

Soft Tissue Tumor Immunohistochemistry Update

Illustrative Examples of Diagnostic Pearls to Avoid Pitfalls

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• **Context.**—Current 2013 World Health Organization classification of tumors of soft tissue arranges these tumors into 12 groups according to their histogenesis. Tumor behavior is classified as benign, intermediate (locally aggressive), intermediate (rarely metastasizing), and malignant. In our practice, a general approach to reaching a definitive diagnosis of soft tissue tumors is to first evaluate clinicoradiologic, histomorphologic, and cytomorphologic features of the tumor to generate some pertinent differential diagnoses. These include the potential line of histogenesis and whether the tumor is benign or malignant, and low or high grade. Although molecular/genetic testing is increasingly finding its applications in characterizing soft tissue tumors, currently immunohistochemistry still not only plays an indispensable role in defining tumor histogenesis, but also serves as a surrogate for underlining molecular/genetic alterations.

Objective.—To provide an overview focusing on the

current concepts in the classification and diagnosis of soft tissue tumors, incorporating immunohistochemistry. This article uses examples to discuss how to use the traditional and new immunohistochemical markers for the diagnosis of soft tissue tumors. Practical diagnostic pearls, summary tables, and figures are used to show how to avoid diagnostic pitfalls.

Data Sources.—Data were obtained from pertinent peer-reviewed English-language literature and the authors' first-hand experience as bone and soft tissue pathologists.

Conclusions.—The ultimate goal for a pathologist is to render a specific diagnosis that provides diagnostic, prognostic, and therapeutic information to guide patient care. Immunohistochemistry is integral to the diagnosis and management of soft tissue tumors.

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Current 2013 World Health Organization (WHO) classification of tumors of soft tissue arranges these tumors into 12 groups according to their histogenesis as follows: adipocytic, fibroblastic/myofibroblastic, fibrohistiocytic, smooth-muscle, pericytic (perivascular), skeletal-muscle, vascular, chondro-osseous, gastrointestinal stromal, nerve sheath, uncertain differentiation, and undifferentiated/unclassified.¹ Tumor behavior is classified as benign, intermediate (locally aggressive), intermediate (rarely metastasizing), and malig-

nant. In our practice, a general approach to reaching a definitive diagnosis when involving soft tissue tumors is to first consider clinicoradiologic, histomorphologic, and cytomorphologic features of the tumor to generate a pertinent differential diagnosis that includes the potential line of histogenesis and whether the tumor is benign or malignant. Sometimes, the line of histologic differentiation is obvious such as smooth-muscle, skeletal-muscle, vascular, neural, and chondro-osseous lineages. However, when the line of differentiation is not obvious, the histomorphologic pattern encountered may help in determining differential diagnoses for further workup (Table 1). Ancillary testing is often required to confirm the tumor histogenesis when working up a soft tissue tumor. Immunohistochemistry (IHC) plays an important role in the diagnosis of soft tissue tumors. The ultimate goal for a pathologist is to render a specific diagnosis that provides diagnostic, prognostic, and therapeutic information to guide patient care.

This article aims to provide an overview focusing on the current concepts in the classification and diagnosis of soft tissue tumors, incorporating IHC. Although there are some recent reviews addressing this topic (Table 2), this article uses illustrative examples to discuss how to incorporate both traditional and new immunohistochemical markers in the diagnosis of soft tissue tumors. Abundant practical diagnostic pearls, summary tables,

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Table 1. Differential Diagnosis by Histologic Pattern

Histologic Pattern	Differential
Adipocytic	Lipoma, hibernoma, spindle cell lipoma, atypical lipomatous tumor/well-differentiated liposarcoma, myxoid liposarcoma, dedifferentiated liposarcoma, and pleomorphic liposarcoma
Spindle cell	Desmoid tumor (fibromatosis), fibroma, nodular fasciitis, low-grade fibromyxoid sarcoma, dermatofibrosarcoma protuberance, solitary fibrous tumor, fibrosarcoma, leiomyoma, leiomyosarcoma, spindle cell/sclerosing rhabdomyosarcoma, gastrointestinal stromal tumor, schwannoma, neurofibroma, malignant peripheral nerve sheath tumor, synovial sarcoma, spindle cell lipoma, dedifferentiated liposarcoma, and undifferentiated spindle cell sarcoma
Myxoid	Myxoma, soft tissue perineurioma, superficial and deep angiomyxoma, myxoid liposarcoma, low-grade fibromyxoid sarcoma, myxofibrosarcoma, myoepithelioma/myoepithelial carcinoma/mixed tumor, extraskeletal myxoid chondrosarcoma, and chordoma
Round cell	Ewing sarcoma, embryonal rhabdomyosarcoma, alveolar rhabdomyosarcoma, myxoid/round cell liposarcoma, extraskeletal myxoid chondrosarcoma, desmoplastic small round cell tumor, and undifferentiated round cell sarcoma
Epithelioid	Sclerosing epithelioid fibrosarcoma, glomus tumor, granular cell tumor, PEComa, rhabdomyoma, myoepithelioma/myoepithelial carcinoma/mixed tumor, epithelioid hemangioendothelioma, epithelioid angiosarcoma, epithelioid leiomyosarcoma, epithelioid sarcoma, clear cell sarcoma, and alveolar soft part sarcoma
Pleomorphic	Pleomorphic liposarcoma, dedifferentiated liposarcoma, pleomorphic rhabdomyosarcoma, myxofibrosarcoma, extraskeletal osteosarcoma, and undifferentiated pleomorphic sarcoma

Abbreviation: PEComa, perivascular epithelioid cell tumor.

and figures are used to demonstrate how to avoid diagnostic pitfalls.

TUMOR WITH ADIPOCYTIC AND/OR SPINDLE CELL MORPHOLOGY

Illustrative Example 1

A 78-year-old man underwent a right-sided forehead mass excision. The initial diagnosis from an outside hospital was dermatofibrosarcoma protuberans (DFSP). The tumor was immunoreactive to cluster of differentiation (CD) 34 while negative for S100 protein, Melan-A, tyrosinase, MART-1 (melanocytic antigen recognized by cytotoxic T lymphocytes 1), human herpesvirus 8, cytokeratin, and

smooth muscle actin (SMA). The tumor exhibited a high proliferation index with Ki-67 (methylation-inhibited binding protein 1 [MIB-1]) positivity in the nuclei of 30% of the tumor cells. Was this really a DFSP? The first impression of the tumor under low magnification was spindle cells infiltrating adipose tissue (Figure 1, A). The spindle tumor cells were CD34⁺ (Figure 1, B). Closer look under higher magnification showed moderate to marked cytologic atypia and frequent mitotic activity (Figure 1, C). Further inquiry about clinical history revealed that the patient had a “lipomatous tumor” excised from this area before. Additional immunohistochemical studies showed diffuse MDM2 (mouse double minute 2 homolog) nuclear immunoreactivity in spindle cells (Figure 1, D). The tumor was also positive

Table 2. Recent Review Articles on Immunohistochemistry (IHC) of Soft Tissue Tumors

Source, y	Markers Discussed	Reference
Hornick, ²⁶ 2014	Seven lineage-restricted transcription factors (myogenin [MYF4], myoD1 [MYF3], FLI1, ERG, Brachyury, SOX10, SATB2), 8 proteins correlating molecular alteration markers (β -catenin, MDM2/CDK4, SMARCB1 [INI1], SDHB, TFE3, ALK, STAT6), and 4 markers identified by gene expression profile (DOG1, TLE1, MUC4, GRIA2)	<i>Mod Pathol.</i> 2014;27(suppl 1):S4–S63
Miettinen, ⁸¹ 2014	Six basic panel markers (CD34, desmin, EMA, keratin cocktail AE1/AE3, S100 protein, α -SMA), and 4 specific tumor-type markers (CD31, ERG, KIT, DOG1/Ano-1)	<i>Histopathology.</i> 2014;64(1):101–118
Parham, ⁸² 2015	Thirty-nine selected cell-type markers (germ cells: α -fetoprotein, OCT3/4, SALL4m, CD30, PLAP; epithelial cells: cytokeratin, EMA; muscle cells: actin, caldesmon, desmin, myoglobin, myogenin, myoD; hematopoietic cells: CD45, CD20, CD79a, CD15, CD1a, CD68, CD3, myeloperoxidase, TdT, CD21, CD23, CD36; endothelial cells: Von Willebrand factor, CD31, CD34, ERG; neuroendocrine cells: neuron-specific endolase, CD56, CD57, PGP5.5, synaptophysin, chromogranin, neuro N, neurofilaments; melanocytic cells: S100, HMB-45, MITF, Melan-A) and 18 fusion gene product markers or surrogates detected by IHC (FLI1, ERG, AP1 β , TLE1, ALK, ROS1, NR4A3, BCL2, WT1, MYC, NUT, BCL6, TFE3, ZAP70, MUC4)	<i>Anal Chem Insights.</i> 2015;10(suppl 1):1–10
Lin and Doyle, ⁸³ 2015	Thirteen new markers (ERG, MYC, MDM2/CDK4, STAT6, MUC4, DOG1, SDHB/A, INI1, TLE1, TFE3, SOX10, NY-ESO-1)	<i>Arch Pathol Lab Med.</i> 2015;139(1):106–121

Abbreviations: EMA, epithelial membrane antigen; ERG, erythroblast transformation-specific transcription factor; MITF, microphthalmia transcription factor; PLAP, placental alkaline phosphatase; SMA, smooth muscle actin.

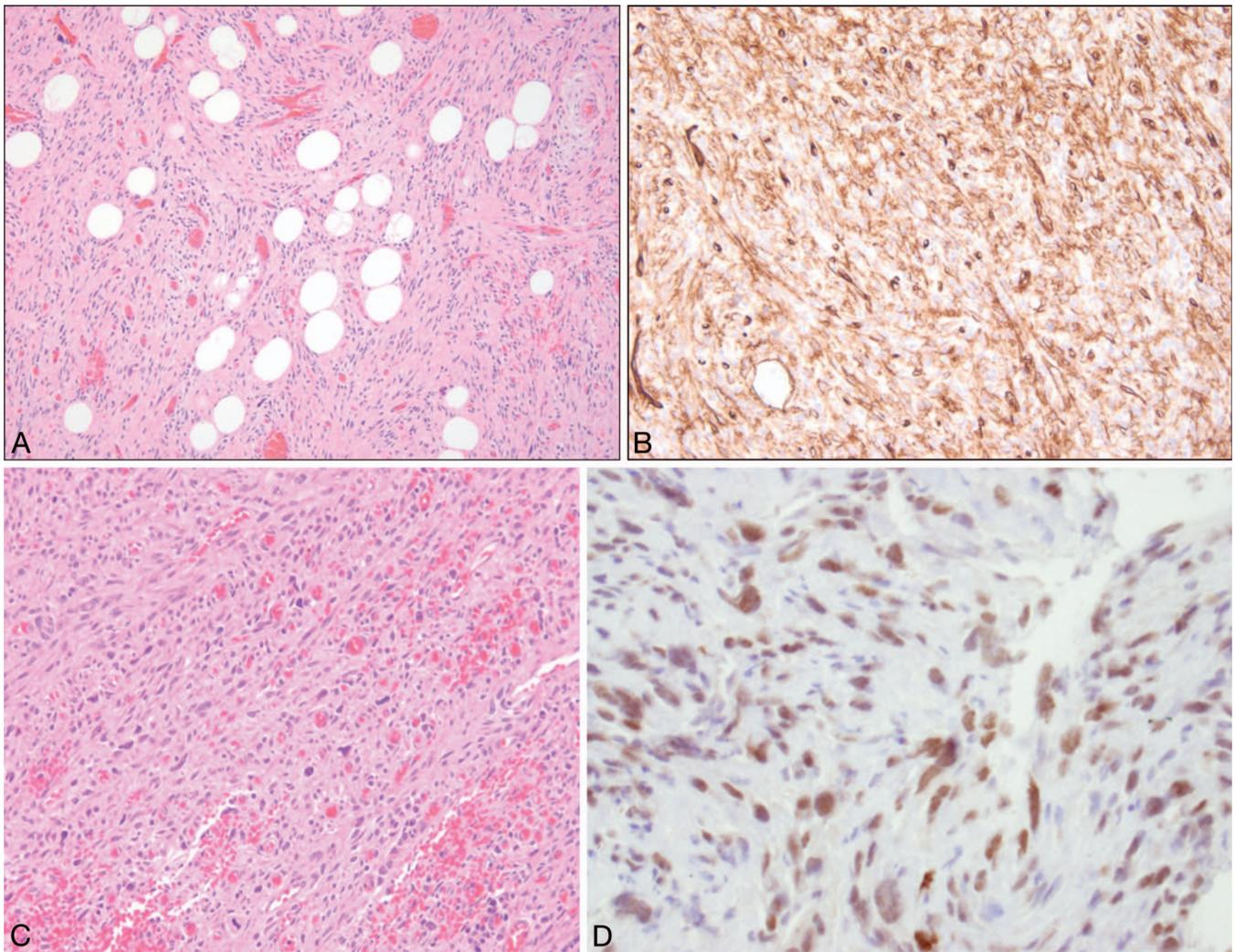


Figure 1. Dedifferentiated liposarcoma misdiagnosed as dermatofibrosarcoma protuberans. *A*, The tumor shows adipocytic and spindle cell morphology mimicking dermatofibrosarcoma protuberans. *B*, The spindle cells are immunoreactive to cluster of differentiation 34 (CD34). *C*, The spindle cells exhibit moderate to severe cytologic atypia and frequent mitotic activity. *D*, Immunohistochemical study is positive for mouse double minute 2 homolog (MDM2) (hematoxylin-eosin, original magnifications $\times 10$ [A] and $\times 20$ [C]; original magnifications $\times 10$ [B] and $\times 40$ [D]).

for amplification of *MDM2* (12q15) by fluorescence in situ hybridization (FISH), not shown here. The diagnosis was then changed to dedifferentiated liposarcoma. The pertinent differential diagnoses and the judicious use of immunohistochemical studies will be discussed.

