Diagnostic Immunohistochemistry for Soft Tissue and Bone Tumors: An Update

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Abstract: Although some soft tissue and bone tumors can be identified based on histologic features alone, immunohistochemistry plays a critical diagnostic role for most mesenchymal tumor types. The discovery of recurrent genomic alterations in many benign and malignant mesenchymal neoplasms has added important biologic insights and expanded the spectrum of some diagnostic subgroups. Some tumors are defined by unique genomic alterations, whereas others share abnormalities that are not tumor-specific and can be observed in a sometimes broad range of biologically unrelated neoplasms. We herein focus on novel immunohistochemical markers, based on molecular genetic alterations, which are particularly useful in the diagnostic workup of selected groups of soft tissue and bone tumors, including recently described entities, specifically round cell sarcomas (Ewing sarcoma, *CIC*-rearranged sarcoma, and *BCOR*-rearranged sarcoma), vascular tumors (epithelioid hemangioma, epithelioid hemangioendothelioma, and pseudomyogenic hemangioendothelioma), SMARCB1-deficient neoplasms, adipocytic tumors (spindle cell/pleomorphic lipoma, atypical spindle cell lipomatous tumor, and conventional atypical lipomatous tumor), giant cell-rich bone tumors (giant cell tumor of bone and chondroblastoma), and biphenotypic sinonasal sarcoma. Given the complex nature of sarcoma classification, and the rarity of many mesenchymal tumor types, careful integration of clinical presentation, imaging features, histology, immunophenotype, and cytogenetic/molecular alterations is crucial for accurate diagnosis of soft tissue and bone tumors.

Key Words: sarcoma, CAMTA1, FOSB, SMARCB1, PAX3, histone 3

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The rarity and yet striking biologic diversity of soft tissue and bone tumors make the diagnostic workup of this group of tumors challenging. Substantial advances in recent years have provided important insights into the genomic underpinnings of many benign and malignant mesenchymal neoplasms and led to the gradual incorporation of immunohistochemistry, cytogenetic, and molecular genetic techniques into routine diagnostics. We herein focus on the most recent advances that have been made in selected groups of soft tissue and bone tumors and highlight important immunohistochemical findings that aid in their diagnostic workup—in correlation with clinical presentation, morphologic features, and genomic alterations.

As notable examples, newly emerging entities in the group of round cell sarcomas and vascular tumors can now be distinguished by the expression of markers that are directly or indirectly linked to the underlying defining cytogenic alteration. The identification of distinct cytogenetic features in subsets of vascular tumors in the past few years has led to the introduction of associated immunohistochemical markers that directly reflect the underlying genetic aberration.

In contrast, the spectrum of benign and malignant mesenchymal (and epithelial) neoplasms that share SMARCB1 deficiency continues to expand, which may lead to diagnostic challenges in tumors with otherwise similar morphologic and immunophenotypic characteristics and yet marked differences in biologic behavior, such as epithelioid schwannoma and epithelioid malignant peripheral nerve sheath tumor (MPNST). In addition, the recently refined classification of adipocytic tumors recognizes atypical spindle cell lipomatous tumor as a distinct entity, thereby expanding the spectrum of adipocytic neoplasms with spindle cell features. The discovery of highly recurrent mutations in histone 3.3 encoding genes in certain giant cell-rich bone tumors led to the introduction of mutation-specific antibodies with high specificity and sensitivity, which directly point to the underlying type of mutation. Finally, biphenotypic sinonasal sarcoma represents a recently described entity defined by distinct cytogenetic aberrations with direct immunohistochemical correlates. Recent advances in the immunohistochemical workup and underlying genetic alterations of these groups of soft tissue and bone tumors are summarized in Table 1.

The increasing use of next-generation sequencing and improved bioinformatics algorithms for structural variant detection in the diagnostic workup of soft tissue and bone tumors has substantially contributed to the field, and is expected to continue to identify novel entities and introduce more nuances into existing classification systems. However, with the increasing discovery of novel molecular and cytogenetic findings of unknown biologic significance, critical correlation with morphologic and immunohistochemical features remains of critical importance in the diagnostic workup of mesenchymal neoplasms.

EMERGING ENTITIES IN THE GROUP OF ROUND CELL SARCOMAS

While Ewing sarcoma represents the prototypical round cell sarcoma, the recent discovery of recurrent cytogenetic alterations in round cell sarcomas lacking *EWSR1* rearrangement has refined the diagnostic spectrum of round cell sarcomas (Table 1). Specifically, round cell sarcomas harboring rearrangements of *CIC* or *BCOR* will be discussed herein.

Ewing Sarcoma

Ewing sarcoma is comprised of poorly differentiated, primitive cells with round nuclei, inconspicuous nucleoli and scant cytoplasm with a sheet-like growth pattern (Fig. 1A). The tumor cells usually display a strikingly monotonous appearance; pleomorphism is absent. However, a variety of

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TABLE 1. Overview of Immunohistochemical Markers and Molecular Correlates in the Differential Diagnosis of Selected Round Cell Sarcomas, Vascular Tumors, SMARCB1-deficient Neoplasms, Adipocytic Tumors, Giant Cell-rich Bone Tumors, and Biphenotypic Sinonasal Sarcoma

		Immunohistochemistry		
Diagnostic Group	**Tumor Type**	**Positive Markers**	**Negative Markers**	**Genetic Alteration**
Round cell sarcomas	Ewing sarcoma	CD99 (100%), NKX2.2 (>90%)	ETV4, WT1, BCOR, CCNB3	*EWSR1-FLI1* fusion (*EWSR1-ERG*, others)
	CIC-rearranged sarcoma	CD99 (20%), ETV4 (>90%), WT1 (>90%)	NKX2.2	*CIC-DUX4* fusion (*CIC-FOXO4*)
	BCOR-rearranged sarcoma	CD99 (80%), BCOR (>90%), CCNB3 (90%)	NKX2.2	*BCOR-CCNB3* fusion (*BCOR-MAML3, ZC3H7B-BCOR7, KMT2D-BCOR*)
Vascular tumors	Epithelioid hemangioma	CD31, ERG, FOSB (50%)	—	*ZFP36-FOSB* fusion, *WWTR1-FOSB* fusion, *FOS* rearrangement
	Epithelioid hemangioendothelioma	CD31, ERG, keratin (25%), CAMTA1 (90%), TFE3 (5%)	—	*WWTR1-CAMTA1* fusion (90%), *YAP1-TFE3* fusion (5%)
	Pseudomyogenic hemangioendothelioma	CD31, ERG, keratin, FOSB (>90%)	—	*SERPINE1-FOSB* fusion
SMARCB1-deficient tumors	Malignant rhabdoid tumor	Keratin, EMA, various others	SMARCB1 (100%)	*SMARCB1* mutation/deletion
	Epithelioid sarcoma	Keratin, EMA, CD34 (55%)	SMARCB1 (90%)	*SMARCB1* deletion (miR-206,-381, 671-5p upregulation)
	Epithelioid schwannoma	S100 (100%), SOX10 (100%), GFAP (40%)	SMARCB1 (40%)	NA
	Epithelioid MPNST	S100 (100%), SOX10 (100%), GFAP (60%)	SMARCB1 (70%)	NA
	Poorly differentiated chordoma	Brachyury (100%), keratin (100%)	SMARCB1 (100%)	NA
	Myoepithelial carcinoma	Myoepithelial markers (p63, SMA, GFAP, S100), keratin	SMARCB1 (10-40%)	NA
	Extraskeletal myxoid chondrosarcoma	S100 (<50%), EMA (30%)	SMARCB1 (17%)	*NR4A3-EWSR1* fusion (*NR4A3-TAF15*)
	Renal medullary carcinoma	PAX8, keratin	SMARCB1 (90%)	*SMARCB1* rearrangement
Adipocytic tumors with spindle cell features	Spindle cell/pleomorphic lipoma	CD34	RB1	13q14 deletion (*RB1*)
	Atypical spindle cell lipomatous tumor	CD34 (60%), S100 (40%), desmin (20%)	RB1 (50%)	13q14 deletion (*RB1*)
	Atypical lipomatous tumor	MDM2, CDK4, HMGA2	—	12q13-15 high-level amplification (*MDM2*, *CDK4*, *HMGA2*)
Giant cell-rich bone tumors	Giant cell tumor of bone	H3G34W (>90%, rarely G34V or G34R)	H3K36M	*H3F3A* (*H3F3B*) G34W mutation (G34V, G34R, G34L, G34M)
	Chondroblastoma	H3K36M (>95%)	H3G34W	*H3F3B* (*H3F3A*) K36M mutation
Sinonasal sarcoma	Biphenotypic sinonasal sarcoma	S100, SMA (desmin, myogenin), PAX3 (PAX8), β-catenin (TLE1)	—	*PAX3-MAML3* fusion (*PAX3-FOXO1*, *PAX3-NCOA1*, others)

EMA indicates epithelial membrane antigen; GFAP, glial fibrillary acidic protein; MPNST, malignant peripheral nerve sheath tumor; NA, not available.

