methods are used.16

Adipose Tumors

Normal adipocytes and lipoblasts express S-100. Although the use of S-100 has been recommended in the differential diagnosis with malignant fibrous histiocytoma, it is important to note that liposarcomas vary in intensity of expression and poorly differentiated tumors may lack expression entirely. Smooth muscle actin may indicate focal muscle differentiation.

Smooth Muscle Tumors

Although the quality of reagents has steadily improved, interpretation of IHC profiles in smooth muscle tumors has to consider the aberrant expression of these markers in non-smooth muscle neoplasias. Muscle-specific actin is consistently expressed in most leiomyosarcomas, but desmin is more variably expressed. Focal expression of any marker should not be interpreted as evidence of muscle differentiation. Furthermore, other antigens such as cytokeratins, EMA, S-100, Leu-7, and even CD34 have been demonstrated in leiomyosarcomas.25

Striated Muscle Tumors

Striated cells express myoglobin, desmin, alpha-smooth muscle actin, and occasionally S-100, vimentin, and Leu-7. Other antigens such as myosin, creatine kinase, beta-enolase, and titin are less sensitive. In general, the intensity of expression correlates with the degree of rhabdomyoblastic differentiation. S-100 and cytokeratins have been found in poorly differentiated tumors.26

Vascular Tumors

Vascular markers such as CD34, factor VIII-associated protein, or Ulex europaeus are variably expressed. Most hemangioendotheliomas express factor VIII, although the kaposiform type may not express either this marker or Ulex europaeus while still expressing CD34. These markers are sometimes not expressed by the more aggressive angiosarcomas. For these tumors, CD31 has been reported to be more specific. Lymphatic endothelium expresses factor VIII, CD31, and Ulex europaeus, thus complicating the differential diagnosis of lymphangioma/lymphangiosarcoma and hemangioma/angiosarcoma.25 Thus, the diagnosis should be made histologically whenever possible.

Perivascular Tumors

Histology is usually very characteristic in this category of tumors, making diagnostic confusion unlikely. Very cellular tumors, however, can be mistaken for adenexal tumors. Adnexal tumors express cytokeratin, carcinoembryonic antigen (CEA), or EMA, markers not found in glomus tumors or hemangiopericytomas. Nevi can be distinguished from perivascular tumors by their expression of S-100. Glomus tumors express vimentin and muscle-actin isoforms and, to a variable degree, desmin. Laminin and collagen IV, constituents of basal lamina, can be found outlining cells or groups of cells. Hemangiopericytomas variably express vimentin, CD34, and factor XIIIa, but they do not express factor VIII, Ulex europaeus, or smooth muscle actin. Occasional expression of S-100, Leu-7, and myelin-associated glycoprotein has also been reported.27

Synovial Tumors

Cells of benign synovial and tenosynovial tumors are most likely related to monocytes and macrophages and express cell surface markers such as HLA-A, -B, -C, -D, -DR, L3T4, and Leu-7. In malignant tumors, these markers may be variably expressed and in some cases may also be positive for smooth muscle actin and factor XIIIa. In general, however, the expression of these markers is less consistent than in other sarcomas. Thus, the diagnosis of synovial sarcoma is usually based on the absence of markers for other lineages. Dermatofibrosarcoma protuberans, for instance, strongly expresses CD34, and S-100 and cytokeratins have been reported.25 Other markers, such as factor VIII, CD31, and Ulex europaeus, are variably expressed. Most hemangioendotheliomas express factor VIII, CD31, and Ulex europaeus, thus complicating the differential diagnosis of lymphangioma/lymphangiosarcoma and hemangioma/angiosarcoma.25 Thus, the diagnosis should be made histologically whenever possible.
and DR, LCA, Leu-M3, and Leu-3. This has been interpreted as a potential osteoclastic lineage. Synovial sarcomas contain various degrees of epithelial and spindle-cell elements. Both elements generally express low- and high-molecular-weight cytokeratins and EMA, found even in monophasic fibrous types where histopathological examination does not reveal evident epithelial elements. Cytokeratin 7 and 19 seem to be specific for this tumor, and Leu-7 and S-100 can also be found. Another tumor, epithelioid sarcoma, also coexpresses epithelial and mesenchymal markers. These two tumors have been found to coexist at the same location and may be somehow related. Histology helps in the differential diagnosis since, in epithelioid sarcoma, epithelial and mesenchymal elements are not clearly demarcated and mucin is not produced. Pure epithelial synovial sarcomas, however, are difficult to distinguish from adnexal or metastatic carcinomas.\textsuperscript{28}