Dermatofibrosarcoma Protuberans.—Conventional DFSP is a low-grade and locally aggressive fibroblastic neoplasm that can be cured by excision with clear surgical margins.² It is characteristically superficially located and consists of spindle-shaped tumor cells infiltrating fat lobules with a collagenous stroma and immunoreactivity to CD34, shown in this case. However, the spindle cells in DFSP are monotonous and bland with a storiform pattern that is not identified in this case. Other variants of DFSP include pigmented (also known as Bednar tumor) DFSP, myxoid DFSP, DFSP with myoid differentiation, plaquelike DFSP, giant cell fibroblastoma (juvenile form of DFSP), and fibrosarcomatous DFSP.³ In 10% to 15% of DFSPs, fibrosarcomatous transformation occurs whereby the tumor exhibits high-grade morphology and loss of CD34 expression, while maintaining the signature *COL1A1-PDGFB* (platelet-derived growth factor, β polypeptide) fusion gene.

Fibrosarcomatous DFSP shows a similar local recurrence rate to ordinary DFSP but 13% of fibrosarcomatous DFSPs develop distant metastases.¹ Identification of *COL1A1-PDGFB* fusion gene is important for managing fibrosarcomatous DFSP because imatinib mesylate, a tyrosine kinase inhibitor, has shown significant activity against PDGFRB (platelet-derived growth factor receptor, β) and benefits the patients with locally advanced and metastatic diseases in clinical trials.⁴

Spindle Cell Lipoma.—Spindle cell lipoma is a benign tumor composed of bland spindle cells admixed with mature adipose tissue in a background of thick and ropey collagen.³ The spindle cells are positive for CD34 stain. The matrix can also be myxoid. The pleomorphic lipoma is a morphologic continuum of this tumor, exhibiting multinucleated and floretlike cells. Important differential diagnoses of spindle cell lipoma include atypical lipomatous tumor and dedifferentiated liposarcoma.

Atypical Lipomatous Tumor.—*Atypical lipomatous tumor* is preferred by *WHO Classification of Tumours of Soft Tissue and Bone*¹ (2013 edition) over the term *well-differentiated liposarcoma* if the tumor occurs in the extremities because

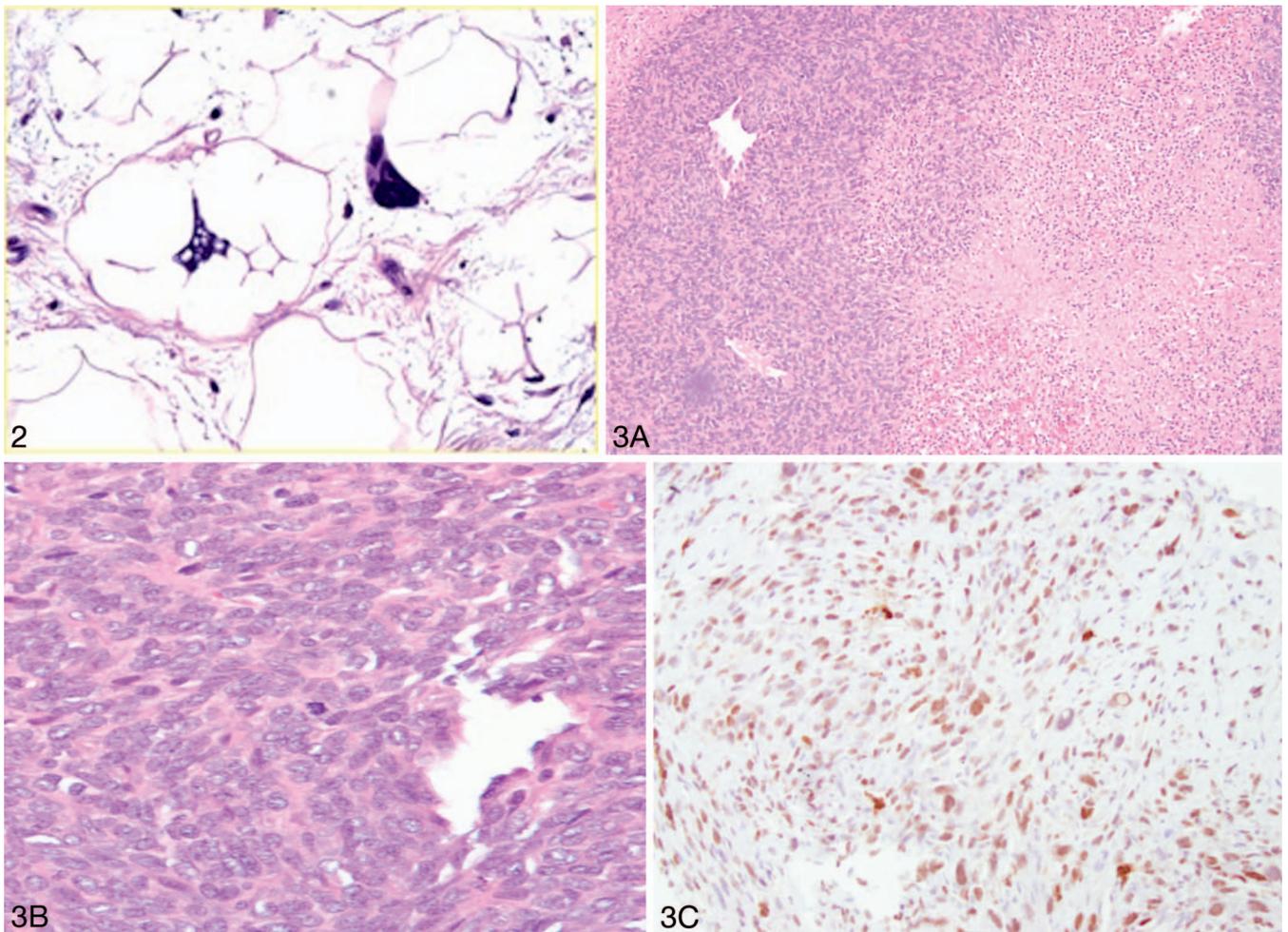


Figure 2. Atypical lipomatous tumor. There is a highly atypical large stromal cell with hyperchromatic nuclei that is also multinucleated. There is a lipoblast that has sharply margined cytoplasmic vacuoles scalloping the large and hyperchromatic nucleus (hematoxylin-eosin, original magnification $\times 40$).

Figure 3. Synovial sarcoma misdiagnosed as malignant solitary fibrous tumor. A, A highly cellular spindle cell neoplasm with a rich vascular network ranging from small vessels to large, ectatic ones with sinusoidal spaces. Areas of necrosis are present. B, The neoplastic cells show pale eosinophilic cytoplasm with inconspicuous borders and round to oval nuclei, granular chromatin, small nucleoli, and numerous atypical mitoses. C, The tumor cells are positive for TLE1 stain (hematoxylin-eosin, original magnifications $\times 10$ [A] and $\times 40$ [B]; original magnification $\times 20$ [C]).

this is a locally aggressive adipocytic neoplasm with no potential for metastasis. We all know that lipomatous tumors are immunoreactive to S100; however, the adipocytic nature of the tumor is usually obvious and does not warrant an S100 stain. The morphologic distinction between benign lipoma and atypical lipomatous tumor relies on the identification of the hallmark diagnostic cells that are the atypical hyperchromatic stromal cells and lipoblasts (Figure 2). The histologic types include adipocytic, sclerosing, spindle cell, and inflammatory variants. When in doubt of this diagnosis, nuclear immunoreactivity of MDM2 and cyclin-dependent kinase 4 (CDK4) are confirmatory, which correspond to amplification of these genes.³

MDM2 and CDK4 Immunohistochemical Stain.—The defining genetic feature of atypical lipomatous tumor is the presence of rings or giant markers of chromosome 12 that contain amplification of the 12q14-15 region. The *MDM2* gene and its neighboring gene *CDK4* are amplified, which can be detected by molecular methods such as reverse transcription-polymerase chain reaction (RT-PCR) and FISH. The resultant MDM2 and CDK4 protein overexpression

can be detected by IHC. However, nuclear staining with MDM2 and CDK4 is not entirely specific for atypical lipomatous tumor (Table 3). For example, MDM2 or CDK4 can show positivity in intimal sarcoma, pleomorphic rhabdomyosarcoma, a subset of malignant peripheral nerve sheath tumor, and myxofibrosarcoma.⁵⁻⁹ Meanwhile, these markers are useful to distinguish atypical lipomatous tumor from lipoma as well as dedifferentiated liposarcoma from undifferentiated sarcoma, especially when both markers show positivity. Please be aware that pleomorphic liposarcoma and myxoid liposarcoma are negative for MDM2 and CDK4.¹⁰

Dedifferentiated Liposarcoma.—Deep-seated, recurrent atypical lipomatous tumor can undergo dedifferentiation with transformation into nonadipocytic high-grade sarcoma. The current case is an example of a recurrent atypical lipomatous tumor with dedifferentiation. The most common location of dedifferentiated liposarcoma is within the retroperitoneum. Dedifferentiated liposarcoma does occur at rare sites, such as head and neck.¹¹ An institutional review of adult retroperitoneal sarcomas within the past 10

Table 3. MDM2 Positivity by IHC and FISH in Soft Tissue and Bone Tumors

Tumor Type	MDM2 by FISH	MDM2 by IHC
ALT/WD liposarcoma	+	+ (nuclear)
Dedifferentiated liposarcoma	+	+ (diffuse, nuclear)
Pleomorphic rhabdomyosarcoma	–	+
Intimal sarcoma	+	+ (up to 70%)
Malignant peripheral nerve sheath tumor	–	+ (subset)
Myxofibrosarcoma	–	+ (subset)
Low-grade central osteosarcoma	+	+
Conventional osteosarcoma	+ (10%)	–
Parosteal osteosarcoma	+ (>85%)	+
Undifferentiated high-grade pleomorphic sarcoma of bone	+ (17%)	–

Abbreviations: ALT/WD, atypical lipomatous tumor/well differentiated; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; MDM2, mouse double minute 2 homolog; +, positive; –, negative.

years shows that liposarcoma is the most common subtype (54.7%; 168 of 307 cases), followed by leiomyosarcoma (26.1%; 80 of 307 cases), which is in keeping with the literature review (45.1% and 21.3% respectively).¹² The incidence rate of dedifferentiated liposarcoma in the retroperitoneum is 15.6% (48 of 307 cases) at Moffitt Cancer Center and 20.9% in the literature. The concurrent atypical lipomatous tumor component may not be sampled by core needle biopsy. Therefore, when facing a high-grade and pleomorphic sarcoma biopsy from the retroperitoneum, judicious use of immunohistochemical stains for MDM2 and CDK4 is helpful in identifying dedifferentiated liposarcoma. We recently summarized a proposed algorithm for the pathology-focused management of retroperitoneal soft tissue sarcoma.¹²

Undifferentiated Sarcoma.—By definition, undifferentiated sarcoma shows no identifiable lineage differentiation. This is a heterogeneous group of tumors that exhibit pleomorphic, round cell, spindle cell, and epithelioid morphology. According to WHO 2013 classification of tumors of soft tissue, “undifferentiated sarcoma” with 12q14-15 amplification (*MDM2/CDK4*) is now classified as dedifferentiated liposarcoma. Why is it important to distinguish among undifferentiated sarcoma, dedifferentiated liposarcoma, and pleomorphic liposarcoma? The answer is that their associated 5-year survival rates are different: 35% to 60% for undifferentiated sarcoma; 65% for dedifferentiated liposarcoma; and 60% for pleomorphic sarcoma.^{13–16}

CD34 Immunostain and Diagnostic Pitfall.—CD34 is a transmembrane glycoprotein expressed by hematopoietic stem cells and endothelial cells with a membranous pattern. It is also typically expressed by solitary fibrous tumor, gastrointestinal stromal tumor, DFSP, epithelioid sarcoma, spindle cell lipoma, synovial sarcoma, and vascular tumors. When the diagnosis is truly one of the above tumors, CD34 will show positivity; however, the converse is not true. For example, the current case is positive for CD34, but the history of “prior removed lipomatous tumor,” the high-grade cytology, and positivity for MDM2 confirmed the diagnosis of a dedifferentiated liposarcoma instead of a conventional DFSP or fibrosarcomatous DFSP.

Illustrative Example 2

A 49-year-old woman presented with a right-sided cheek mass. The clinician performed an incisional biopsy to rule out a salivary gland neoplasm. The initial diagnosis from an outside hospital was high-grade solitary fibrous tumor (SFT). The tumor was positive for vimentin and exhibited a high Ki-67 proliferation rate, while it was negative for

cytokeratins (AE1/AE3 and CAM 5.2), epithelial membrane antigen (EMA), S100 protein, neuron-specific enolase, chromogranin, SMA, and muscle-specific actin. Immunostaining for STAT6 (signal transducer and activator of transcription 6), CD34, CD99, or B-cell CLL/lymphoma 2 (Bcl-2) was not performed. Was this really a malignant SFT? The histology was that of a high-grade malignancy composed of spindle cells arranged in SFT-like patternless pattern with tumor necrosis and frequent mitotic activity (Figure 3, A and B). The SFT morphologic pattern is shared by synovial sarcoma. Further IHC and molecular studies confirmed the diagnosis of synovial sarcoma. The pertinent differential diagnoses and the judicious use of immunohistochemical studies will be discussed.