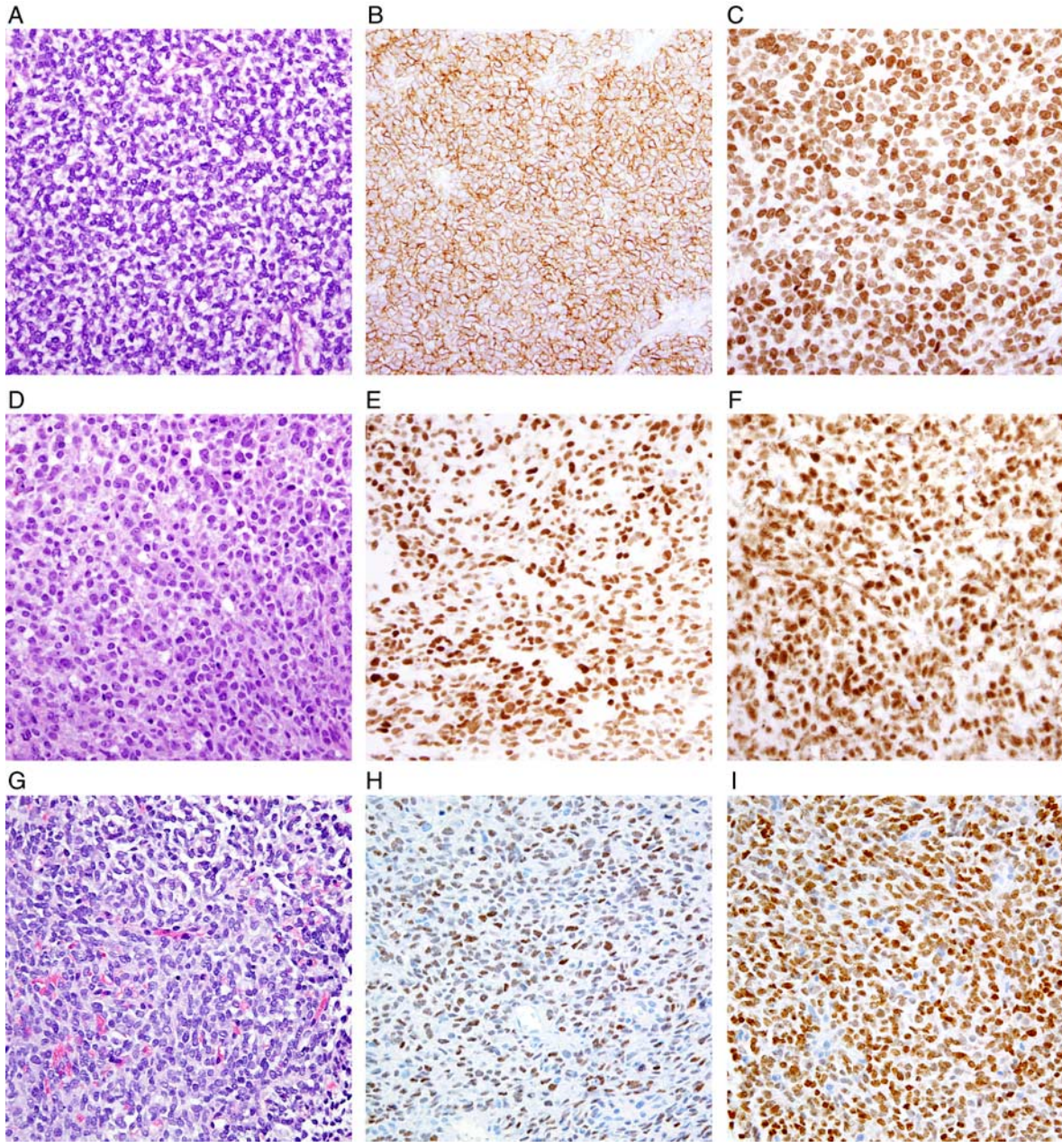

FIGURE 1. Emerging entities in the group of round cell sarcomas. Ewing sarcoma is characterized by sheets of uniform tumor cells with rounded nuclei and inconspicuous nucleoli (A) with diffuse membranous expression of CD99 (B) and nuclear expression of the transcription factor NKX2.2 (C). *CIC*-rearranged sarcoma is comprised of a morphologically more heterogeneous population of primitive round to ovoid or spindled tumor cells (D) with nuclear expression of ETV4 (E) and WT1 (F). *BCOR*-rearranged sarcoma features primitive appearing round to ovoid and occasionally spindled tumor cells, arranged in fascicles or showing a patternless architecture with variably prominent myxoid stroma (G). These tumors show expression of BCOR (H) and CCNB3 (I) in most cases.

rare morphologic variants has been described.[1] Approximately 90% of Ewing sarcomas harbor t(11;22)(q24;q12) leading to *EWSR1-FLI1* fusion. The remainder of cases show *EWSR1* rearrangement with other fusion partners or unknown genes.

Ewing sarcoma typically shows strong and diffuse membranous expression of CD99 (Fig. 1B), which is generally not observed to this extent in other neoplasms; this pattern is therefore relatively specific. As shown recently, nuclear expression of the transcription factor NKX2.2 is found in around 95% of Ewing sarcomas (Fig. 1C), but is also expressed in a subset of histologic mimics such as mesenchymal chondrosarcoma (in 75% of cases) and is therefore not specific for Ewing sarcoma.[2] However, the combination of CD99 and NKX2.2 is diagnostically useful.[3,4]

CIC-rearranged Sarcoma

In recent years, a novel subset of round cell sarcomas was identified that lacked *EWSR1* rearrangement and instead harbored recurrent *CIC* rearrangement, with

CIC-DUX4 fusion resulting from t(4;19)(q35;q13) or t(10;19)(q26;q13) as the most common aberration, followed by rare alternate *CIC-FOXO4* fusion.[5–7] *CIC*-rearranged sarcomas were subsequently shown to exhibit distinct transcriptional and immunohistochemical profiles that set them apart from Ewing sarcoma and further support their classification as a separate entity.[8]

CIC-rearranged sarcoma shows a predilection for the soft tissues of the trunk and extremities of young adults with a mean age of 32 years and a slight male predominance.[9] Histologically, *CIC*-rearranged sarcoma displays a higher degree of nuclear heterogeneity than observed in Ewing sarcoma, including irregular nuclear contours, variation in nuclear size, and prominent nucleoli, as well as more abundant palely eosinophilic cytoplasm, frequent mitoses, and necrosis (Fig. 1D). Expression of CD99 is variable but usually more limited in extent, being diffusely positive in only 20% of cases. In contrast to Ewing sarcoma, *CIC*-rearranged sarcomas exhibit diffuse nuclear expression of ETV4 (Fig. 1E) and WT1 (using the conventional monoclonal antibodies directed against the N-terminus of the protein; Fig. 1F) in >90% of cases. Staining for NKX2.2 is negative in the majority of cases. Of note, *CIC*-rearranged sarcoma behaves more aggressively than Ewing sarcoma, with overall survival rates of 43% versus 77%, and shows worse response to Ewing sarcoma–based chemotherapy regimens.[9] The distinction of *CIC*-rearranged sarcoma from Ewing sarcoma therefore significantly impacts prognostication.

BCOR-rearranged Sarcoma

Another subset of round cell sarcomas lacking *EWSR1* and *CIC* rearrangements was recently identified to harbor recurrent *BCOR* rearrangement, including *BCOR-CCNB3* fusion resulting from inv(X)(p11) in most cases,[10] and rare alternate rearrangement with *MAML3*, *ZC3H7B*[8] or *KMT2D*, as well as *BCOR* internal tandem duplication.[11] *BCOR*-rearranged sarcomas arise most frequently in bone and soft tissue of children with a mean age of 13 to 15 years and are more common in male patients.[11,12] Histologically, *BCOR*-rearranged sarcomas are variably cellular and are often comprised of an admixture of round and spindled cells with monomorphic nuclei embedded in a myxoid or collagenous stroma (Fig. 1G). The tumor cells show variable expression of CD99, and positive staining for BCOR[13] (Fig. 1H) and CCNB3[12] (Fig. 1I) in >90% of cases. Staining for NKX2.2 is negative. Of note, BCOR and CCNB3 immunohistochemistry is generally negative in Ewing sarcoma.[11]

The 5-year overall survival rates of *BCOR*-rearranged sarcomas are 72% to 77% and are comparable with Ewing sarcoma but significantly better than survival rates reported for *CIC-DUX4* sarcomas (see above).[11,12] *BCOR*-rearranged sarcoma has been shown to harbor transcriptional profiles distinct from Ewing sarcoma and *CIC*-rearranged sarcoma, further supporting its recognition as a separate entity.[11]

IMMUNOHISTOCHEMICAL CORRELATES OF RECURRENT CYTOGENETIC ALTERATIONS IN VASCULAR TUMORS

The discovery of recurrent cytogenetic alterations in select epithelioid (and spindle cell) vascular tumors, including epithelioid hemangioma, epithelioid hemangioendothelioma, and pseudomyogenic hemangioendothelioma, have provided insights into the genetic underpinnings of these neoplasms and have led to a more refined classification system in recent years (Table 1). For these distinctive vascular neoplasms, novel immunohistochemical markers that closely reflect underlying genetic alterations have subsequently been introduced into surgical pathology practice.

Epithelioid Hemangioendothelioma

Classified as a low-grade malignant vascular tumor, epithelioid hemangioendothelioma often arises in association with a large vein and is commonly found in soft tissues of the limbs and trunk but also occurs in lung, liver, and bone, where the tumor is often multifocal.[14] Local recurrences have been reported in 15% of cases and distant metastases in 30% of cases. Histologically, epithelioid hemangioendothelioma is characterized by a variably cellular proliferation of epithelioid endothelial cells with palely eosinophilic to glassy cytoplasm and intracytoplasmic vacuoles, arranged in cords and strands, embedded in a characteristic myxoid to hyalinized or collagenous stroma (Fig. 2A). Vascular markers such as CD31 and ERG are generally expressed by the tumor cells, and a subset of cases show positive staining for keratins. Until recently, specific immunohistochemical markers did not exist and the differential diagnosis of epithelioid hemangioendothelioma—which includes a broad range of epithelioid mesenchymal tumors and even carcinomas—was challenging in certain instances, such as highly cellular examples. However, the identification of a recurrent t(1;3)(p36.3;q25)[15] leading to *WWTR1-CAMTA1* fusion in 90% of cases,[16,17] prompted the introduction of CAMTA1 immunohistochemistry, which demonstrates diffuse nuclear expression in most cases and is highly sensitive and specific for the diagnosis of epithelioid hemangioendothelioma (Fig. 2B).[18]

Approximately 5% of cases lack *WWTR1-CAMTA1* fusion and instead harbor alternate t(X;11)(p11;q22), resulting in *YAP1-TFE3* fusion.[19] This subset of epithelioid hemangioendothelioma is characterized by distinct morphologic features and displays more abundant eosinophilic cytoplasm and sometimes prominent vasoformative features (Fig. 2C). Immunohistochemical staining for CAMTA1 is negative in these tumors, which instead show diffuse nuclear staining for TFE3 (Fig. 2D).[18]

Future studies will show whether these tumors belong to the spectrum of epithelioid hemangioendothelioma or warrant separate classification.