### Mesothelial Tumors

Mesotheliomas can present a variety of histological patterns (epithelial, fibrous, biphasic) and therefore often present diagnostic problems, particularly with sarcomas and adenocarcinomas. In general, an extensive panel is necessary to support the diagnosis of mesothelioma on the basis of negative expression to several of these markers. A recommended panel includes vimentin, cytokeratin CEA, EMA, Leu-M1, Ber-EP4, and B72.3. Other antibodies are still under evaluation (HMFG2, ME1, ME2, calretinin, and K1) (Table 4).\textsuperscript{29}

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Mesothelioma (%)</th>
<th>Carcinoma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Epithelial membrane antigen</td>
<td>80</td>
<td>100</td>
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<tr>
<td>Thrombomodulin</td>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td>Leu-M1</td>
<td>100</td>
<td>75</td>
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<tr>
<td>S-100</td>
<td>100</td>
<td>85</td>
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<tr>
<td>Carcinoembryonic antigen</td>
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<tr>
<td>Placental alkaline phosphatase</td>
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<td>65</td>
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<tr>
<td>Vimentin</td>
<td>40</td>
<td>90</td>
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<tr>
<td>Ber-EP4</td>
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<td>Calretinin</td>
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<td>90</td>
</tr>
<tr>
<td>B72.3</td>
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<td>70</td>
</tr>
</tbody>
</table>

Data on two series of 128 and 68 patients.

Table 4. -- Expression of Markers in Mesothelioma and Carcinoma

### Peripheral Nerve Tumors

In contrast to neurofibromas, which contain a mixture of cells, neurilemmomas consist predominantly of Schwann cells (schwanoma) and therefore express S-100 protein, variably Leu-7, and occasionally glial fibrillary acidic protein. Leiomyosarcomas may show some histological resemblance, but they generally do not express S-100 protein. Neurofilament protein helps in the distinction of neurilemmomas and neurofibromas. Malignant tumors arising from nerves or showing nerve sheath differentiation are better designated as "malignant peripheral nerve sheath tumors" since they often recapitulate Schwann cells, perineural fibroblasts, or fibroblasts. Nerve sheath differentiation can be identified using markers such as S-100 protein, Leu-7, and myelin–basic protein. S-100 is expressed, albeit focally, in 50% to 90% of malignant peripheral nerve sheath tumors, Leu-7 in approximately 50%, and myelin–basic protein in 40%. None of these markers is specific; therefore, it is better to use the entire panel. With the exception of rare forms of glandular schwannomas, cytokeratin is not expressed. A potential confusion may arise with synovial sarcomas, but these tumors only rarely express S-100. S-100 is only focally expressed in neurilemmomas, while neurofibromas express S-100 diffusely.

Clear-cell sarcomas, or melanomas of the soft parts, are unique tumors that may produce melanin and are intimately associated to tendons or aponeuroses. They express S-100 and often HMB45, neuron–specific enolase, and Leu-7. The absence of mucin and the presence of melanin distinguish them from synovial sarcomas.\textsuperscript{30}

### Primitive Neuroectodermal Tumors and Related Lesions

Molecular studies have recently revealed that peripheral neuroepithelioma (primitive neuroectodermal tumor or PNET), and Ewing’s sarcoma, entities that were once considered unrelated, are perhaps best considered as members of the same family. In fact, Ewing’s sarcoma and peripheral neuroepithelioma share a common 11/22 chromosomal translocation and are currently considered different degrees of differentiation of the same neoplasia. Desmoplastic small round–cell tumors, malignant ectomesenchymomas, clear-cell sarcomas, and extraskeletal myxoid chondrosarcomas are also considered members of the Ewing’s sarcoma family.\textsuperscript{31}

Peripheral neuroepitheliomas express neuron–specific enolase and the cell surface antigen p30/32 (CD99), encoded by the n-cad gene and detected with the HBA71 and 013 antibodies. This antigen is also detected in Ewing’s sarcomas and in some lymphomas and rhabdomyosarcomas. Although Leu-7, synaptophysin, S-100 protein, neurofilament protein, and chromogranin are variably expressed, glial fibrillary acidic protein is consistently negative. Isolated reports indicate that Ewing’s sarcoma may even express low-molecular-weight cytokeratin.\textsuperscript{31}