Vimentin and Cytokeratin Immunostains and Their Diagnostic Pitfall.—Vimentin is a type II intermediate filament protein encoded by the *VIM* gene. Vimentin is expressed in mesenchymal cells but is not specific for mesenchymal cells. It is also expressed in certain types of carcinomas (eg, renal cell carcinoma, spindle cell carcinoma), as well as lymphomas and melanomas. Vimentin positivity has a limited value in the diagnosis of soft tissue tumors; however, if the mesenchymal tissue is negative for vimentin, it may indicate that the tissue is suboptimal for IHC or not of a mesenchymal (soft tissue) differentiation.

Cytokeratins are proteins of keratin-containing intermediate filaments found in the intracytoplasmic cytoskeleton of epithelial tissue, therefore, markers for carcinomas. However, they are also frequently expressed in many sarcomas: synovial sarcoma, epithelioid sarcoma, epithelioid hemangioendothelioma, angiosarcoma, and desmoplastic small round cell tumor. Diffuse broad-spectrum keratin expression is seen in more than 90% of soft tissue myoepithelial tumors. Occasional aberrant expression of keratin is reported in melanomas and certain sarcomas, such as Ewing sarcoma and leiomyosarcoma.^{17,18}

Solitary Fibrous Tumor and STAT6 Immunostain.—Solitary fibrous tumor can occur in extrapleural soft tissue. Subcutaneous tissue (40%) and deep tissue of extremities and head and neck area are common sites. It is a spindle cell tumor with fibrous stroma and branching thin-walled vessels. The malignant SFT exhibits hypercellularity, increased mitotic activity (>4/10 high-power fields), tumor necrosis, visible cytologic atypia, and infiltrative margins. Although SFT is immunoreactive to CD34 (90%–95%), EMA, SMA, CD99, and Bcl-2, it is not until recently that STAT6 has been identified as a sensitive and specific marker for diagnosing SFT.^{19–21} Overexpression of nuclear STAT6 results from *NAB2-STAT6* fusion identified in SFTs. The

fusion partners are in close proximity on chromosome band 12q13, precluding identification by conventional FISH analysis. One should be cautious to call an “SFT-look-alike” tumor an SFT without a positive STAT6 immunostain result. The current tumor was subsequently tested for STAT6 and was negative.

Malignant Peripheral Nerve Sheath Tumor.—Malignant peripheral nerve sheath tumor (MPNST) also shares the SFT morphologic pattern. It is often seen in the setting of neurofibromatosis type 1. It may exhibit an SFT-like appearance. Immunohistochemical staining of S100 protein, Sry-related HMG-BOX gene 10 (SOX-10), and p75 neurotrophin receptor (p75NTR) can be helpful to suggest the diagnosis of MPNST. SOX-10 is a member of the SOX family of transcription factors and is relatively specific for neuroectodermal neoplasms. It is expressed in benign nerve sheath tumors, clear cell sarcoma, and melanoma (including desmoplastic and spindle cell variants). It is more sensitive and specific for the diagnosis of melanocytic and schwannian tumors than S100 protein. It is a relatively more specific marker than S100 in diagnosing MPNST. SOX-10 reactivity can also be seen in astrocytomas, myoepithelial tumors, granular cell tumors, and a subset of breast carcinomas.

Differentiating benign and malignant peripheral nerve sheath neoplasms can be diagnostically challenging. Features favoring malignancy include increased size, rapid growth, infiltrative border, internal necrosis, increased vascularity, and frequent mitotic activity with atypical mitoses. In addition, perivascular hypercellularity, tumor herniation into vascular lumens, necrosis, and expression of p75NTR is more frequently associated with MPNST than cellular schwannoma in a large study.²² Recently, loss of anti-histone H3 acetyl K27 (H3K27) trimethylation has been reported in 50% of MPNSTs, predominantly in high-grade MPNST.²³

Synovial Sarcoma and TLE1 Immunostain.—Synovial sarcoma may exhibit an SFT-like appearance with hemangiopericytoma-like vessels and shares the positive immunostain pattern of EMA, CD99, and Bcl-2. However, synovial sarcoma is typically negative for CD34, which is helpful to distinguish it from SFT.³ Although cytokeratins generally show positivity in synovial sarcoma, the monophasic spindle cell variant of synovial sarcoma is less frequently positive (50%–80%) than its biphasic counterpart. In this case, perhaps the negative cytokeratin and EMA staining patterns misdirected the workup and precluded synovial sarcoma as a critical differential diagnosis. Transducin-like enhancer of split 1 (TLE1) is a new marker for synovial sarcoma. It is a transcriptional repressor essential to hematopoiesis, neuronal differentiation, and terminal epithelial differentiation. It also plays an important role in the synovial sarcoma-associated Wnt/ β -catenin signaling pathway.²⁴ TLE1 positivity is seen in 85% to 97% of synovial sarcomas; however, it has also been reported in endometrial stromal sarcoma, SFT, malignant peripheral nerve sheath tumor, Ewing sarcoma, schwannoma, and epithelioid sarcoma.^{25,26} The diagnosis of synovial sarcoma should be further verified by molecular testing. In this case, the tumor is TLE1 positive by IHC (Figure 3, C) and positive for SYT rearrangement by FISH (not shown here), confirming the diagnosis of synovial sarcoma.

Given both are malignant, what is the clinical significance in distinguishing malignant SFT from synovial sarcoma? Synovial sarcoma is one of those sarcomas that are chemosensitive, while conventional chemotherapy is less

effective for malignant SFT. In general, precise subclassification would greatly benefit patients with chemosensitive sarcomas, such as synovial sarcoma, Ewing sarcoma, osteosarcoma, rhabdomyosarcoma, desmoplastic small round cell tumor, angiosarcoma, myxoid/round cell liposarcoma, and uterine leiomyosarcoma.

TUMORS WITH A PROMINENT EPITHELIOID MORPHOLOGY

Illustrative Example 3

A 17-year-old boy presented with a 2.5-month history of a gradually enlarging mass at the base of the right side of penis. He underwent an excisional biopsy under the clinical impression of a hematoma or aneurysm. During the operation, the mass was apparently adherent to the corporal tissue. Microscopic examination of the lesion demonstrated an epithelioid neoplasm with tumor necrosis and brisk mitotic activity. The lesional cells were focally positive for pancytokeratin but negative for cytokeratin 5/6 and p63. The tumor was diffusely immunoreactive for vimentin and focally positive for CD31 and erythroblast transformation-specific transcription factor (ERG). Loss of integrase interactor 1 (INI1) expression was seen in most of the tumor cells (Figure 4, A through D). A diagnosis of epithelioid sarcoma of the “proximal type” was rendered.

Epithelioid Sarcoma.—Epithelioid sarcomas are tumors of unknown histogenesis, accounting for less than 1% of all soft tissue sarcomas. They are usually slow growing, with peak incidence in young adult men, and occur predominantly in the extremities.²⁷ Histologically, the tumor frequently demonstrates a nodular growth pattern, with epithelioid cells surrounding areas of central necrosis/hyalinization and peripheral spindling, thus reminiscent of granulomas.²⁸ In contrast to the conventional, “distal-type” epithelioid sarcoma, the proximal variant occurs more commonly in the pelvic and perineal regions, and is characterized by a predominantly large-cell, epithelioid histomorphology, marked cytologic atypia, frequent rhabdoid features, and lack of a granuloma-like pattern in most cases.^{29,30} The “proximal type” demonstrates a more aggressive clinical behavior.³⁰

INI1 (also known as hSNF5 and SMARCB1) is a member of the SWI/SNF chromatin remodeling complex located on chromosome band 22q11.2. Loss of INI1 expression is observed in more than 90% of both conventional and proximal-type epithelioid sarcomas but not seen in most of its mimickers, thus it is characteristic of these tumors.³¹ Notably, other INI1-deficient tumors reportedly include renal medullary carcinomas and a subset of epithelioid malignant peripheral nerve sheath tumors, myoepithelial carcinomas, and extraskeletal myxoid chondrosarcomas.³²

In addition to cytokeratins, expression of vascular markers, including CD31, CD34, ERG, and Friend leukemia integration 1 transcription factor (FLI1), is a frequent finding in epithelioid sarcomas, with the latter two being reportedly observed in 60% and 70% of cases, respectively.^{33–36} It is extremely important to be aware that coexpression of cytokeratin and vascular markers is common in both epithelioid vascular tumors and epithelioid sarcoma when classifying a soft tissue tumor with an epithelioid pattern.

Epithelioid Vascular Tumors.—Epithelioid hemangiomas most frequently occur in the craniofacial regions, especially the forehead, preauricular area and scalp, followed by distal extremities.³⁷ The penis is an uncommon

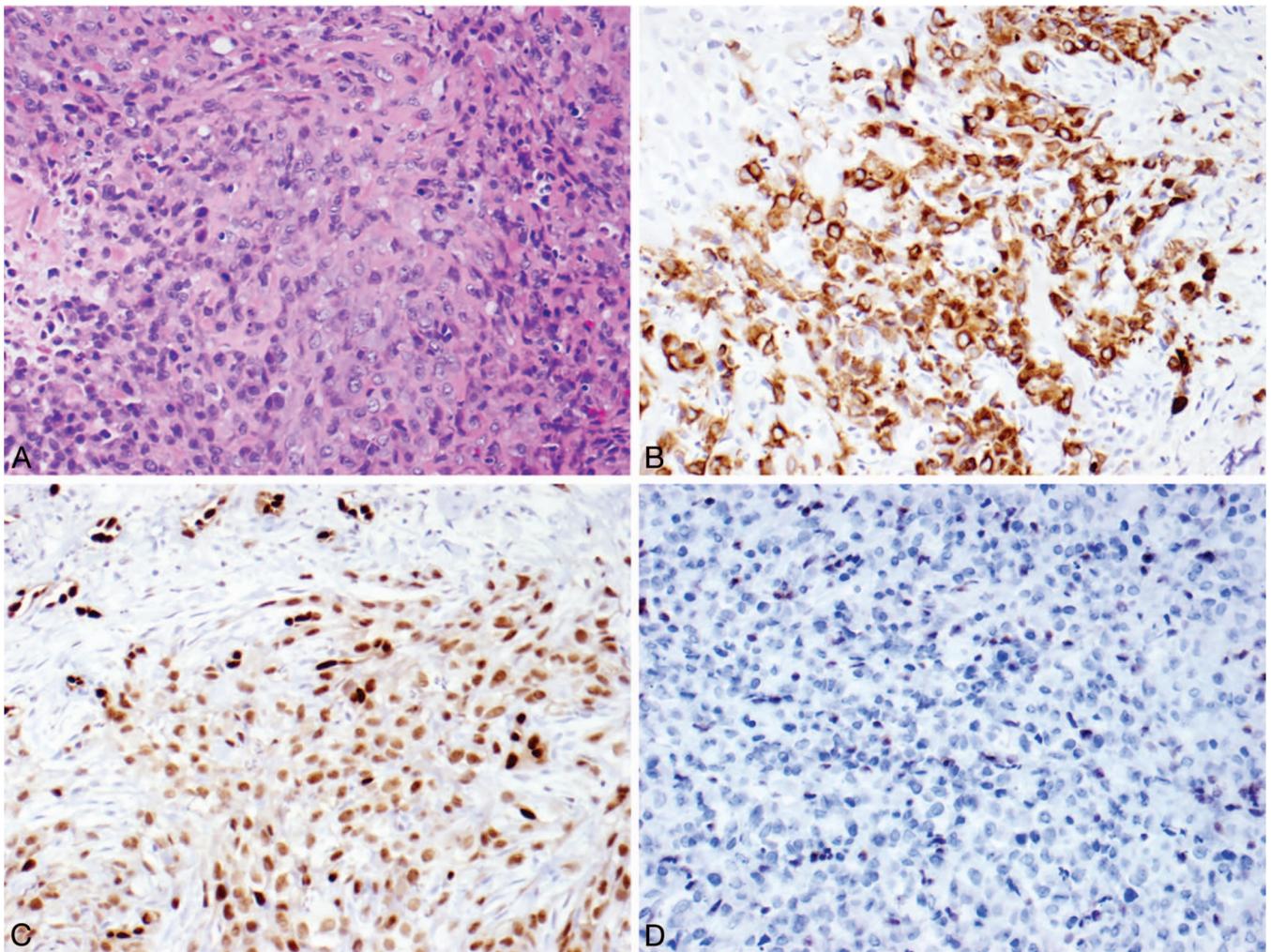


Figure 4. Epithelioid sarcoma. A, The tumor shows epithelioid morphology and moderate to severe cytologic atypia. The lesional cells are positive for cytokeratin (B) and erythroid transformation-specific transcription factor (ERG) (C) and demonstrate loss of nuclear integrase interactor 1 (INI1) (D) (hematoxylin-eosin, original magnification $\times 200$ [A]; original magnifications $\times 200$ [B through D]).

site of involvement but lesions in this location may be confused with epithelioid hemangioendothelioma or epithelioid angiosarcoma.³⁸ Histologically, these lesions typically demonstrate well-formed vessels lined by plump, epithelioid cells with copious amphophilic or eosinophilic cytoplasm, often in a nodular or lobular configuration (Figure 5, A through D). These tumors are also referred to as *histiocytoid hemangioma* given their prominent histiocytoid cytomorphology. Numerous eosinophils and lymphocytes are often present, hence it is also known as *angiolymphoid hyperplasia with eosinophilia*. The epithelioid endothelial cells may be immunoreactive for keratin, typically in a focal pattern, in addition to vascular markers CD31, ERG, and, to a lesser extent, CD34.³⁷