Pseudomyogenic Hemangioendothelioma

A vascular tumor of intermediate biological potential, pseudomyogenic hemangioendothelioma characteristically presents with multiple synchronous tumors that involve multiple tissue planes in one anatomic region and most commonly affects young to middle-aged male patients.[20] Pseudomyogenic hemangioendothelioma is associated with a low risk for distant metastasis. Histologically, the tumor is comprised of plump spindled and epithelioid cells with prominent eosinophilic cytoplasm and scattered cells with rhabdomyoblast-like cytomorphology arranged in fascicles, often accompanied by neutrophil infiltrates (Fig. 2E). Immunohistochemical staining reveals expression of vascular markers such as CD31 and ERG as well as keratins. A recently discovered recurrent t(7;19)(q22;q13), leading to

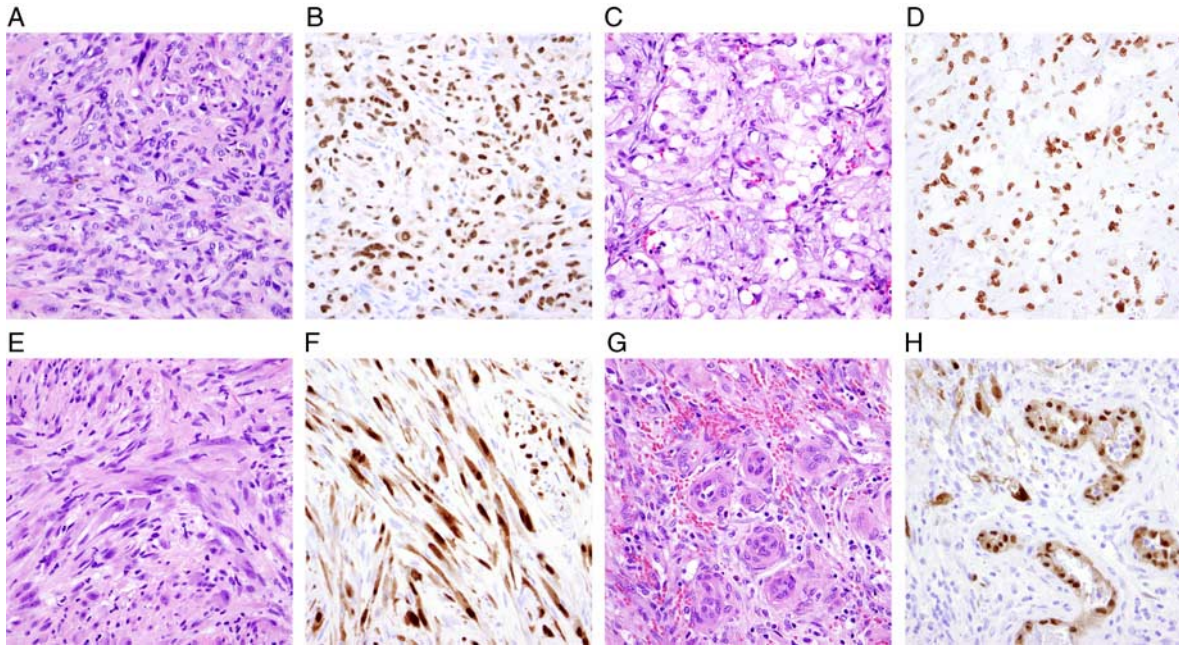

FIGURE 2. Immunohistochemical correlates of recurrent cytogenetic alterations in vascular tumors. Epithelioid hemangioendothelioma is comprised of cords and strands of epithelioid endothelial cells with palely eosinophilic to glassy cytoplasm and intracytoplasmic vacuoles, which are embedded in a myxohyaline stroma (A). Most cases show nuclear expression of CAMTA1 (B) resulting from *WWTR1-CAMTA1* fusion. A small subset of epithelioid hemangioendotheliomas with more abundant eosinophilic cytoplasm and prominent vasoformative features (C) harbors alternate *YAP1-TFE3* fusion leading to nuclear TFE3 expression (D). Pseudomyogenic hemangioendothelioma is characterized by plump spindled and epithelioid cells with prominent eosinophilic cytoplasm and occasional rhabdomyoblast-like cells arranged in fascicles (E). The tumor cells show nuclear expression of FOSB in most cases (F), resulting from *SERPINE1-FOSB* fusion. Epithelioid hemangioma exhibits prominent epithelioid endothelial cells (G). A subset of predominantly cellular epithelioid hemangiomas harbors *ZFP36-FOSB* or *WWTR1-FOSB* fusion, leading to FOSB expression (H).

SERPINE-FOSB fusion, is a defining feature of pseudomyogenic hemangioendothelioma and detected in the majority of cases.[21] Consequent FOSB expression is demonstrated by immunohistochemistry in >90% of cases; this is a useful diagnostic marker. However, FOSB expression is not specific for pseudomyogenic hemangioendothelioma and can also be observed in some epithelioid hemangiomas (see below).[22]

Epithelioid Hemangioma

Epithelioid hemangioma is a benign vascular tumor that commonly occurs in the head neck region, trunk, limbs, and deep soft tissues of middle-aged adults. Sometimes arising in association with a blood vessel, epithelioid hemangioma usually appears as a well-circumscribed and lobular mass. Histologically, the tumor is comprised of epithelioid endothelial cells with hobnail features (Fig. 2G) and a distinctive zonation of well-formed vessels at the periphery of the lesion and more compressed vessels in the center. Nuclear atypia is usually absent or mild, mitoses are rare, and nuclear pleomorphism is uncommon. Although around 20% of cases are multifocal at presentation and local recurrence is observed in 30% of cases, epithelioid hemangiomas do not metastasize.

A subset of epithelioid hemangiomas preferentially occurring in bone and penis is characterized by increased cellularity and often worrisome radiologic features. These "cellular" epithelioid hemangiomas are multifocal in 25% of cases, show less vasoformative features and instead a more prominent solid, sheet-like architecture, making their distinction from malignant vascular neoplasms difficult in some instances. Recurrent t(19;19)(q13.2;q13.2) or interstitial del19(q13.2-3) resulting in *ZFP36-FOSB* gene fusion as well as alternate t(3;19)(q25;q12) resulting in *WWTR1-FOSB* gene fusion have been identified in around 20% of cases.[23] Another subset of both conventional and cellular epithelioid hemangiomas (up to 20%) harbors *FOS* rearrangement, resulting from t(1;14)(q22;q24), t(10;14)(p13;q24), or t(3;14)(q25;q24).[24,25]

Epithelioid hemangioma shows universal expression of vascular markers such as CD31 or ERG. In addition, underlying *FOSB* rearrangement can be inferred by diffuse nuclear expression of FOSB by immunohistochemistry (Fig. 2H) in about half of cases.[22] As outlined above, FOSB expression is also observed in most pseudomyogenic hemangioendotheliomas and is therefore not tumor-specific. However, clinical presentation, tumor site, and disparate morphologic appearances are sufficient for a clear diagnostic distinction between these two entities.

THE BIOLOGIC SPECTRUM OF SMARCB1-DEFICIENT TUMORS

An increasing number of biologically unrelated benign and malignant mesenchymal tumors (and rare carcinomas) exhibit loss of SMARCB1 (INI1) expression (Table 1), and in certain instances, the differential diagnosis of a SMARCB1-deficient epithelioid neoplasm may be challenging, especially when evaluating small biopsies.

Malignant rhabdoid tumor is the prototypical malignant neoplasm defined by genomic inactivation of

SMARCB1 on 22q11.23, either by mutations and/or deletions, and associated loss of SMARCB1 expression in tumor cells.[26] Epithelioid sarcoma is another neoplasm in which genomic *SMARCB1* inactivation is observed in the vast majority of cases, either through homozygous deletion or upregulation of miR-206, miR-381, and 671-5p, with associated loss of SMARCB1 expression in around 90% of cases.[27] In addition, SMARCB1 deficiency is found in 10% to 40% of myoepithelial tumors (mostly in pediatric patients)[27] and in 17% of extraskeletal myxoid chondrosarcomas, classically harboring *NR4A3* rearrangement,[27] although the genomic mechanisms leading to SMARCB1 loss remain to be identified in this tumor type. In addition, nearly all renal medullary carcinomas, a highly aggressive renal neoplasm occurring in young patients with sickle cell trait or disease,[28] show SMARCB1 loss resulting from a balanced translocation disrupting *SMARCB1*.[29]

More recently, epithelioid schwannoma, epithelioid MPNST, and poorly differentiated chordoma have been added to the list of SMARCB1-deficient neoplasms and will be discussed in more detail.

Epithelioid Schwannoma

These tumors constitute a rare variant of schwannoma, occur over a wide age range from 13 to 75 years with a mean age of 45 years with an equal sex distribution, and are generally not associated with neurofibromatosis type 1 (NF1) or 2.[30] Most epithelioid schwannomas arise in the limbs and trunk, where they are usually superficially located, but may also rarely be found at visceral locations. Histologically, the tumors show a multilobular architecture and consist of uniform cells with round vesicular nuclei and abundant palely eosinophilic cytoplasm, arranged in sheets or singly dispersed within a myxoid to hyalinized stroma (Fig. 3A). Nuclear pleomorphism, hyperchromasia, and an increased mitotic rate are usually absent. The presence of atypical nuclei has been described in rare cases with malignant transformation to epithelioid MPNST.[30]

Immunohistochemical staining demonstrates diffuse positivity for S100 protein (Fig. 3B) and SOX10 in all cases, as well as expression of glial fibrillary acidic protein in around 40% of cases. Loss of SMARCB1 expression is identified in 42% of epithelioid schwannomas (Fig. 3C).[30]

Although malignant transformation and local recurrences have been described in rare cases, metastatic spread has not been reported.[30]

Epithelioid Malignant Peripheral Nerve Sheath Tumor

In contrast to conventional MPNST which arises in association with NF1 in about half of cases, epithelioid MPNST generally does not occur in a background of NF1.[31] Epithelioid MPNST affects patients over a wide age range from 6 to 80 years with a mean age of 44 years and relatively equal distribution among women and men.[31] The most common site is the lower limb followed by the trunk, and most tumors are superficially located. Like epithelioid schwannoma, epithelioid MPNST shows a multilobular growth pattern and is comprised of epithelioid tumor cells with round, vesicular nuclei and abundant amphophilic to palely eosinophilic cytoplasm, arranged in sheets or nests surrounded by myxoid or fibrous stroma (Fig. 3D). High cellularity and marked nuclear atypia, as well as a high mitotic rate and foci of necrosis distinguish epithelioid MPNST from epithelioid schwannoma.

Expression of S100 protein is strong and diffuse in around 90% of cases (Fig. 3E), to a degree that is unusual for conventional MPNST, which usually shows limited expression in the 40% of cases in which staining is detected.[31] Glial fibrillary acidic protein is positive in 60% of cases, whereas melanocytic markers (eg, melan A, HMB45, and MiTF) are negative. Loss of SMARCB1 expression is found in 67% of epithelioid MPNST (Fig. 3F).

Epithelioid MPNST appears to show a relatively low risk of disease progression—independent of anatomic site or depth—with local recurrences reported in around 30% of cases and distant metastases in 17% of cases.[31]

Poorly Differentiated Chordoma

A rare aggressive variant of chordoma, poorly differentiated chordoma occurs in the skull base/clivus, cervical spine, and sacrum/coccyx of children and young adults between 1 and 29 years with a mean age of 11 years.[32] Histologically, poorly differentiated chordoma bears little resemblance to conventional chordoma and consists of sheets of atypical epithelioid cells with nuclear atypia, abundant eosinophilic cytoplasm, and frequent mitoses (Fig. 3G).[32] Diffuse nuclear expression of the transcription factor brachyury is found in all cases (Fig. 3H), as is staining for keratins. Poorly differentiated chordoma shows consistent loss of SMARCB1 expression (Fig. 3I).