### Neuroblastoma

Although a number of antigens can be found on neuroblastomas, neuron–specific enolase is the most sensitive. It is present, at least focally, in almost all neuroblastomas and with greater intensity in the most differentiated tumors. However, it is also expressed in many other small round-cell tumors such as Ewing’s sarcoma and rhabdomyoblastoma, thereby limiting its use in differential diagnosis. Another marker found in neuroblastoma whose expression depends on the degree of differentiation and fixation conditions is neurofilament protein, which localizes preferentially to cells that show ganglionic differentiation. S-100 is found in areas of ganglioneuromatous differentiation, allowing perhaps the grading of tumors according to pattern of expression as a correlate of differentiation. Other markers such as chromogranin, synaptophysin, and vasointestinal peptide can also be found in the most differentiated tumors.

### Paragangliomas

The paraganglia are a collection of neural crest cells arising in association with autonomic ganglia. Paragangliomas express neuron–specific enolase, S-100 and, in some cases, glial fibrillary acidic protein, gastrin, and serotonin. In paragangliomas, S-100 is expressed at the periphery of the cell groups. Several peptides can also be variably identified, including leu-enkephalin (76%), met-enkephalin (75%), substance P (31%), vasointestinal peptide (30%), pancreatic polypeptide (51%), somatostatin (67%), bombesin (15%), calcitonin (23%), neurotensin (12%), and corticotropin (28%).\textsuperscript{32}
Extraskeletal Cartilaginous and Osseous Tumors

Myxoid chondrosarcoma (chordoid sarcoma), a form of extraskeletal chondrosarcoma, expresses S-100 protein and, rarely, cytokeratin and Leu-7. In general, however, the role of IHC in the differential diagnosis of cartilaginous neoplasms relies in the demonstration of the absence of specific markers for other lineages. The role of IHC in extraskeletal osseous tumors is also limited in ruling out other neoplastic lineages, particularly in the context of a small round-cell neoplasm, where the diagnoses of small-cell osteosarcoma and Ewing’s sarcoma are considered.

Tumors of Uncertain Origin

Myxoid neoplasms such as myxoma must be distinguished from other myxoid entities, eg, myxoid liposarcoma, myxoid cartilaginous lesions, or myxoid malignant fibrous histiocytoma. Myxomas do not express S-100 protein. S-100 protein is expressed by lipoblasts and chondroblasts. Alveolar soft-part sarcomas are considered to be of striated muscle origin, given their expression of vimentin, muscle-specific actin, MyoD1, desmin, and the lack of markers for other lineages. Epithelioid sarcoma shares some characteristics with synovial sarcoma, discussed previously, and although the exact cell of origin is unknown, an origin in synovioblastic mesenchyme is likely. IHC plays an important role in the diagnostic workup of another entity of dubious origin, malignant extrarenal rhabdoid tumor, which must be differentiated from other poorly differentiated neoplasms such as rhabdomyosarcoma, synovial sarcoma, mesothelioma, epithelioid sarcoma, carcinoma, and melanoma. Desmoplastic small-cell tumor of childhood expresses cytokeratin, EMA, desmin, vimentin and, variably, S-100 protein, Leu-7, Leu-M1, and synaptophysin. Neurofilament protein, chromogranin, and CEA are generally not expressed. The cell of origin is unknown.

Conclusions

The availability of high-quality reagents and the improvement, simplification, and automation of procedures have made IHC an indispensable tool for the solution of the diagnostic challenges facing the pathologist. In the field of soft-tissue pathology, IHC, on the one hand, has confirmed the diagnostic accuracy of previous generations of pathologists who, based on morphology alone, have made sense of a frightening number of entities. On the other hand, it has revealed the inherent capabilities of histogenetically unrelated tumors to adopt variable and often overlapping morphological features. Today, the role of IHC is so firmly established that pathologists often tend to rely on its results in detriment of careful histopathological analysis. The increase in the number of applications and the use of an unnecessarily large number of markers may lead to unnecessary costs. These can be reduced by gaining a good knowledge of the sensitivity, specificity, and potential pitfalls of reagents and by optimizing the application and proper interpretation of results. In general, it is advisable to use a limited number of markers as a first step and then expand the number of tests accordingly (Tables 5-7).

References