Epithelioid hemangioendothelioma (EHE) is an intermediate-grade malignant angiocentric vascular neoplasm. While it arises more commonly in the superficial or deep soft tissue of the extremities, the tumor can be seen in virtually any body site.³⁹ Microscopically, EHE is distinctively composed of cords or chains of epithelioid endothelial cells in the background of a myxoid or hyalinized stroma (Figure 6, A and B). The cells typically have abundant eosinophilic cytoplasm that often contains vacuoles (so-called blister cells). The lesional cells are of low nuclear

grade but may rarely demonstrate high-grade features (thus named *malignant epithelioid hemangioendothelioma* by some authorities). Epithelioid hemangioendothelioma expresses typical vascular markers including CD31, CD34, FLI1, and ERG. Epithelial antigens (CK7, CK8, CK18, and EMA) are also expressed in some tumors.³⁹ A small subset of EHEs are positive for TFE3. Importantly, EHE has a t(1;3)(p36;q23-25) translocation that leads to *WWTR1-CAMTA1* fusion in virtually all cases.⁴⁰ Nuclear expression of CAMTA1 is extremely helpful in confirming the diagnosis of EHE.⁴¹

While well-differentiated angiosarcomas typically show vasoformative characteristics, it is not uncommon that these tumors present with a predominantly (or exclusively) epithelioid appearance (Figure 6, C through F). This unique morphologic variant most often arises in the deep soft tissues of the extremities, but a variety of other primary sites have been reported, including the thyroid gland, skin, adrenal glands, and bone.⁴² Epithelioid angiosarcoma is highly aggressive, often with early nodal and solid organ metastasis. Histologically, focal areas of irregularly anastomosing vascular formation are typically discernible. Purely epithelioid tumors are uncommon although foci with completely epithelioid appearance may be present. This may become extremely challenging in biopsy specimens with scant

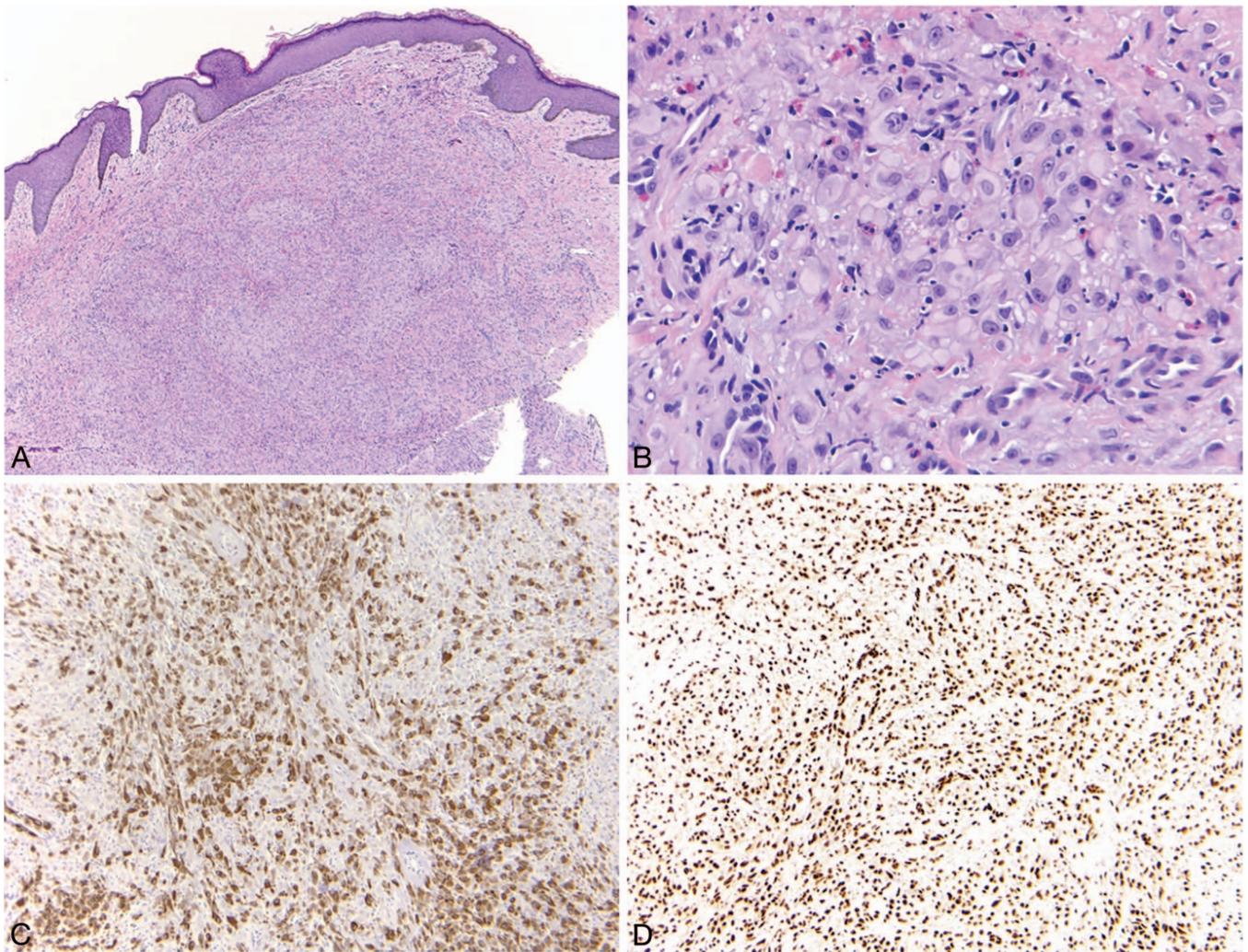


Figure 5. Epithelioid hemangioma of the penis. *A*, Sections show a multinodular lesion in the dermis. *B*, A high-power view demonstrates pleomorphic epithelioid cells with eosinophils. *C*, The lesional cells are focally positive for pancytokeratin (not shown), and they are diffusely immunoreactive for erythroblast transformation-specific transcription factor (ERG) (*D*), with a low Ki-67 proliferation index (<5%) and (not shown) retained nuclear integrase interactor 1 (INI1) (hematoxylin-eosin, original magnifications $\times 20$ [*A*] and $\times 200$ [*B*]; original magnification $\times 40$ [*C* and *D*]).

pathologic material available. Expression of cytokeratin has been reportedly seen in 35% of cases.⁴³ The most frequently encountered differential diagnosis includes metastatic carcinoma and, less frequently, epithelioid sarcoma, given the sometime indistinguishable cytomorphology and expression of cytokeratin. A useful histologic hint is that the vasoformative nature (ie, extravasation of blood) is almost always identifiable in angiosarcomas but not in other epithelioid tumors. A panel of IHC stains to include at least vascular markers and INI1 is necessary when working up a malignant epithelioid soft tissue tumor.

It is important to note that while transcription factors FLI1 and ERG have been increasingly used in practice as endothelial markers given their nuclear expression (thus a cleaner staining background), these markers are less specific than CD31. FLI1 is expressed in Ewing sarcoma, subsets of wide range of mesenchymal tumors, subsets of high-grade lymphomas including lymphoblastic lymphomas and diffuse large B-cell lymphomas, and even subsets of carcinomas and melanomas, thus it has limited utility by itself owing to its low specificity. Similarly, ERG is expressed in a subset of

Ewing sarcomas (5%–10%), prostatic adenocarcinoma, and a small subset of acute myeloid leukemia. Its specificity depends upon the antibody clone.²⁶ Thus, these markers should be used and interpreted in the appropriate clinicopathologic settings.

Sclerosing Epithelioid Fibrosarcoma.—Sclerosing epithelioid fibrosarcoma (SEF) is a distinctive fibroblastic neoplasm characterized by epithelioid tumor cells arranged in nests, cords, or sheets embedded within a sclerotic collagenized matrix. This entity most commonly arises in the deep soft tissue of extremities, followed by shoulder, trunk, and head and neck regions, but may rarely occur in the visceral organs or bone.^{44,45} The lesional cells demonstrate relatively small, uniform, round/ovoid nuclei and eosinophilic or clear cytoplasm, and lack significant cytologic atypia, thus sometimes closely resembling metastatic lobular carcinoma of the breast (Figure 7, *A*). The most distinctive immunophenotype of SEF is the expression of mucin 4 (MUC4) (up to 70% of cases), similar to that in low-grade fibromyxoid sarcoma, while staining for cytokeratins is typically negative. Moreover, the t(7;16)(q33;p11) transloca-

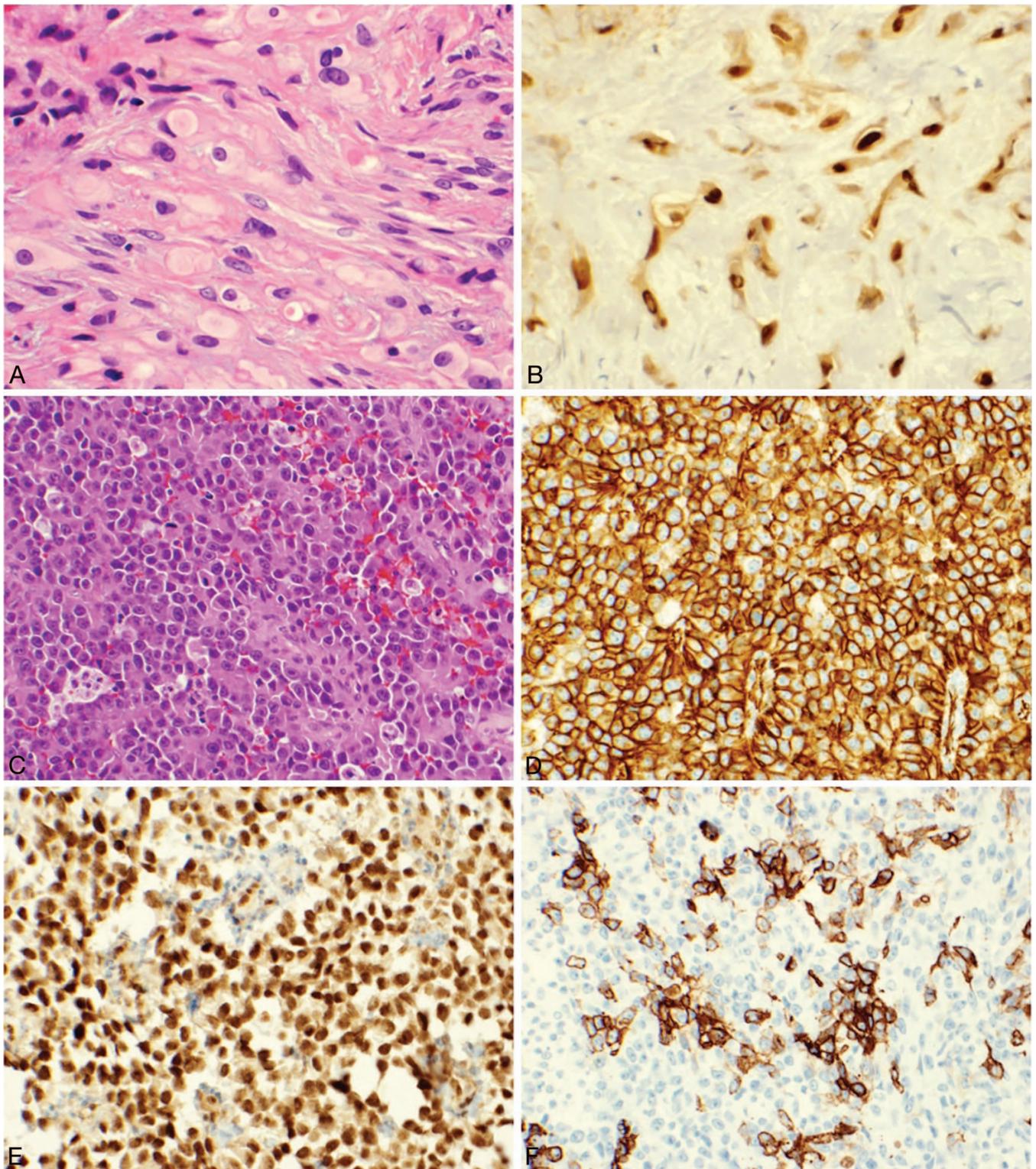


Figure 6. Epithelioid vascular tumors. A, Epithelioid hemangioendothelioma is composed of epithelioid cells with abundant eosinophilic cytoplasm that often contains vacuoles. B, The endothelial nature of the cells is confirmed by erythroblast transformation-specific transcription factor (ERG) staining. C, Epithelioid angiosarcoma demonstrates focal vasoformative features. The tumor is diffusely positive for CD31 (D) and Friend leukemia integration 1 transcription factor (FLI1) (E), with a variable CD34 expression (F) (hematoxylin-eosin, original magnification $\times 200$ [A] and $\times 100$ [C]; original magnification $\times 200$ [B]; original magnification $\times 20$ [D through F]).