This tumor type follows an aggressive clinical course with a mean overall survival of only 53 months, compared with 109 months for conventional chordoma, and requires aggressive multimodality treatment.[32]

Despite sharing common SMARCB1 deficiency, epithelioid schwannoma, epithelioid MPNST, and poorly differentiated chordoma represent neoplasms that differ substantially in terms of clinical behavior and prognosis, highlighting the importance of correctly diagnosing the various types of epithelioid neoplasms with SMARCB1 loss for optimal patient management.

EMERGING SUBTYPES OF ADIPOCYTIC TUMORS

The diagnostic spectrum of adipocytic tumors with spindle cell features includes spindle cell/pleomorphic lipoma, atypical spindle cell lipomatous tumor, and conventional atypical lipomatous tumor (ALT)/well-differentiated liposarcoma, and, due to overlapping morphologic appearances, correct classification of tumors in this group may be challenging (Table 1).

Spindle Cell/Pleomorphic Lipoma

These tumors are benign adipocytic neoplasms that mostly present as a circumscribed subcutaneous mass in the neck and upper back of middle-aged men. Spindle cell/pleomorphic lipoma is comprised of a bland uniform spindle cell population with an admixed variably prominent component of mature fat. The tumor cells exhibit characteristic short "stubby" nuclei, lack nuclear atypia or pleomorphism, and are embedded in a variably myxoid stroma, often with prominent "ropey" collagen bundles and scattered mast cells (Fig. 4A). The tumor cells in spindle cell/pleomorphic lipoma typically show expression of CD34[33] (Fig. 4B) and loss of nuclear RB1 (Fig. 4C) in most cases, resulting from genomic inactivation of *RB1* at 13q14,[34] which is also a typical feature of cellular angiofibroma and (mammary

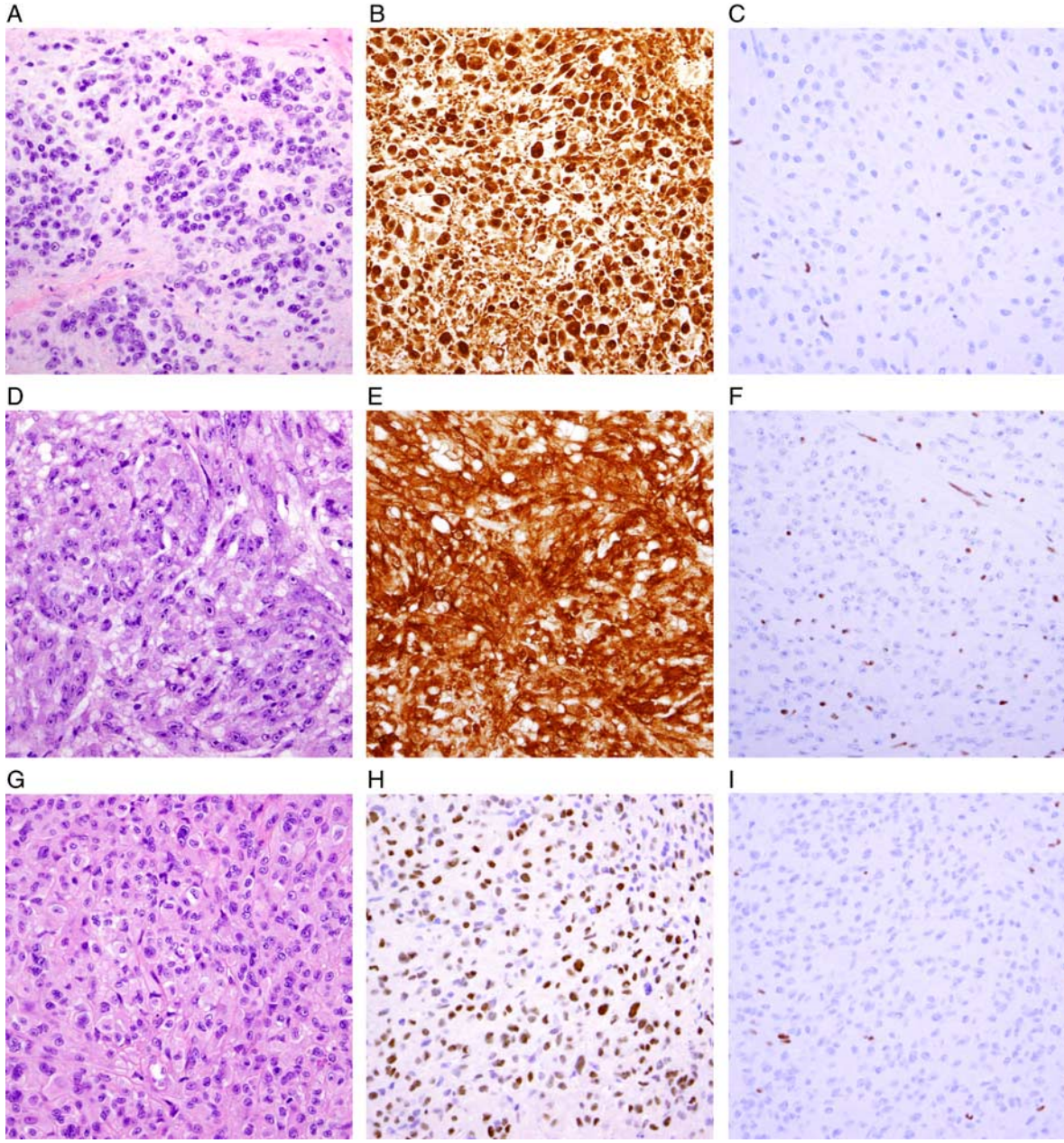

FIGURE 3. The biological spectrum of SMARCB1-deficient tumors. Epithelioid schwannoma is comprised of uniform epithelioid cells with round vesicular nuclei and abundant palely eosinophilic cytoplasm, arranged in sheets or singly dispersed within a myxoid to hyalinized stroma (A). The tumor cells are strongly positivity for S100 protein (B), and a subset of epithelioid schwannomas shows loss of SMARCB1 expression in tumor cells (C); endothelial and inflammatory cells serve as internal control. Epithelioid MPNST is characterized by atypical epithelioid tumor cells with round vesicular nuclei in a lobular or nested growth pattern (D) which, like epithelioid schwannoma, typically exhibit strong and diffuse S100 protein staining (E) to a degree that is rare for conventional MPNST. Most epithelioid MPNST lacks SMARCB1 expression (F). Poorly differentiated chordoma is comprised of epithelioid cells growing in sheets with nuclear atypia and abundant eosinophilic cytoplasm (G). The tumor cells show nuclear expression of the transcription factor brachyury (H) and loss of SMARCB1 expression (I). MPNST indicates malignant peripheral nerve sheath tumor.

type) myofibroblastoma; many experts believe these tumor types are related.

Atypical Spindle Cell Lipomatous Tumor

Over the past few decades, it has become clear that there exists a group of adipocytic neoplasms that do not fit into existing diagnostic categories; such tumors have been variably referred to as "atypical spindle cell lipomatous tumor,"[35] "fibrosarcoma-like lipomatous neoplasm,"[36] and "atypical spindle cell lipoma."[37] Atypical spindle cell lipomatous tumor is a low-grade neoplasm that shows a wide age distribution ranging from 6 to 87 years with a mean age of 54 years and a male predominance. Most cases occur in the limbs and limb girdle, followed by the hands and feet,

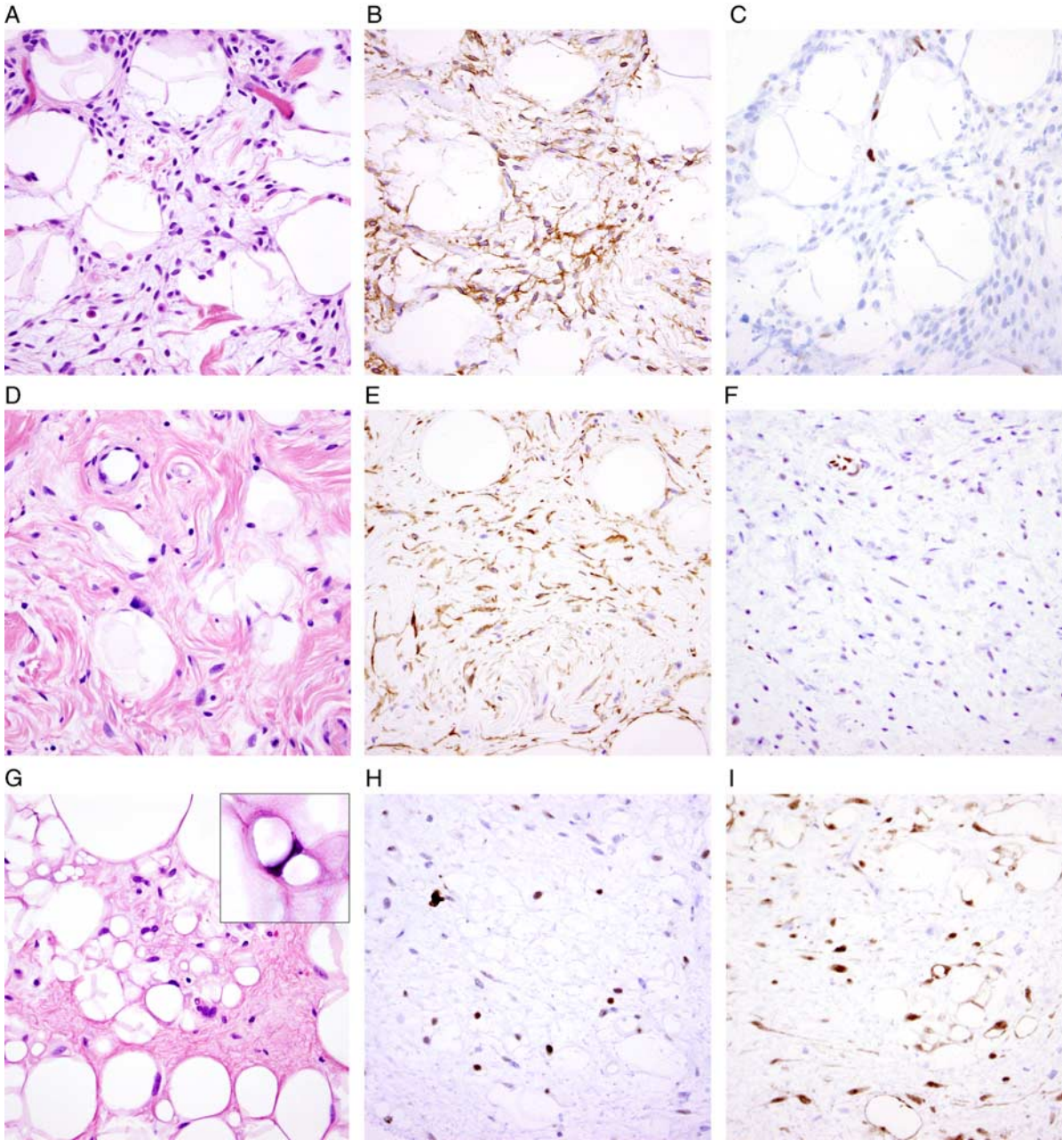

FIGURE 4. Adipocytic tumors with spindle cell morphology. Spindle cell lipoma is comprised of a mature adipocytic component and a bland spindle cell population with characteristic short "stubby" nuclei without atypia, embedded in a variably myxoid to collagenous background often with prominent ropey collagen bundles and admixed mast cells (A). Spindle cell lipomas usually express CD34 (B), and most cases show loss of RB1 expression (C), resulting from genomic inactivation of *RB1* on chromosome 13q14. Atypical spindle cell lipomatous tumor is comprised of mildly atypical spindle cells embedded in a collagenous to myxoid stroma, with focal nuclear atypia (D). As in spindle cell lipoma, ropey collagen bundles and scattered mast cells are often present. Atypical spindle cell lipomatous tumor also shows expression of CD34 (E) and loss of RB1 (F) in many cases. Conventional atypical lipomatous tumor is composed of an adipocytic proliferation with variation in adipocyte size and focal nuclear atypia and hyperchromasia in adipocytes and/or stromal cells (G). Lipoblasts may be present (G, inset) but are not required for the diagnosis. The tumor cells show nuclear expression of MDM2 (H) and CDK4 (I), resulting from high-level chromosome 12q13-15 amplification.