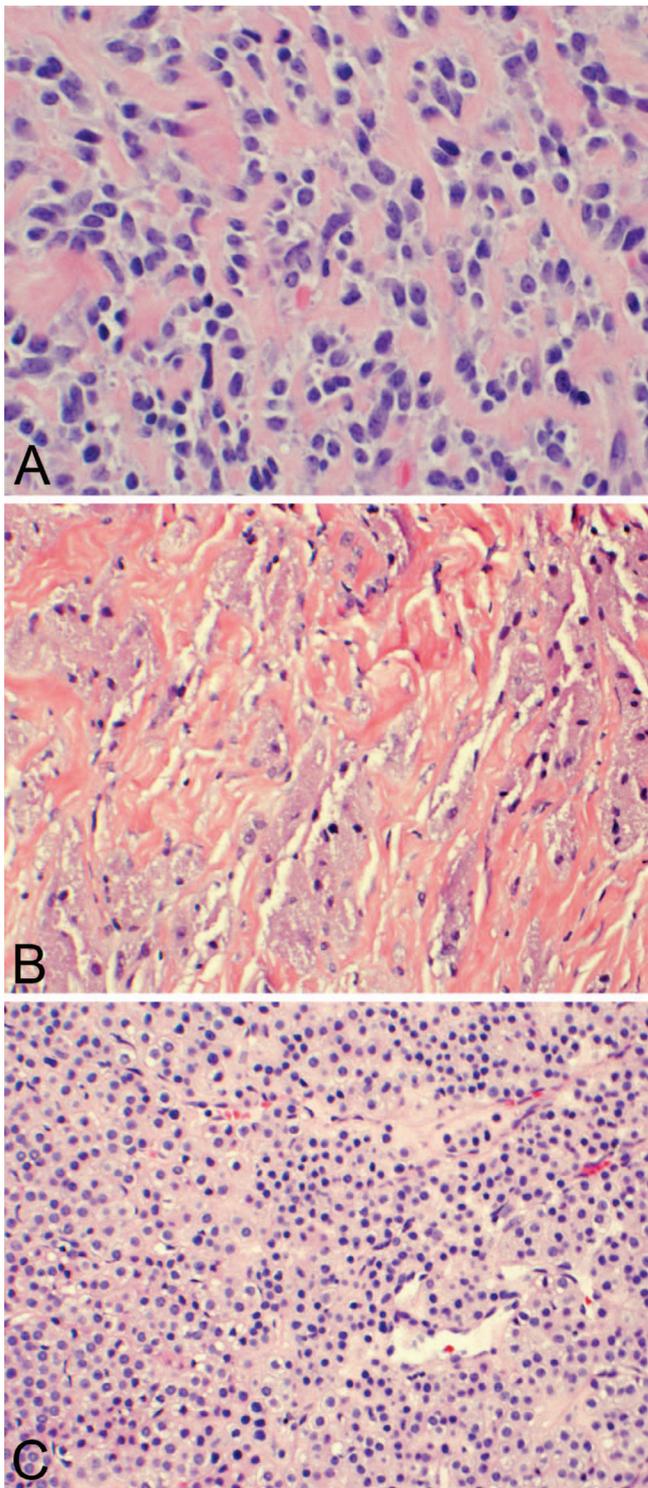


Figure 7. Selected epithelioid tumors. A, Sclerosing epithelioid fibrosarcoma shows cytologically atypical cells in the background of collagenized stroma. B, Granular cell tumor demonstrates large cells with small nuclei and abundant granular cytoplasm. C, Glomus tumor shows small, uniform cells (hematoxylin-eosin, original magnifications $\times 200$ [A and B] and $\times 100$ [C]).

tion resulting in a *FUS-CREB3L2* fusion gene characteristic of low-grade fibromyxoid sarcoma has been detected in some SEF cases.⁴⁴ This observation has led some authorities to propose a potential relationship between these 2 tumors.

Other Selected Epithelioid Tumors.—In addition to the aforementioned entities, several benign and malignant soft tissue tumors may demonstrate an epithelioid morphology.

Granular cell tumor commonly affects head and neck regions but can occur in any anatomic site. Granular cell tumor is thought to have neuroectodermal differentiation that is likely schwannian in type, thus is generally positive for S100 protein and SOX-10 (Figure 7, B).⁴⁶ The tumor cells are also variably reactive for CD68, neuron-specific enolase, microphthalmia-associated transcription factor (MITF), and transcription factor E3 (TFE3), while negative for MART-1 and human melanoma black 45 (HMB-45).

Glomus tumors are most frequently seen in the distal extremities and consist of cells resembling modified smooth muscle cells of the normal glomus body, thus typically expressing SMA and h-caldesmon. Abundant pericellular production of type IV collagen is another classic feature (Figure 7, C).

Neoplasms with perivascular epithelioid differentiation (PEComas) include angiomyolipoma, clear cell “sugar” tumor of the lung, lymphangioliomyomatosis, and a group of other tumors with similar histomorphology and immunophenotype. These tumors show a variety of anatomic distributions but most often arise in the retroperitoneal and abdominopelvic regions. They are usually composed of uniform epithelioid cells with round nuclei and abundant granular eosinophilic or clear cytoplasm. The distinctive immunophenotype is the expression of melanocytic markers such as HMB-45 (most sensitive), MART-1 (Melan-A), and MITF, and muscle markers such as SMA and calponin. TFE3 reportedly shows positivity in about 10% of cases.⁴⁷

Alveolar soft part sarcoma (ASPS) most commonly occurs in the deep soft tissue of the thigh or buttock in adults and the head and neck region in children. It is characteristically composed of large, polygonal, uniform epithelioid cells with abundant eosinophilic, granular cytoplasm and is arranged in a distinctive organoid or nesting pattern. The distinguishing phenotype of ASPS is its strong nuclear staining with an antibody raised against the carboxy terminal portion of TFE3 retained in the fusion protein resulting from the *ASPSCR1-TFE3* fusion gene. Other nonspecific immunorepression includes desmin and S100 protein but is usually focal.

Succinate dehydrogenase (SDH)-deficient gastrointestinal stromal tumors (GISTs) are a subgroup of GISTs that occur exclusively in the stomach (so far) with loss of the SDH complex function as its oncogenic mechanism, instead of KIT- or PDGFRA (platelet-derived growth factor A)-activating mutations, as seen in most GISTs. They account for most pediatric GISTs and GISTs in association with 2 previously described syndromes: Carney-Stratakis syndrome and Carney triad.⁴⁸ Succinate dehydrogenase-deficient GISTs are histologically distinctive with a multinodular architecture and an epithelioid cytology. Loss of SDH subunit B (SDHB) expression by IHC effectively identifies SDH-deficient GISTs, some of which have loss-of-function germline mutations in one of the SDH subunits (A, B, C, or D). Like conventional GISTs, they are usually immunoreactive for CD117 and discovered with GIST-1 (DOG1). However, conventional GIST risk stratification based on mitotic activity and tumor size fails to predict progression of this special group of epithelioid GISTs.⁴⁹ Therefore, immunohistochemical analysis for SDHB is highly recommended for all epithelioid GISTs to identify this clinically and biologically distinctive group of GISTs.

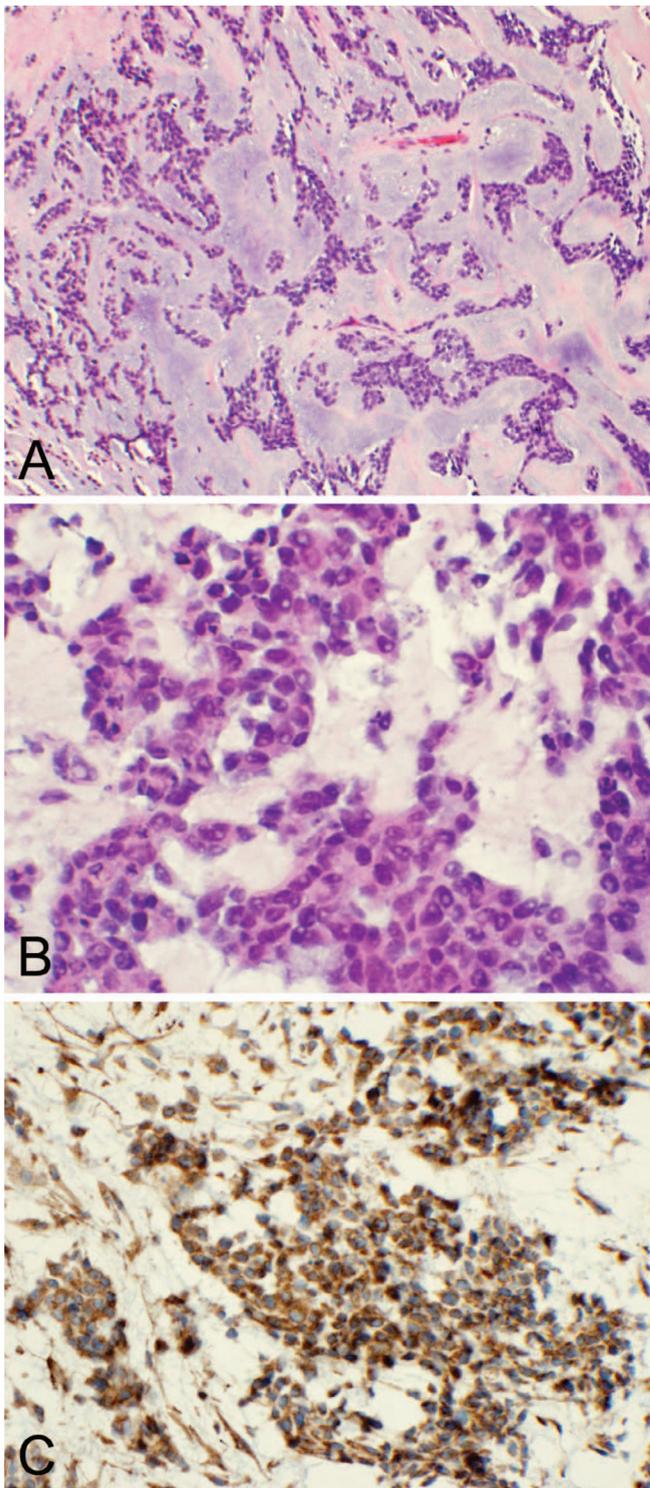


Figure 8. Myoepithelial tumors. A, Myoepithelioma exhibits nests of myoepithelial cells in a chondromyxoid stroma. B, Myoepithelial carcinoma shows significant cytologic atypia, frequent mitoses, and calponin expression (C) (hematoxylin-eosin, original magnifications $\times 40$ [A] and $\times 200$ [B]; original magnification $\times 200$ [C]).

Malignant epithelioid soft tissue tumors include, but are not limited to, epithelioid MPNST, epithelioid leiomyosarcoma, epithelioid rhabdomyosarcoma, and clear cell sarcoma. Epithelioid MPNST is mostly not associated with NF1. This rare variant (<5%) is unique in that it shows strong and

diffuse expression of S100 protein, can be positive for epithelial markers (cytokeratin/EMA), but demonstrates INI1 loss (67%) and lacks staining for melanoma markers. In contrast, conventional (spindle cell) MPNST is positive for S100 protein in less than 50% of cases.⁵⁰ SOX-10 reportedly shows positivity in two-thirds of epithelioid MPNSTs.⁵¹ It is noteworthy that glandular differentiation seen in conventional MPNST, particularly in patients with NF1, should not be regarded as epithelioid MPNST.⁵² Most clear cell sarcomas display predominant epithelioid morphology, but spindle cell areas are commonly present. Epithelioid leiomyosarcoma mostly occurs in the uterus but can be rarely seen in the external deep soft tissue.⁵³ Epithelioid rhabdomyosarcoma is a morphologic variant recently described in adults and children that may closely mimic carcinoma or melanoma. Histologically, epithelioid rhabdomyosarcoma displays sheets of large cells with or without rhabdomyoblastic differentiation. The cells invariably express skeletal muscle markers including desmin and myogenin, may show positivity for keratin and EMA, and lack *PAX3/7-FOXO1* transcripts characteristic of alveolar rhabdomyosarcoma.^{54,55}

Soft tissue tumors showing purely myoepithelial differentiation (myoepithelioma/myoepithelial carcinoma) and those with a mixed epithelial and myoepithelial component (mixed tumor) arise from eccrine sweat glands of the skin, analogous to their salivary gland counterparts. The former may be part of a continuum with mixed tumors (ductal structures but few myoepithelial cells). The cells constituting myoepithelial tumors demonstrate a spectrum of cytomorphology including epithelioid, histiocytoid, plasmacytoid, or spindled, with little matrix or in the background of a chondromyxoid or collagenous/hyalinized stroma. The cells with myoepithelial differentiation may express epithelial markers (cytokeratin and/or EMA), a variety of myoepithelial markers such as S100 protein and glial fibrillary acidic protein (50%), and muscle markers including calponin, SMA, and desmin.⁵⁶ SOX10, a panschwannian and melanocytic marker, may also show positivity in myoepithelial cell tumors (Figure 8, A through C).⁵⁷ Mixed tumor of skin is morphologically identical to pleomorphic adenoma of the salivary gland, exhibiting secondary structures such as glands/ducts, cysts, keratinous cysts, and foci of squamous differentiation, with a mucoid stroma typically showing cartilaginous metaplasia (hence also known as *chondroid syringoma*). The inner luminal epithelial cells lack expression of the aforementioned myoepithelial markers.

In summary, epithelioid morphology is a frequent finding in soft tissue tumors and can be seen in mesenchymal neoplasms of virtually all lineages. One should always bear this in mind when working up an unknown soft tissue tumor. Lastly, it is important to note that metastatic carcinoma is far more common than epithelioid mesenchymal tumors, especially in the elderly patients. The histologic features and key immunophenotypes of selective epithelioid soft tissue tumors are summarized in Table 4.

TUMORS WITH MYXOID STROMA

Illustrative Example 4

A 52-year-old woman presented with left lower quadrant abdominal pain. She was found to have cholelithiasis, hiatal hernia, and gastroesophageal reflux. A computed tomography scan was recommended as part of her evaluation for possible laparoscopic cholecystectomy. In this study, a 6-cm

Table 4. Salient Histologic Features and Key Immunophenotypes of Selected Epithelioid Soft Tissue Tumors

Tumor Type	Clinical Characteristics	Histologic Hints	Key IHC/Molecular Studies
Epithelioid sarcoma	Extremities (distal) Pelvic and perineal regions (proximal)	Granulomatous (distal type)	<ul style="list-style-type: none"> • Keratin • Vascular marks • INI1 loss
Epithelioid hemangioma	Head and neck, distal extremities, penis	Well-formed vascular channels with a prominent inflammatory infiltrate including eosinophils	<ul style="list-style-type: none"> • Vascular markers • Keratin (focal)
Epithelioid hemangioendothelioma	Any anatomic site	Angiocentric, cords or chains of cells with a myxoid or hyalinized stroma	<ul style="list-style-type: none"> • Vascular markers • Keratin (less common) • TFE3 (small subset) • t(1;3)(p36;q23-25) <i>WWTR1-CAMTA1</i> fusion
Epithelioid angiosarcoma	Highly aggressive	At least focally vasoformative	<ul style="list-style-type: none"> • Vascular markers • Keratin (35%)
Sclerosing epithelioid fibrosarcoma		Small epithelioid cells with a sclerotic collagenized stroma	<ul style="list-style-type: none"> • MUC4 • t(7;16)(q33;p11) <i>FUS-CREB3L2</i> fusion
Glomus tumor	Mostly distal extremities	Small, uniform cells	<ul style="list-style-type: none"> • SMA, h-caldesmon • Type IV collagen
Granular cell tumor	More common in head and neck	Abundant granular cytoplasm	<ul style="list-style-type: none"> • S100, SOX10 • CD68, NSE, MITF, TFE3
PEComa	More common in the retroperitoneal and abdominopelvic regions	Uniform cells with round nuclei and abundant granular eosinophilic or clear cytoplasm	<ul style="list-style-type: none"> • HMB-45, Mart-1, MITF • SMA, calponin. • TFE3 (10%)
Alveolar soft part sarcoma	Most common in deep soft tissue of the thigh/buttock	Large, uniform cells with abundant eosinophilic, granular cytoplasm, arranged in an organoid or nesting pattern	<ul style="list-style-type: none"> • TFE3 (C-terminal) • t(X;17)(p11;q25) <i>ASPCRT1-TFE3</i> fusion
Myoepithelioma/ myoepithelial carcinoma	Cutaneous	Epithelioid, histiocytoid, plasmacytoid, or spindled cells, with little matrix or a chondromyxoid or collagenous stroma	<ul style="list-style-type: none"> • Myoepithelial markers (S100, GFAP) • Muscle markers (calponin, SMA, desmin) • Keratin and EMA • SOX10
Epithelioid MPNST	Not associated with NF1	Uniform cells with abundant eosinophilic cytoplasm, rounded nuclei with vesicular chromatin, arranged in sheets, nests, or cords within a collagenous or myxoid stroma	<ul style="list-style-type: none"> • S100 (strong and diffuse), SOX10 • INI1 loss • Can be positive for keratin/EMA
Clear cell sarcoma	Young adults, mostly distal extremities (ankle or foot)	Spindle cell areas commonly present	<ul style="list-style-type: none"> • Melanocytic markers and SOX10 • t(12;22)(q13;q12) <i>EWSR1-ATF1</i> fusion
Epithelioid RMS	Wide age range	Large cells with or without rhabdomyoblastic differentiation	<ul style="list-style-type: none"> • Skeletal muscle markers (ie, desmin and myogenin)
SDH-deficient GIST	Stomach	Multinodular	<ul style="list-style-type: none"> • CD117, DOG1, SDHB loss

Abbreviations: EMA, epithelial membrane antigen; GFAP, glial fibrillary acidic protein; GIST, gastrointestinal stromal tumor; IHC, immunohistochemistry; MPNST, malignant peripheral nerve sheath tumor; MUC4, mucin 4; NSE, neuron-specific enolase; PEComa, perivascular epithelioid cell differentiation; RMS, rhabdomyosarcoma; SDH, succinate dehydrogenase; SDHB, SDH subunit B; SMA, smooth muscle actin.

mass in the proximal right thigh was discovered. The patient underwent a needle biopsy followed by surgical excision of the right-sided thigh mass. The histologic sections showed a low-grade spindle cell lesion with alternating fibrous and myxoid areas. The lesional cells were diffusely positive for MUC4, thus resulting in a diagnosis of low-grade fibromyxoid sarcoma (Figure 9, A through C).

Myxoid tumors encompass a group of soft tissue neoplasms with a “myxoid” stroma composed of clear, mucuslike substance. The diagnosis of a myxoid tumor is often challenging, as many soft tissue tumors show myxoid changes, and some myxoid tumors are extremely uncommon. Moreover, there is a significant overlap across different entities, especially among the spindle cell tumors with myxoid change for which a recognizable histologic pattern is often lacking. Correlation with clinical and radiologic information and awareness of the entities are crucial in

the differential diagnosis. Strategies in the differential diagnosis of myxoid tumors are summarized in Table 5.

Low-Grade Fibromyxoid Sarcoma and MUC4.—Also known as Evans tumor, low-grade fibromyxoid sarcoma (LGFMS) consists of bland fusiform or spindled cells, often in a whirling pattern. The tumor typically demonstrates alternating fibrous and myxoid areas. Hyalinizing spindle cell tumor with giant rosettes is a unique morphologic pattern seen in some LGFMSs that may simulate palisaded granulomas. MUC4 is a highly sensitive (100%) and quite specific marker for LGFMS.⁵⁸ A t(7;16)(q32-34;p11) translocation resulting in *FUS-CREB3L2* fusion has been found in more than 90% of the cases, with an alternative translocation t(11;16)(p11;p11) *FUS-CREB3L1* fusion found in rare tumors.⁵⁹ Thus, LGFMS is thought to be closely related to SEF given the same translocations and immunophenotype.

Myxoma.—Myxomas more commonly occur in the extremities (intramuscular, juxtaarticular). They are mostly

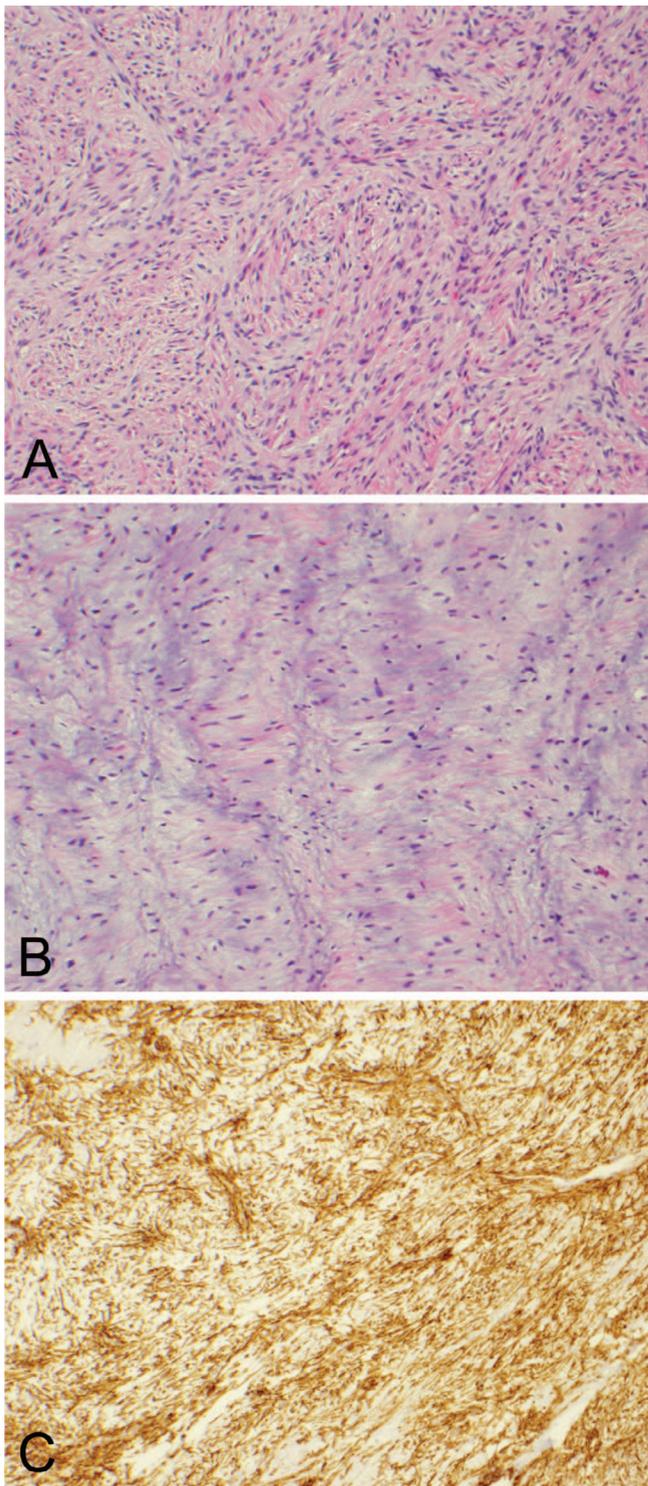


Figure 9. Low-grade fibromyxoid sarcoma (LGFMS). A and B, LGFMS exhibits alternating fibrous and myxoid areas and mucin 4 (MUC4) expression (C) (hematoxylin-eosin, original magnification $\times 100$ [A and B]; original magnification $\times 100$ [C]).

sporadic but may be rarely associated with other diseases (ie, Mazabraud syndrome, Carney complex). The diagnosis of myxomas is mostly straightforward given their imaging characteristics and histologic appearance of paucicellularity and abundant granular myxoid stroma (Figure 10, A). Cellular myxomas contain similar bland spindle cells and

may be difficult to distinguish from other low-grade myxoid lesions, such as low-grade fibromyxoid sarcoma and low-grade myxofibrosarcoma, especially in a small biopsy specimen (Figure 10, B).

Soft Tissue Perineurioma.—Soft tissue perineurioma is a rare benign peripheral nerve sheath tumor showing perineurial cell differentiation. It occurs predominantly in middle-aged adults and arises mainly in subcutaneous tissue in the limbs. Histologically, it is composed of bland spindled cells, with delicate, elongated bipolar cytoplasmic processes arranged in a whorled or storiform architectural pattern. Prominent myxoid stroma is common. Like normal perineurial cells, tumor cells in perineuriomas usually express EMA and claudin-1, the commonly used perineurial markers. However, these markers are unfortunately nonspecific and can be seen in up to 50% of LGFMSs, its major malignant mimic.^{60,61}

Nodular Fasciitis.—Nodular fasciitis is a rapidly growing lesion that is almost always smaller than 5 cm. It is composed of variably cellular fibroblasts and myofibroblasts (thus typically strongly and diffusely positive for SMA and muscle-specific actin) in a myxoid stroma, which may be variably collagenized in longstanding lesions (Figure 10, C). The proliferating cells commonly display a tissue culturelike growth pattern, with frequent mitotic figures but no atypical forms. Extravasated red blood cells, lymphocytes, and giant cells are frequently discernible. Nodular fasciitis has been historically regarded as a reactive process, given its self-limiting nature, but is now thought to be neoplastic owing to the identification of recurrent translocation t(17;22)(p13;q13) that results in *MYH9-USP6* fusion.⁶²

Schwannoma.—Schwannoma is a frequently encountered tumor with a myxoid matrix. Recognition of its biphasic growth pattern characterized by hypercellular Antoni A and myxoid, hypocellular Antoni B areas, in combination with its strong and diffuse S100 immunoreactivity, is typically diagnostic (Figure 10, D).

Myxofibrosarcoma.—Myxofibrosarcoma demonstrates a broad spectrum of cellularity and nuclear pleomorphism, but invariably possesses a curvilinear vascular pattern. The cellularity dictates tumor grade, although the latter does not predict the clinical behavior.⁶³ The low-grade lesions show prominent elongated, curvilinear, thin-walled blood vessels with perivascular condensation of tumor cells (Figure 10, E), whereas the high-grade neoplasms (previously known as *myxoid malignant fibrous histiocytoma*) (Figure 10, F) comprise solid sheets of pleomorphic cells but also focally show features of low-grade lesions. There are currently no unique immunophenotypes or molecular genetic abnormalities.

Extraskeletal Myxoid Chondrosarcoma.—Extraskeletal myxoid chondrosarcoma (EMC) is characterized by the abundant chondromyxoid matrix and small, uniform cells with round to oval nuclei. The tumor typically has a multilobular growth pattern, in which the neoplastic cells are interconnected with each other to form cords, chains, or clusters (Figure 11, A and B). Extraskeletal myxoid chondrosarcoma is distinctively hypovascular and lacks well-developed hyaline cartilage. There is no specific IHC marker for EMC. These tumors may express S100 protein (20%), CD117 (30%), and rarely, cytokeratins; and those with rhabdoid features may show loss of INI1. The t(9;22)(q22;q12) translocation resulting in *EWSR1-NR4A3* fusion has been found as the sole anomaly, while a number of other rare fusion partners for *NR4A3* have been recently identified, including t(9;17)(q22;q11) and t(9;15)(q22;q21).⁶⁴

Table 5. Strategies in the Differential Diagnosis of Myxoid Soft Tissue Tumors

Cellularity	Cytomorphology	Useful Histologic Clues	Key IHC/Molecular Studies	Tumor Type
Low	Spindle	Paucicellular/slightly cellular Lack of curvilinear vessels	<i>GNAS</i> point mutations	Myxoma/cellular myxoma
	Spindle	Infiltrative margin Dilated, thick-walled vessels	CD34/HMGA2	Aggressive angiomyxoma
Moderate	Spindle	Small (<5 cm) Tissue culturelike pattern Extravasated blood cells	SMA, MSA t(17;22)(p13;q13) <i>MYH9-USP6</i> fusion	Nodular fasciitis
	Spindle	Alternating fibrous and myxoid areas	MUC4 t(7;16)(q33;p11) <i>FUS-CREB3L2</i> fusion	Low-grade fibromyxoid sarcoma
	Spindle	Slender cells with bipolar cytoplasmic processes in a whorled or storiform pattern	EMA, claudin1	Soft tissue perineurioma
	Spindle	Antoni A and Antoni B Hyalinized vessels	S100	Schwannoma
	Round-spindle	Incomplete peripheral metaplastic bone	S100	Ossifying fibromyxoid tumor
	Round	Chicken-wire vessels Lipoblasts	S100 (variable) t(12;16)(q13;p11) <i>FUS-DDIT3</i> fusion t(12;22)(q13;q12) <i>EWSR1-DDIT3</i> fusion	Myxoid liposarcoma
	Round	Cells arranged in cords, chains, or small clusters	S100 (~20%) CD117 (30%) <i>EWSR1-NR4A3</i> fusion	Extraskeletal myxoid chondrosarcoma
	Epithelioid	Physaliferous cells	Keratins, EMA, S100, Brachyury	Chordoma
High	Epithelioid	Macronucleoli Mixed inflammatory infiltrate	t(1;10)(p22-31;q24-25) <i>TGFBR3-MGEA5</i> fusion	Myxoinflammatory fibroblastic sarcoma
	Spindle	Curvilinear vessels, pleomorphic (cellularity dictates grade)	N/A	Myxofibrosarcoma

Abbreviations: EMA, epithelial membrane antigen; IHC, immunohistochemistry; MSA, muscle-specific actin; MUC4, mucin 4; N/A, not applicable; SMA, smooth muscle actin.