occurring at both superficial and deep locations.[38] Most atypical spindle cell lipomatous tumors are poorly circumscribed with infiltrative margins. The tumors show a wide spectrum of histologic appearances and are characterized by mildly atypical spindle cells in a fibrous or fibromyxoid stroma and a variably prominent adipocytic component with variation in adipocyte size and scattered nuclear atypia (Fig. 4D). Univacuolated or multivacuolated lipoblasts are often present. The immunophenotype is somewhat similar to that of spindle cell/pleomorphic lipoma, including

expression of CD34 in 64% of cases (Fig. 4E), S100 protein in 40%, and, less commonly, desmin (23%). Loss of RB1 expression is found in about half of cases (Fig. 4F). Of note, MDM2 and CDK4 are not overexpressed, and these tumors lack high-level amplification of *MDM2*, which is an important finding in the distinction from conventional ALT. Although atypical spindle cell lipomatous tumors may recur locally in around 10% of cases, they are not associated with dedifferentiation or distant metastasis.[38]

Atypical Lipomatous Tumor

ALT (termed "well-differentiated liposarcoma" when occurring at deep, central body cavity locations, not amenable to complete surgical excision) frequently occurs in the extremities of middle-aged adults and is divided into adipocytic, sclerosing, inflammatory, and spindle cell subtypes.[39,40] Histologically, ALT comprises a mature adipocytic proliferation (to varying extent, depending on histologic subtype) with variation in adipocyte size, and contains atypical adipocytes and stromal cells, which are often enriched in fibrous septa (Fig. 4G). Univacuolated or multivacuolated lipoblasts may be present but are not a requirement for the diagnosis. ALT harbors giant marker or ring chromosomes that contain amplified material from 12q13-15 including the *MDM2*, *CDK4*, and *HMGA2* loci,[41,42] which leads to overexpression of MDM2 (Fig. 4H), CDK4 (Fig. 4I), and HMGA2 by immunohistochemistry.[43]

Spindle cell features are found in rare cases of conventional ALT, overlapping morphologically with atypical spindle cell lipomatous tumor. However, in contrast to atypical spindle cell lipomatous tumor, ALT with spindle cell features shows consistent expression of MDM2 and CDK4 and retained expression of RB1.

MUTATION-SPECIFIC IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF GIANT CELL-RICH BONE TUMORS

The differential diagnosis of giant cell-rich bone tumors comprises a broad spectrum of entities that includes giant cell tumor of bone, chondroblastoma, aneurysmal bone cyst, and osteosarcoma—with substantial differences in biological behavior and clinical management. Although information about patient age, anatomic location of the tumor, and radiologic impression is very important in the diagnostic workup of bone tumors and often helps narrow the differential diagnosis, certain cases with unusual clinical presentation may be diagnostically challenging, especially in small biopsy specimens.

The recent discovery of highly recurrent oncogenic mutations in the *H3F3A* and *H3F3B* genes in subsets of giant cell-rich bone tumors has provided important insights into the genetic underpinnings of these rare bone tumors and has led to the introduction of novel markers that aid in their diagnostic workup (Table 1).[44–49] Of note, *H3F3A* and *H3F3B* are located on different chromosomes but encode histone 3.3 (H3.3) proteins of identical amino acid sequence; oncogenic mutations in these genes are mutually exclusive.

Giant Cell Tumor of Bone

Giant cell tumor of bone most frequently arises in young adults with a mature skeleton and usually develops in the epiphysis of long bones. Malignant transformation is rare but distant metastasis is observed in up to 10% of cases. Histologically, the tumor contains an admixture of ovoid to spindly mononuclear tumor cells, non-neoplastic mononuclear cells, and numerous reactive osteoclast-like giant cells (Fig. 5A).

Approximately 92% of giant cell tumors of bone harbor *H3F3A* (and, rarely, *H3F3B*) mutations, which target codon 34 of H3.3.[44] G34W is the most frequent type of mutation, found in around 85% of cases, followed by alternate G34V, G34R, G34M, or G34L mutations.[44,45,50,51] A mutation-specific antibody directed against mutant H3G34W demonstrates high specificity and sensitivity for the diagnosis of giant cell tumor of bone using immunohistochemistry (Fig. 5B); diffuse nuclear staining is observed in 91% of cases, but not in other giant cell-rich bone tumors.[45] In contrast, staining for H3K36M (see below) is negative in giant cell tumor of bone (Fig. 5C).

As the mutation-specific H3G34W antibody fails to detect alternate mutations involving codon 34, negative H3G34W immunohistochemistry does not preclude the diagnosis of giant cell tumor of bone; additional antibodies specifically directed at other amino acid exchanges at codon 34 may be helpful.[50] Alternatively, genomic sequencing may be performed to detect an underlying mutation; reported detection rates range from 69% for Sanger sequencing[47] to 96% for targeted next-generation sequencing.[48] However, such studies are rarely needed in clinical practice, since the combination of histologic and radiologic features is usually sufficient for diagnosis. Of note, previous denosumab treatment, decalcification, and malignant transformation do not significantly affect the results obtained by immunohistochemistry or sequencing.

Chondroblastoma

Chondroblastoma affects mostly children and adolescents with an immature skeleton and usually involves the epiphysis of long bones, with or without extension to the articular cartilage. Histologically, chondroblastoma is comprised of mononuclear cells and admixed multinucleated giant cells embedded in a dense eosinophilic matrix (Fig. 5D). Occasionally, characteristic "chicken-wire" calcification is observed.

In contrast to giant cell tumor of bone, chondroblastoma lacks H3G34W expression (Fig. 5E). Instead, this tumor is characterized by oncogenic *H3F3B* (and, rarely, *H3F3A*) mutations that encode H3.3 K36M, which can be detected by sequencing in 70% to 100% of cases, depending on the method used.[44,47–49,52] A mutation-specific H3K36M antibody demonstrates diffuse nuclear expression in 96% of chondroblastomas by immunohistochemistry (Fig. 5F) but not in histologic mimics.[46]

The high specificity of H3G34W and H3K36M immunohistochemistry in the diagnosis of giant cell tumor of bone and chondroblastoma, respectively, highlights the value of these markers in the often challenging differential diagnosis of bone tumors, especially when only limited biopsy material is available.[51] However, it is important to emphasize that the heterozygous oncogenic *H3F3A* and *H3F3B* mutations are restricted to the neoplastic mononuclear cells, often accounting for <50% of cells. The relatively low mutant allele fraction of around 25% may therefore lead to false-negative results when Sanger sequencing is performed;[47,51] careful correlation between radiologic and morphologic features is required.

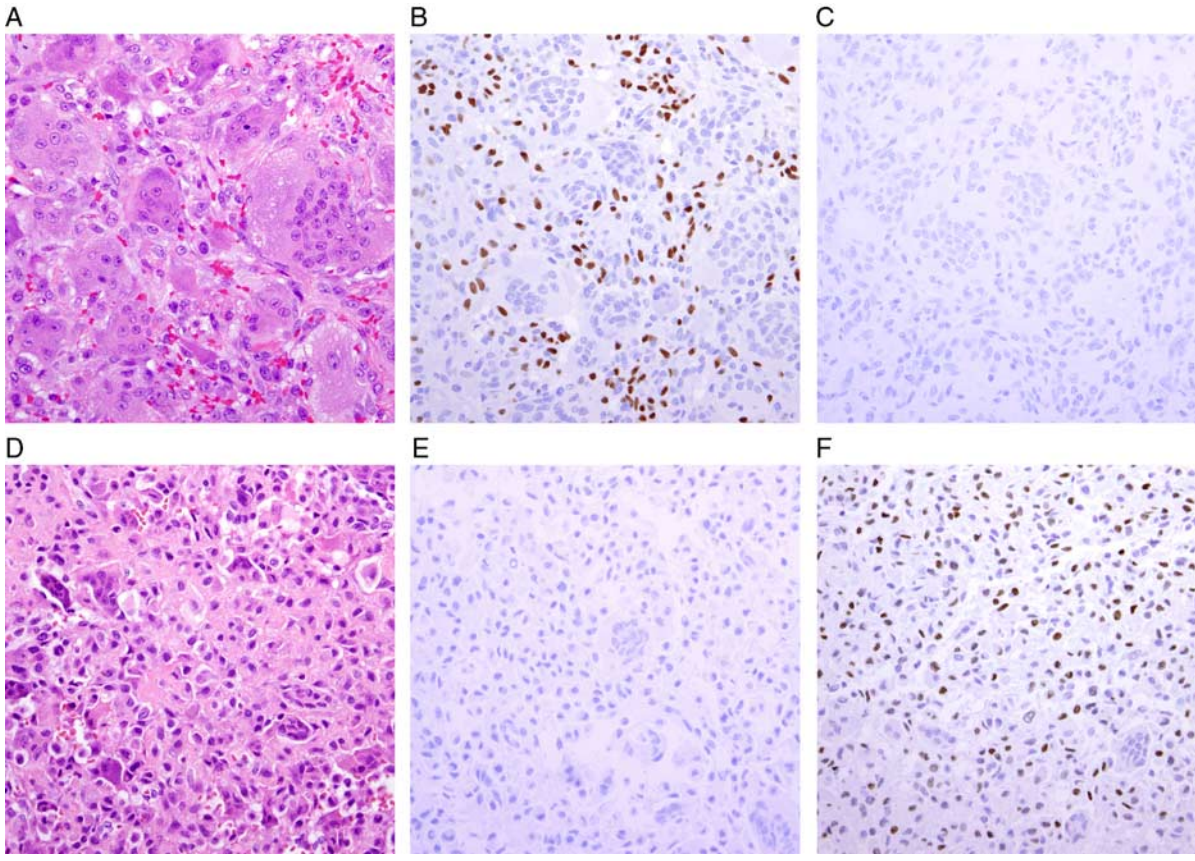

FIGURE 5. Mutation-specific immunohistochemistry in the diagnosis of giant cell-rich bone tumors. Giant cell tumor of bone is comprised of ovoid to spindled mononuclear tumor cells with numerous admixed non-neoplastic multinucleated osteoclast-like giant cells (A). Mutation-specific immunohistochemistry for H3G34W encoded by mutant *H3F3A* (or, less frequently, *H3F3B*) shows diffuse nuclear expression in tumor cells in most cases (B), whereas staining for H3K36M is negative (C). Chondroblastoma is comprised of mononuclear cells and admixed multinucleated giant cells embedded in a dense eosinophilic matrix (D). Mutation-specific immunohistochemistry for H3G34W is negative (E), whereas staining for H3K36M, encoded by mutant *H3F3B* (or, less frequently, *H3F3A*), shows diffuse nuclear staining in tumor cells (F). Note that admixed giant cells are non-neoplastic in giant cell tumor of bone and chondroblastoma and stain negative for H3G34W and H3K36M.