Chordoma.—Chordoma is a malignant midline bone tumor arising from fetal notochord. It typically affects the base of skull, the vertebral bodies, and the sacrococcygeal bone, but may be rarely seen in the extraaxial skeleton, such as intervertebral discs and presacral soft tissue. The morphologic hallmark is the presence of cords and lobules of “physaliferous cells” separated by fibrous septa in abundant myxoid matrix. The stroma may less commonly have a chondromyxoid appearance, thus resembling a hyaline cartilage tumor (chondroid chordoma). The tumor cells are typically immunoreactive for cytokeratins, EMA, S100 protein, and Brachyury, with the latter being highly specific (Figure 12, A through D).⁶⁵ It is noteworthy that the cells of intraosseous benign notochordal cell tumor have the same immunophenotype as chordoma, but the former typically lacks a lobular architecture, fibrous bands, and myxoid matrix. Thus, correlation with imaging studies is crucial when dealing with a limited biopsy specimen.

Other Myxoid Tumors.—Ossifying fibromyxoid tumor is typically a well-circumscribed mass that is characteristically composed of incomplete peripheral metaplastic bone tissue. The tumor contains cords of bland, round or short spindled cells in a stroma ranging from predominantly myxoid to hyalinized. The lesional cells are typically positive for S100 protein and, to a lesser degree, desmin.

Myxoinflammatory fibroblastic sarcoma (MIFS) typically affects lower extremities. It is histologically characterized by epithelioid fibroblasts with macronucleoli mimicking virocytes or Reed-Stenberg cells and a prominent mixed inflammatory infiltrate in a variably myxoid stroma. The

lesional cells may be immunoreactive for SMA and CD34. Most MIFSs carry t(1;10)(p22;q24), which results in *TGFBR3-MGEA5* fusion.⁶⁶

Aggressive angiomyxoma mostly affects women and arises in the perineal and pelvic regions. It is typically circumscribed but demonstrates peripheral infiltrative margins with extension into adjacent structures. The tumor is hypocellular and composed of monotonous small spindled and stellate fibroblastic cells in the background of myxoid stroma and prominent, dilated, thick-walled vessels. The cells express CD34 but immunohistochemical studies are usually not needed.

Recently, HMGA2, a sensitive but nonspecific marker for aggressive angiomyxoma, has been shown to be useful in evaluating margins and in re-excision specimens when the foci of aggressive angiomyxoma are morphologically subtle.⁶⁷

ROUND CELL TUMORS

Illustrative Example 5

A 10-year-old girl presented with a few months’ history of increasing pain and swelling in the left shoulder region. A computed tomography scan showed a large intracapsular, heterogeneous mass with no involvement of bone. A biopsy revealed a small blue round cell tumor that was immunoreactive for CD99 and FLI1. FISH analysis demonstrated *EWSR1* gene rearrangement, thus confirming the diagnosis of Ewing sarcoma.

Round cell tumors of soft tissue constitute a divergent group of neoplasms, largely including Ewing sarcoma,

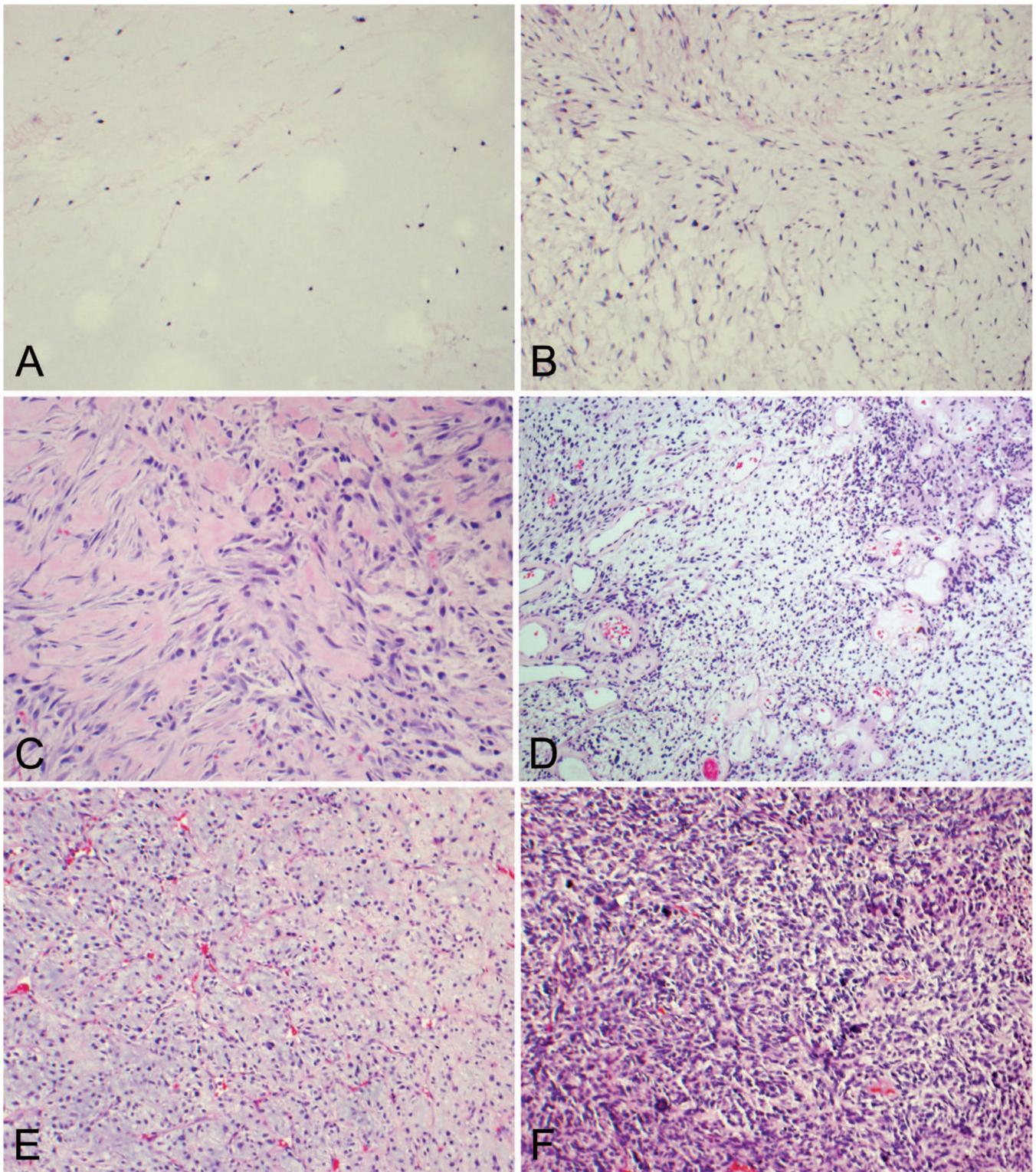


Figure 10. Selected myxoid tumors: myxoma (A), cellular myxoma (B), nodular fasciitis (C), schwannoma (D), low-grade myxofibrosarcoma (E), and high-grade myxofibrosarcoma (F) (hematoxylin-eosin, original magnification $\times 100$ [A through F]).

rhabdomyosarcoma, desmoplastic small round cell tumor, neuroblastoma, and recently characterized CIC (capicua transcriptional suppressor)-rearranged sarcoma and BCOR (Bcl-6 corepressor)-rearranged sarcoma.⁶⁸⁻⁷⁰ These tumors morphologically look alike and may share some immunophenotypes but many harbor specific molecular genetic

abnormalities. Moreover, hematologic malignancies and metastatic small cell carcinoma should always be within the differential diagnosis during the workup of round cell tumors in the soft tissue.

Ewing Sarcoma.—Ewing sarcoma and primitive neuroectodermal tumor (PNET) were historically thought to be

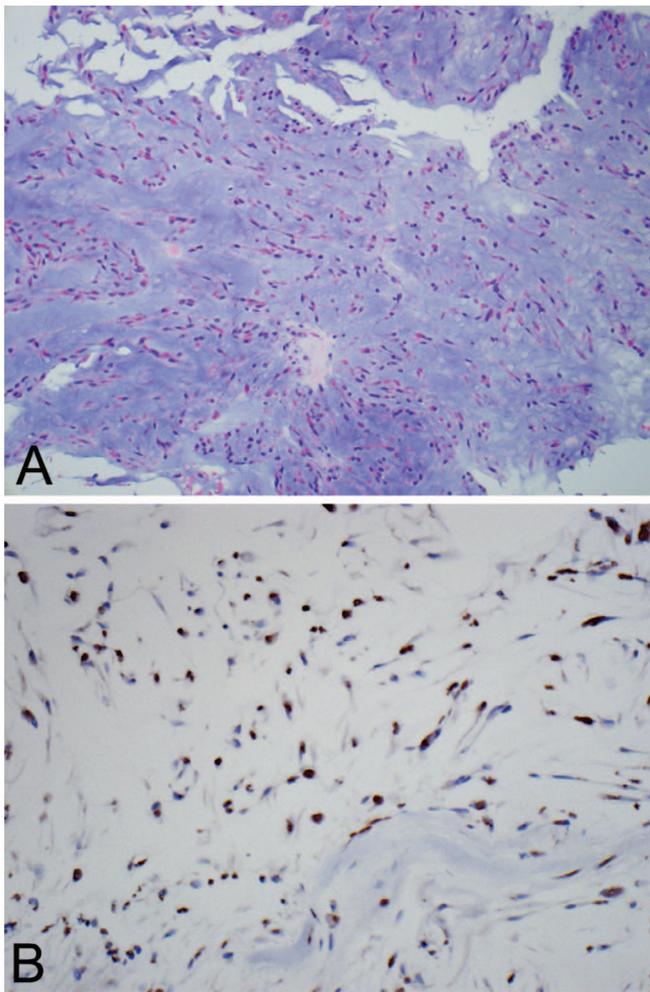


Figure 11. Extraskeletal myxoid chondrosarcoma. *A*, Tumor shows cords and chains of small, uniform cells with round to oval nuclei and abundant chondromyxoid matrix. *B*, The lesional cells may occasionally express cytokeratin (hematoxylin-eosin, original magnification $\times 200$ [A]; original magnification $\times 400$ [B]).

different entities, with the latter demonstrating neuroectodermal differentiation. It is now generally accepted that Ewing sarcoma of bone, extraosseous Ewing sarcoma, PNET, and Askin tumor (PNET of the thoracopulmonary region) are histologic variants of the same tumor spectrum. As many other round cell tumors, Ewing sarcoma typically appears as solid sheets of uniform, small blue round cells with minimal cytoplasm and little extracellular matrix. Diffuse membranous expression of CD99 is characteristic for Ewing sarcoma but not specific. Nuclear FLI1 expression is seen in most Ewing sarcoma cases with the t(11;22)(q24;q12) translocation, whereas ERG immunoreactivity is seen in a small subset of cases harboring the t(21;22)(q22;q12) translocation (Figure 13, A and B). In addition, ERG is expressed in both normal and neoplastic endothelial cells (see previous discussion). It also shows positivity in acute myeloid leukemia and a subset of prostate carcinomas. Focal expression of cytokeratin can also be seen.⁷¹ As previously mentioned, FLI1 is not specific for Ewing sarcoma and is also expressed in most lymphoblastic lymphomas (also CD99⁺), as well as anaplastic large cell lymphoma and angioimmunoblastic T-cell lymphoma, in addition to its utility as an endothelial marker. The protein is also

expressed in a small subset of melanomas, Merkel cell carcinomas, synovial sarcomas, and carcinomas of lung and breast.⁷² A recent study⁷³ has demonstrated that NKX2.2, a homeodomain-containing transcription factor that plays a critical role in neuroendocrine/gliar differentiation, is a target of *EWSR1-FLI1*, a valuable marker for Ewing sarcoma, with a sensitivity of 93% and a specificity of 89%. Moreover, protein kinase C- β (PRKCB) activation is directly regulated by the chimeric oncogene *EWSR1-FLI1*. PRKCB expression has also been shown to be a sensitive and specific marker for *EWSR1* rearrangements and a potential therapeutic target, thus warranting further investigation.⁷⁴ While most Ewing sarcomas harbor *EWSR1-FLI1* (85%) or *EWSR1-ERG* (~10%) fusion gene, there is a growing list of additional rare transcripts identified.⁷¹ FISH analysis using break-apart probe is highly sensitive (>90%) in detecting *EWSR1* rearrangements but does not identify its translocation partner, whereas RT-PCR analysis is a specific test in identifying the *EWSR1-FLI1* fusion gene but has a suboptimal sensitivity (54%) in formalin-fixed paraffin-embedded tissue.⁷⁵ The latter also requires abundant lesional tissue, good quality of RNA, and a longer turnaround time.

Rhabdomyosarcoma.—Rhabdomyosarcoma constitutes the single largest category of soft tissue sarcomas in children and young adults. The histologic subtypes with prominent round cell morphology include embryonal and alveolar forms, whereas other rare variants demonstrate either a spindle cell or pleomorphic cytology (spindle cell/sclerosing rhabdomyosarcoma and pleomorphic rhabdomyosarcoma, respectively).