BIPHENOTYPIC SINONASAL SARCOMA SHOWS CHARACTERISTIC IMMUNOPHENOTYPIC AND CYTOGENETIC FEATURES

Biphenotypic sinonasal sarcoma—a low-grade spindle cell sarcoma arising in the upper sinonasal tract of middle-aged adults—was initially described in 2012 by Lewis et al[53], including two cases with identical t(2;4). Biphenotypic sinonasal sarcoma is defined by characteristic co-expression of neural and myogenic markers, and, as discovered more recently, harbors recurrent *PAX3* rearrangement, with *PAX3-MAML3* fusion resulting from t(2;4)(q35;q31) being most frequent,[54,55] followed by alternate *PAX3-FOXO1*[56] or *PAX3-NCOA1*[57] fusions.

Histologically, biphenotypic sinonasal sarcoma is comprised of a homogeneously cellular spindle cell population arranged in short fascicles (Fig. 6A). The tumor cells show bland elongated nuclei and scant cytoplasm without significant nuclear atypia or pleomorphism. Necrosis and mitotic figures are uncommon. Concomitant neural and myogenic differentiation is reflected by its immunophenotype (Table 1): co-expression of S100 protein (Fig. 6B) and SMA (Fig. 6C) or calponin, and, less commonly, desmin and myogenin (the latter only in rare cells), is characteristic of biphenotypic sinonasal sarcoma; a subset of cases also expresses TLE1. In addition, most biphenotypic sinonasal sarcomas show nuclear expression of β-catenin, whereas SOX10 is negative.[58]

The differential diagnosis of biphenotypic sinonasal sarcoma includes other spindle cell sarcomas, chiefly low-grade MPNST and monophasic synovial sarcoma, which rarely occur in this anatomic location. Before its recognition as a distinct entity, most cases of biphenotypic sinonasal sarcoma were presumably diagnosed as either MPNST or synovial sarcoma based on morphologic and immunophenotypic similarities. In contrast to MPNST, biphenotypic sinonasal sarcoma lacks alternation of hypercellular and hypocellular areas and accentuation of tumor cells around blood vessels. Prominent hemangiopericytoma-like blood vessels—which are often found in synovial sarcomas—may sometimes be observed. Of note, the immunophenotype of biphenotypic sinonasal sarcoma is not tumor-specific and may pose diagnostic challenges: cases with positive staining for TLE1 may be confused with synovial sarcoma; a subset of MPNST also show expression of TLE1. Expression of S100 protein is not only a feature of biphenotypic sinonasal sarcoma but can also be found in up to 40% of MPNST

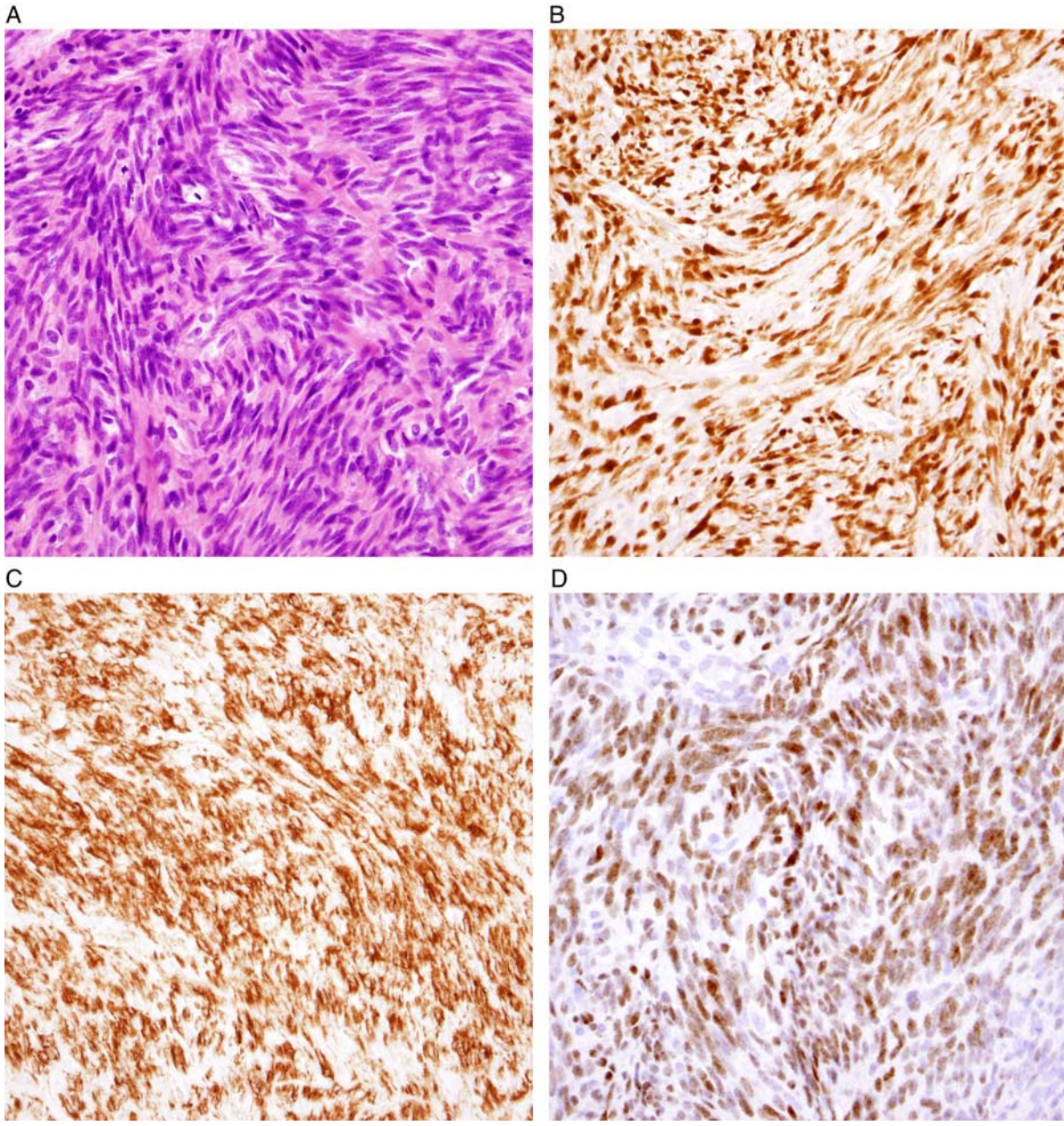

FIGURE 6. Biphenotypic sinonasal sarcoma with characteristic immunophenotypic and cytogenetic features. Biphenotypic sinonasal sarcoma is a low-grade sarcoma composed of cellular fascicles of uniform spindle cells with bland elongated nuclei and scant cytoplasm (A). The tumor cells show characteristic co-expression of S100 protein (B) and myogenic markers, such as SMA (C). Nuclear expression of PAX3, resulting from *PAX3* rearrangement, is detected in most cases (D).

(usually limited in extent, however) and up to 30% of synovial sarcomas.[56] Likewise, absence of SOX10 expression does not aid in the distinction from MPNST, which is negative for this marker in more than half of cases.

A recent study evaluated PAX3 expression in biphenotypic sinonasal sarcoma and demonstrated positive staining in all cases tested. Histologic mimics were largely negative, except for 1 case (10%) of spindle cell rhabdomyosarcoma; alveolar rhabdomyosarcomas were also positive (80%), as might be expected from underlying *PAX3* gene rearrangement. The high sensitivity of 100% and specificity of 98% suggest that PAX3 may serve as a helpful diagnostic marker in this context.[59] Of note, due to cross-reactivity with PAX3, biphenotypic sinonasal sarcomas also show positive staining with polyclonal PAX8 antibodies in most cases.[59]

As demonstrated in a large study of 44 cases, biphenotypic sinonasal sarcomas harbor *PAX3-MAML3* fusion in 55% of cases, and less frequently, alternate *PAX3-FOXO1* or *PAX3-NCOA1* fusion.[55] Rare cases show *MAML3* rearrangement with an unknown fusion partner or lack a detectable structural rearrangement.[55] These findings suggest that absence of *PAX3* rearrangement (and concurrent PAX3 expression) do not necessarily rule out a

diagnosis of biphenotypic sinonasal sarcoma; correlation of clinical presentation with morphologic appearances and immunophenotype remains important in such cases.

Despite bearing the same *PAX3-FOXO1* fusion as a subset of alveolar rhabdomyosarcomas, biphenotypic sinonasal sarcoma is considered a low-grade sarcoma that may show locally aggressive behavior but rarely metastasizes.

CONCLUSIONS

The integrated use of conventional cytogenetics, targeted next-generation sequencing, and immunohistochemistry has led to marked improvements in the diagnostic workup of soft tissue tumors in recent years. Advances in the diagnosis of round cell sarcomas have led to the introduction of *CIC*-rearranged and *BCOR*-rearranged sarcomas as entities distinct from Ewing sarcoma into current classification systems. In addition, identification of specific cytogenetic alterations in vascular tumors distinguishes subtypes within the groups of epithelioid hemangioendothelioma and epithelioid hemangioma, and identified a characteristic aberration in pseudomyogenic hemangioendothelioma. However, many immunohistochemical markers that relate to an underlying genomic alteration are not completely tumor specific and need to be interpreted with caution, but are nonetheless more sensitive and specific than conventional lineage-associated markers. SMARCB1 deficiency is observed in a diverse range of neoplasms but may—in conjunction with tumor site, presentation, and other immunohistochemical markers—be of substantial diagnostic value. Existing immunohistochemical stains in the group of adipocytic neoplasms further help separate the recently described atypical spindle cell lipomatous tumor from conventional ALT. The recent discovery of highly recurrent oncogenic *H3F3A* and *H3F3B* mutations that define giant cell tumor of bone and chondroblastoma, respectively, prompted the introduction of highly sensitive mutation-specific antibodies that provide additional insights into the oncogenic mechanisms that drive these rare benign bone tumors. Finally, biphenotypic sinonasal sarcoma sets an example of how the recognition of a unique immunophenotype, identification of *PAX3* rearrangement and PAX3 expression helped delineate a novel diagnostic entity.

Nonetheless, the careful integration of all available information and critical interpretation of established and novel diagnostic markers remains crucial in the diagnosis of soft tissue tumors.

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An Update on the Application of Newly Described Immunohistochemical Markers in Soft Tissue Pathology

George Lin, MD, PhD; Leona A. Doyle, MD

• ***Context.***—**During the last 5 to 10 years, significant progress has been made in the molecular characterization of soft tissue tumors, predominantly with the identification of recurrent translocations or amplification of certain genes in different tumor types. Alongside this, translational efforts have identified many novel and diagnostically useful immunohistochemical markers for many of these tumor types.**

Objective.—**This article reviews a select group of recently described immunohistochemical markers of particular use in the evaluation of mesenchymal neoplasms; the underlying biology of the protein product, practical utility, and limitations of each marker are discussed in detail.**

Data Sources.—**Literature review, authors' research data, and personal practice experience serve as sources.**

Conclusions.—**There are many diagnostically useful immunohistochemical markers to help confirm the diagnosis of many different soft tissue tumor types, some of which have reduced the need for additional, and more costly, studies, such as fluorescence in situ hybridization. However, no one marker is 100% specific for a given tumor, and knowledge of potential pitfalls and overlap in patterns of staining among other tumor types is crucial to ensure the appropriate application of these markers in clinical practice.**

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The last 10 years have seen the description of many immunohistochemical markers in the field of soft tissue tumor pathology. As expected, some of these markers prove to be more useful in clinical practice than others, and with time it is generally appreciated that significant overlap in staining patterns can be seen in different tumor types, some of which share similar biology or can be explained by known biologic mechanisms. Perhaps the most interesting aspect pertaining to many of these recently described markers is the method of discovery and the speed at which they have been translated into clinical practice; for example, gene expression profiling studies have identified the protein products TLE1, DOG1, and MUC4 as clinically useful markers for synovial sarcoma, gastrointestinal stromal tumor (GIST), and low-grade fibromyxoid sarcoma, respectively.

In addition, new insights into the biology of several different tumor types are reflected in many recently described immunohistochemical markers, such as tumors with INI-1 loss; tumors with amplification in the region of chromosome 12q13-15, which generally show overexpression of MDM2 and CDK4 (and occasionally STAT6); *MYC* amplification in postradiation angiosarcoma; and metabolic enzyme pathway disturbances in the clinicopathologically distinct group of succinate dehydrogenase–deficient GIST. Fusion protein products resulting from recurrent translocations may also be useful markers, such as STAT6 for solitary fibrous tumor and TFE3 for alveolar soft part sarcoma and a subset of epithelioid hemangioendothelioma. Finally, lineage-specific markers, which tend to show nuclear staining, include ERG as a marker of endothelial differentiation and SOX10 as a marker of neuroectodermal differentiation. The utility of these markers, along with their limitations and potential pitfalls, are discussed in detail in this review and are summarized in the Table.

ERG (Avian v-ets Erythroblastosis Virus E26 Oncogene Homolog)

ERG is a member of the ETS family of transcription factors, which also include ETS-1; Friend leukemia integration site 1 (Fli-1); NERF-2; and TEL; and which are expressed by vascular endothelial cells and defined by a conserved DNA-binding ETS domain that forms a winged helix-turn-helix structural motif.[1] ERG regulates endothelial cell differentiation, angiogenesis, and expression of several endothelial-specific antigens, and it is also required for embryonic stem cells to differentiate into endothelial cells.[2–6] Detection of ERG expression by immunohistochemistry was first described in a subset of prostatic adenocarcinomas, where it was shown to correlate with ERG overexpression via chimeric fusion proteins generated from oncogenic translocations, with *TMPRSS2-ERG* being the most common variant, seen in approximately 50% of all prostate-specific antigen–screened prostate cancers detected in the United States.[7–12]

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Recently Described Immunohistochemical Markers of Soft Tissue Tumors			
Antibody	**Expression Pattern**	**Main Use(s)**	**Comments**
ERG	Nuclear	Confirm endothelial differentiation	Also stains a subset of epithelioid sarcomas, subset of Ewing sarcoma, and 45% of prostatic carcinomas (117 of 261 cases[17]; 30 of 66 cases[13])
MYC	Nuclear	Differentiate postradiation angiosarcoma from APRVP	Expressed in a small subset of primary angiosarcomas (usually head and neck)
MDM2/CDK4	Nuclear	Coexpressed in most well-differentiated and dedifferentiated liposarcomas Coexpressed in intimal sarcoma and parosteal and low-grade central osteosarcoma	MDM2 is positive in up to 64% of MPNSTs (21 of 33 cases[46]), 42% of myxofibrosarcomas (10 of 24 cases[46]), and 29% of embryonal rhabdomyosarcomas (12 of 41 cases[46]); MDM2 positivity is also seen in histiocytes
STAT6	Nuclear (+/− cytoplasmic)	Positive in >97% of SFTs (59 of 60 cases[60]; 54 of 54 cases[61]; 34 of 35 cases[62]; 49 of 49 cases[63])	Expressed in 15% of dedifferentiated liposarcomas (3 of 21 cases[60])
MUC4	Cytoplasmic	Expressed in vast majority of low-grade fibromyxoid sarcoma (49 of 49 cases[72]) and sclerosing epithelioid fibrosarcoma (32 of 41 cases[82]; 14 of 15 cases[83])	Focal positivity seen in synovial sarcoma, ossifying fibromyxoid tumors, and epithelioid GIST Expressed in many carcinomas
DOG1	Cytoplasmic + membranous	Positive in >87% of GISTs (136 of 139 cases[89]; 986 of 1040 cases[90]; 370 of 425 cases[91]) Useful to confirm a diagnosis of GIST in gastric KIT-negative tumors	Various other tumor types reported to show focal staining
SDHB/A	Cytoplasmic, granular	Loss of SDHB expression confirms diagnosis of SDH-deficient GIST Loss of SDHA expression indicative of *SDHA* mutations	Loss of SDHB staining also seen in pheochromocytoma/paraganglioma and rare renal cell carcinomas associated with SDH complex dysfunction
INI1	Nuclear	Loss of expression in 93% of epithelioid sarcomas (127 of 136 cases[131]) and virtually all malignant rhabdoid tumors	Loss of staining also seen in 50% of epithelioid MPNSTs (12 of 24 cases[131]) and 40% of pediatric myoepithelial carcinomas (9 of 22 cases[133])
TLE1	Nuclear	Expression seen in >90% of synovial sarcomas (91 of 94 cases[150]; 249 of 259 cases[155]; 39 of 43 cases[156]; 35 of 35 cases[157]; 18 of 20 cases[158])	Expression often seen in MPNST and SFT (usually, but not always, less diffuse than in synovial sarcoma)
TFE3	Nuclear	Positive in virtually all ASPS	Also positive in Xp11 translocation–type renal cell carcinomas, subset of epithelioid hemangioendotheliomas (with *YAP1-TFE3* fusion gene), and a subset of PEComas
SOX10	Nuclear	Confirm neural crest differentiation—melanocytic tumors, clear cell sarcoma, most benign nerve sheath tumors and 27%–50% of MPNSTs (21 of 78 cases[179]; 38 of 77 cases[182])	Also stains a subset of myoepithelial and salivary gland tumors, and subset of carcinoid tumors and breast carcinomas
NY-ESO-1	Cytoplasmic and nuclear	Expressed in up to 80% of synovial sarcomas (20 of 25 cases[188]; 38 of 50 cases[189]) and 95% of myxoid liposarcomas (36 of 38 cases[192])	Positivity is also seen in melanoma and a variety of carcinomas

Abbreviations: APRVP, atypical postradiation vascular proliferation; ASPS, alveolar soft part sarcoma; GIST, gastrointestinal stromal tumor; MPNST, malignant peripheral nerve sheath tumor; PEComa, perivascular epithelioid cell tumor; SDH, succinate dehydrogenase; SFT, solitary fibrous tumor.

A large study evaluating ERG as an immunohistochemical marker for vascular tumors showed that nuclear positivity for ERG was present in the endothelia of all hemangiomas and lymphangiomas examined.[13] In addition, virtually all subtypes of hemangioendotheliomas were positive for ERG, including kaposiform hemangioendotheliomas, retiform hemangioendotheliomas, and epithelioid hemangioendotheliomas. Expression of ERG was also seen in all Kaposi sarcomas evaluated, and in 96 of 100 angiosarcomas (Figure 1).[13] A wide variety of other nonvascular and nonepithelial mesenchymal, neuroectodermal, and hematopoietic tumors were also evaluated, and the vast majority were negative for ERG, with the exception of 7 of 10 blastic extramedullary myeloid tumors (70%) and 2 of 29 Ewing sarcomas (7%).[13] Another study evaluated ERG in the context of differentiating cutaneous angiosarcoma from other cutaneous neoplasms that arise in sun-damaged skin and may enter the histologic differential diagnosis with angiosarcoma, specifically squamous cell carcinoma, malignant melanoma, and atypical fibroxanthoma. In that study, nuclear ERG expression was 100% sensitive and specific for angiosarcoma, with all 23 cases of angiosarcoma showing distinct nuclear staining, whereas all other tumors evaluated (15 poorly differentiated squamous cell carcinomas, 17 melanomas, 12 atypical fibroxanthomas, and 5 leiomyosarcomas) were negative.[14] From these studies,[13,14] ERG is positive in greater