Embryonal rhabdomyosarcoma is the most common subtype, typically affecting children younger than 10 years and occasionally occurring in adolescents. Head and neck region and genitourinary system are the common sites of involvement. The tumor contains primitive mesenchymal cells admixed with a variable content of rhabdomyoblasts, which demonstrate elongation, more cytoplasmic eosinophilia, and sometimes cross-striation (Figure 13, C). A tumor may be composed exclusively of solid sheets of round cells, thus inviting confusion with alveolar rhabdomyosarcoma. There are no unique molecular genetic abnormalities identified for this variant.

Alveolar rhabdomyosarcoma occurs more commonly in adolescents and young adults and more often affects extremities. The tumor is typically densely cellular and consisting of a monotonous population of primitive round, blue cells (Figure 13, D). Rhabdomyoblastic differentiation may be seen but often to a smaller extent. A t(2;13)(q35;q14) or t(1;13)(p36;q14) translocation resulting in *PAX3-FOXO1* or *PAX7-FOXO1* fusion genes occurs in most cases. This subtype is clinically more aggressive than the embryonal variant, thus is important to identify.

Desmin is a muscle-specific protein and a key subunit of the intermediate filament in cardiac, skeletal, and smooth muscles. It shows reasonable sensitivity but not specificity for skeletal muscle tumors. Myogenin and myoD1 are transcription factors involved in myogenesis, thus are highly specific for rhabdomyosarcoma (Figure 13, E). It is noteworthy that nonspecific cytoplasmic myoD1 staining is not uncommon and may be misinterpreted as positive. Cytokeratin, neuroendocrine markers, CD20, and S100 protein expression can be occasionally seen, thus it may cause diagnostic confusion.

Desmoplastic Small Round Cell Tumor.—Desmoplastic small round cell tumor (DSRCT) primarily occurs in children

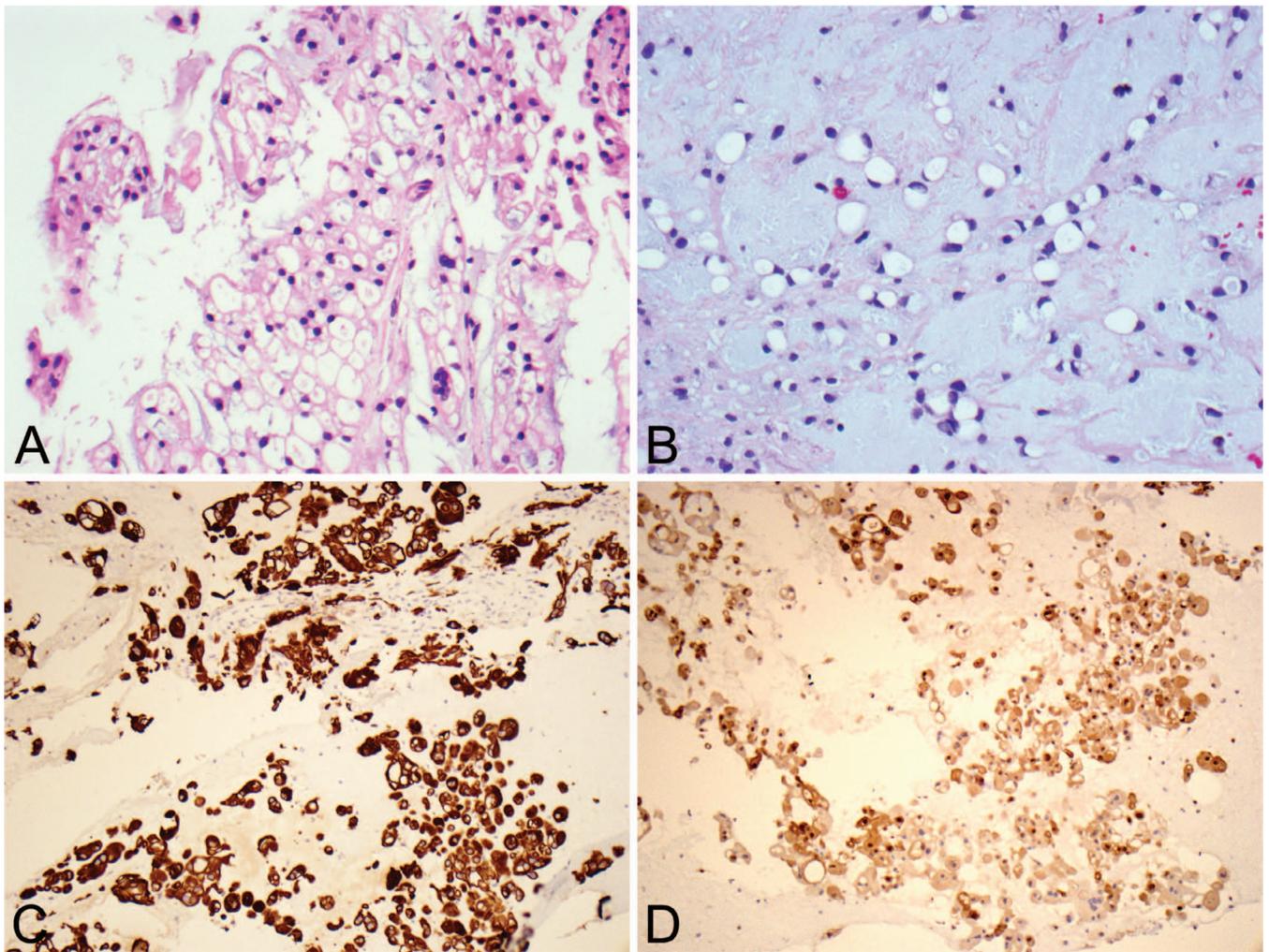


Figure 12. Chordoma. A, This tumor shows characteristic “physaliferous cells.” The stroma may less commonly have a chondromyxoid appearance (chondroid chordoma) (B). The tumor cells are typically immunoreactive for cytokeratin (C) and S100 protein (D) (hematoxylin-eosin, original magnification $\times 200$ [A and B]; original magnification $\times 200$ [C and D]).

and young adults, with a striking predilection for boys. It usually arises in abdomen, retroperitoneum, or pelvis, with widespread serosal implants. The tumor is so named because of prominent stromal desmoplasia (Figure 13, F). Tumor necrosis, frequent mitoses, and cystic degeneration are common. Glandular and pseudorosette formations may be seen. Multiphenotypic differentiation is a distinctive feature of DSRCT, thus epithelial, muscular, and neural markers may have variable immunoreactivity. Nuclear expression of WT1, the hallmark immunophenotype of DSRCT, is characteristically seen when using antibodies raised against the carboxy-terminus, but not the amino-terminus, of WT1.^{76,77} Of note, dotlike perinuclear reactivity of desmin and coexpression of cytokeratin can be seen in both DSRCT and Wilms tumor. Thus, detection of an *EWSR1-WT1* rearrangement resulting from t(11;22)(p13;q12) translocation and selective WT1 carboxy-terminus immunoreactivity (characteristic of DSRCT), but not dual immunoreactivity for the WT1 amino-terminus and carboxy-terminus (characteristic of Wilms tumor), are the most discriminating diagnostic tools for the 2 tumors with overlapping histomorphology.⁷⁸

New Emerging Ewing-Like Sarcomas.—A small subset of round cell sarcomas clinically and histologically mimic

Ewing sarcoma but fail to demonstrate any of the reported cytogenetic abnormalities described above. These tumors have also been historically labeled as Ewing-like sarcomas. In 2006, two cases of “Ewing-like sarcoma” were found to harbor a recurrent t(4;19)(q35;q13) translocation, which resulted in fusion between *CIC*, a human homolog of *Drosophila capicua*, which encodes a high-mobility group box transcription factor, and *DUX4*, a double homeodomain gene.⁷⁹ To date, *CIC-DUX4* fusion is the most frequent genetic alteration in *EWSR1/FUS*-negative undifferentiated small round cell tumors, while a number of other fusion partners for *CIC* have been recently identified.⁸⁰ *CIC*-rearranged sarcomas primarily occur in soft tissue but may rarely affect bone. These tumors may have variable CD99 immunoreactivity, ranging from negative to focal and/or weak, and to diffuse and/or strong.

More recently, a new subtype of Ewing-like sarcomas has been defined by the fusion of the *BCOR* (*BCL6* corepressor) and *CCNB3* genes, which are nonadjacent genes on the X chromosome.⁷⁰ Additional fusion partners for *BCOR* have also been found.⁸⁰ The *BCOR*-rearranged sarcomas more frequently arise in bone than soft tissue, and demonstrate variable CD99 expression as other Ewing-like sarcomas.

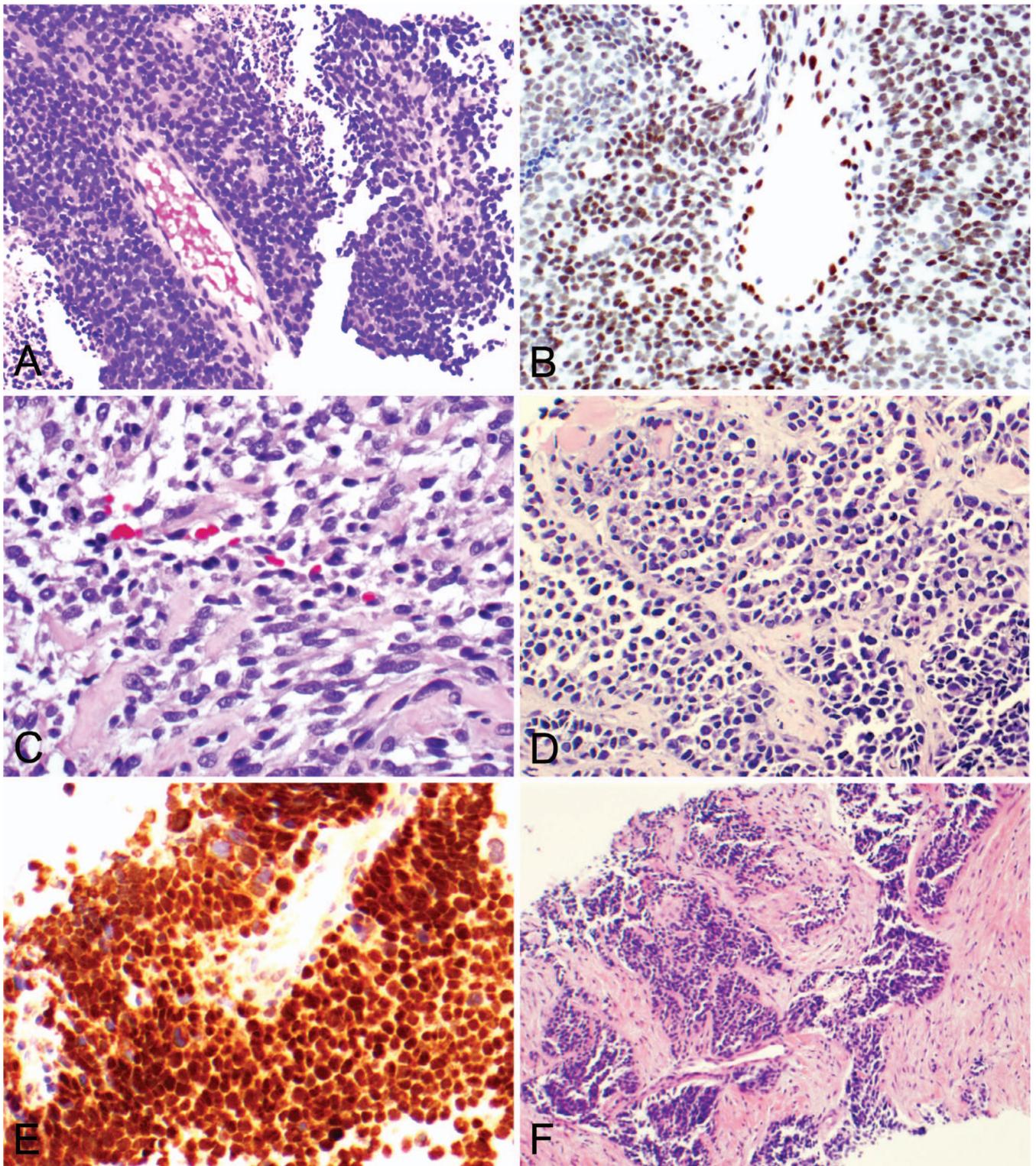


Figure 13. Selected round cell tumors. A, Ewing sarcoma consists of solid sheets of small, blue, round cells with geographic necrosis and nuclear expression of Friend leukemia integration 1 transcription factor (FLI1) (B). C, Embryonal rhabdomyosarcoma demonstrates primitive mesenchymal cells with round and spindled nuclei as well as numerous rhabdomyoblasts. D, Alveolar rhabdomyosarcoma shows monotonous round cells with an “alveolar” growth pattern and strong myoD1 nuclear expression (E). F, Desmoplastic small round cell tumor exhibits nests of round cells separated by a prominent, densely collagenized stroma (hematoxylin-eosin, original magnifications $\times 200$ [A and D], $\times 400$ [C], and $\times 100$ [F]; original magnifications $\times 200$ [B] and $\times 400$ [E]).

Given the overlapping clinical, histologic, and immunophenotypic features of the abovementioned Ewing sarcoma family tumors, *CIC*- and *BCOR*-rearranged sarcomas, molecular cytogenetic studies are required to achieve a correct diagnosis, thus allowing prospective therapeutic management in the pursuit of precision medicine.

SUMMARY

Soft tissue tumors represent a heterogeneous group of neoplasms exhibiting a spectrum of histomorphologies, some with overlapping features, and numerous molecular alterations contributing to their diversity. The classification and diagnosis of soft tissue tumors have improved with recent molecular techniques and IHC. It is important to understand not only the diagnostic utility of these recent technologies but also their potential limits and pitfalls. Clinical and radiologic correlation is still a must to render accurate diagnostic, prognostic, and therapeutic information to guide patient care.

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