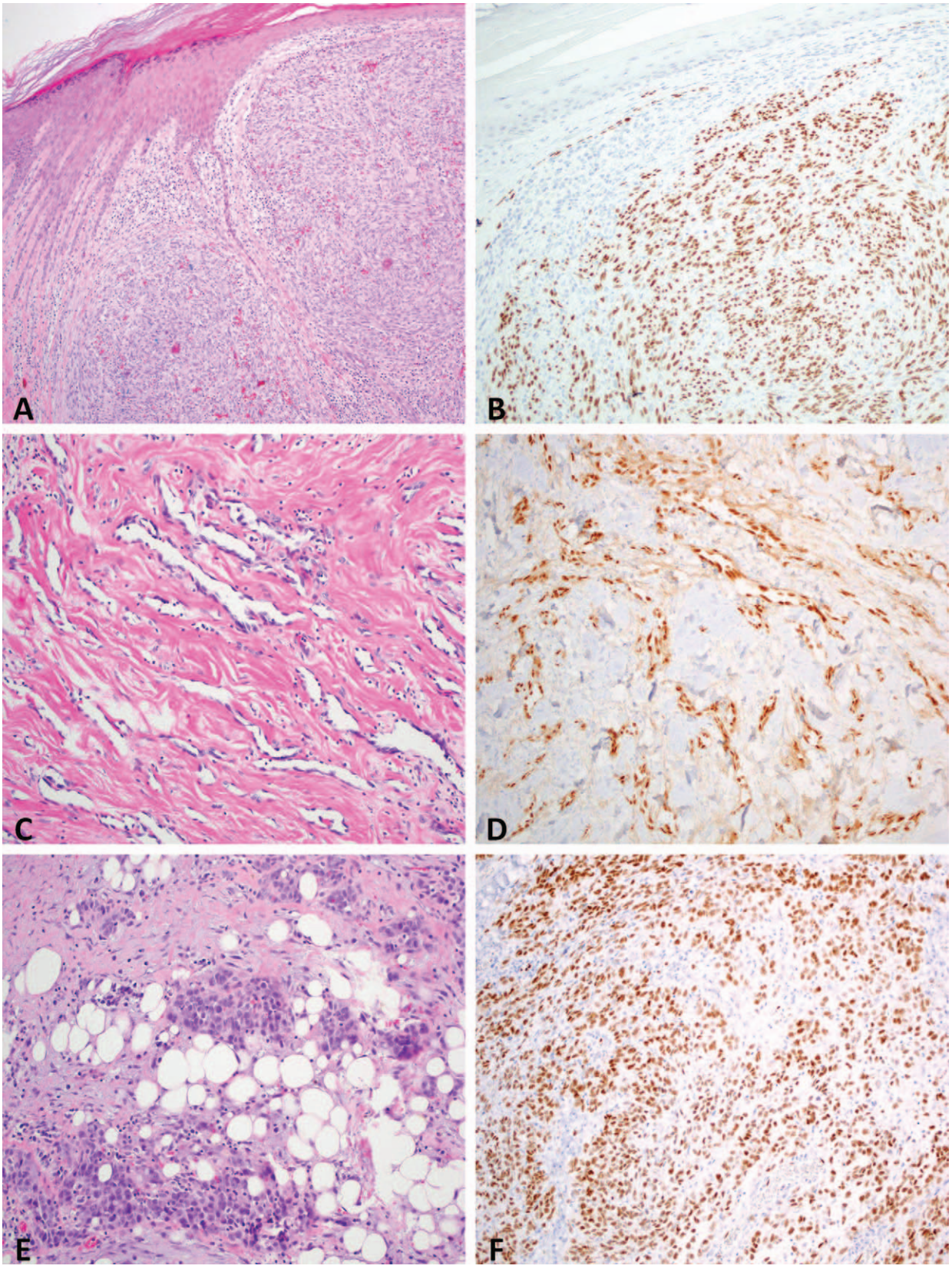

Figure 1. *Nodular Kaposi sarcoma involving dermis composed of fascicles of spindle cells with numerous extravasated red blood cells (A). The tumor cells show diffuse nuclear expression of ERG (B). Vasoformative angiosarcoma composed of complex anastomosing vessels lined by hyperchromatic atypical endothelial cells (C); again, tumor cells show diffuse nuclear expression of ERG (D). Epithelioid angiosarcoma (E), which may mimic poorly differentiated carcinoma or malignant melanoma, is positive for ERG (F), unlike the latter two tumor types (hematoxylin-eosin, original magnifications ×100 [A] and ×200 [C and E]; original magnifications ×100 [B] and ×200 [D and F]).*

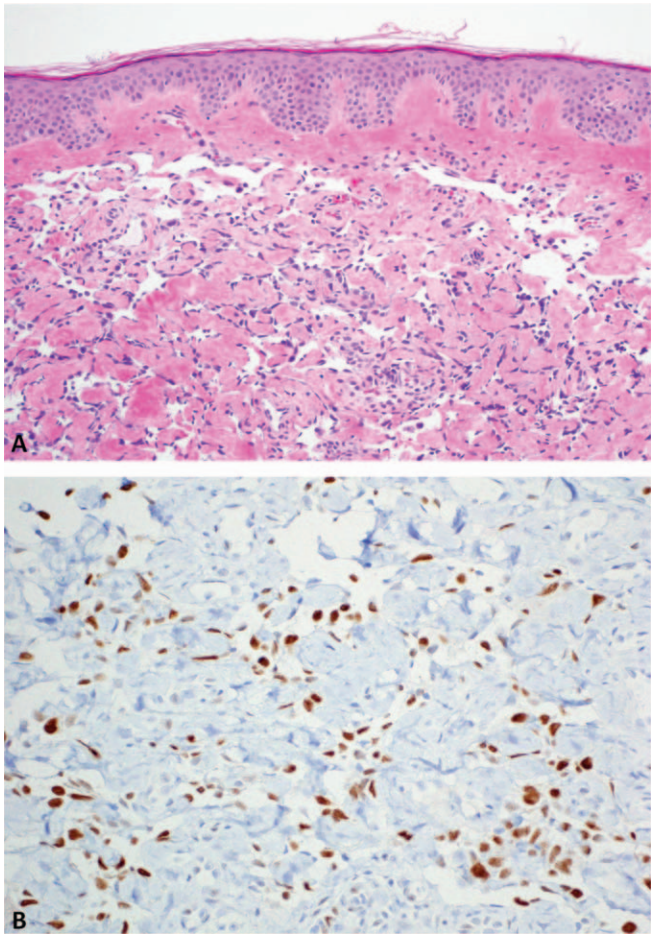

Figure 2. *Postradiation angiosarcoma with a vasoformative growth pattern (A). The tumor cells show diffuse strong nuclear positivity for MYC (B) (hematoxylin-eosin, original magnification ×200 [A]; original magnification ×400 [B]).*

than 95% of angiosarcomas, with a greater sensitivity for angiosarcoma than CD31 and CD34, which are markers routinely used to evaluate for angiosarcoma, and ERG usually shows a diffuse pattern of nuclear staining, which facilitates its interpretation in this context.

Expression of ERG occurs in a small subset of Ewing sarcomas (see above), and strong nuclear ERG expression has been found to correlate with *EWSR1-ERG* rearrangement, which is present in a small percentage of Ewing sarcomas compared with the more characteristic *EWSR1-FLI1* rearrangement.[15] *TLS/FUS-ERG* fusion transcripts occur in acute myeloid leukemia and likely account for the detection of ERG positivity in acute myeloid leukemia tissue infiltrates.[13,16,17] Positivity for ERG has also been reported to occur in a significant percentage of epithelioid sarcomas, with 41 of 109 cases (38%) showing ERG positivity in one study.[18] However, ERG reactivity in epithelioid sarcoma is likely dependent on antibody choice, as a subsequent study showed that an antibody to the N-terminus of ERG resulted in positivity in 19 of 28 cases (68%), whereas an antibody to the C-terminus showed focal positivity in only 1 of 29 cases (3%).[19] Whereas CD34 expression is frequent in epithelioid sarcoma, other more specific endothelial markers, such as CD31, are negative, and the characteristic loss of INI1/SMARCB1 expression typically found in epithelioid sarcomas should allow for the distinction of epithelioid sarcoma from vascular tumors in the vast majority of cases.[18]

Other than prostatic adenocarcinoma, very few epithelial malignancies have been reported to show ERG immunoreactivity—1 (of 42) pulmonary large cell undifferentiated carcinoma and 1 (of 27) pleural epithelioid type mesothelioma.[13] *ERG* gene fusions have also been found in myxoid liposarcoma, although ERG immunoreactivity in myxoid liposarcomas has not been detected thus far.[13,16]

ERG is therefore a useful marker for confirming endothelial differentiation in both benign and malignant neoplasms, but expression can also be seen in a subset of epithelioid sarcomas and a small percentage of Ewing sarcomas, as well as approximately 45% to 50% of prostatic carcinomas.

MYC (v-myc Avian Myelocytomatosis Viral Oncogene Homolog)

Cutaneous angiosarcoma arises in four typical clinical settings—chronically sun-damaged skin, particularly the scalp or face; sporadic visceral angiosarcoma; in the setting of chronic lymphedema (eg, after mastectomy in Stewart-Treves syndrome); and in areas of prior therapeutic radiation, such as for the management of breast carcinoma.[20–29] The last two groups are considered "secondary" angiosarcoma. Atypical vascular proliferations, which have been described under various nomenclature designations, occur in areas of prior radiation, are often seen in association with angiosarcoma, and in some cases may be difficult to distinguish from vasoformative angiosarcoma.[22,23,30–40] *MYC* proto-oncogene is a transcription factor located on the long arm of chromosome 8 (8q24.21) and is implicated in cellular proliferation, differentiation, and apoptosis.[41] Nuclear expression of MYC occurs in the vast majority of secondary angiosarcomas (Figure 2), but it is only very rarely seen in primary angiosarcoma, and it is not detected in atypical or benign vascular lesions occurring in irradiated skin.[42–45]

An array-based comparative genomic hybridization study of 22 cases of angiosarcoma (8 primary; 14 secondary to irradiation or chronic lymphedema) found that 10 secondary angiosarcomas (9 associated with radiation therapy and 1 with lymphedema) showed 16 recurrent alterations, with high-level amplifications on 5q35.3 (2), 8q24.21 (8), and 10p12.33 (6), whereas no recurrent alterations were seen in primary angiosarcomas.[42] Given that *MYC* in the region of 8q24.21 was a likely candidate for amplification, in the same study, fluorescence in situ hybridization (FISH) analysis of 28 primary angiosarcomas and 33 secondary angiosarcomas showed high-level *MYC* gene amplification in 18 secondary angiosarcomas (55%; 16 irradiated, 2 lymphedema) but not in primary angiosarcomas ($P < .001$).[42] Another study has shown high-level *MYC* amplification in 100% (20 of 20) of secondary angiosarcomas, as well as coamplification of *FLT4* in 25% (5 of 20) of cases.[43] Atypical postradiation vascular proliferations and other benign vascular lesions of the breast are negative for both *MYC* amplification and MYC overexpression by immunohistochemistry, whereas MYC positivity is consistently detected in postradiation angiosarcomas.[44] A very recent study found that a subset of non–radiation-associated cutaneous angiosarcomas (9 of 38 cases evaluated) showed detectable expression of MYC by immunohistochemistry and that a subset of these cases showed high-level *MYC* gene amplification by FISH,

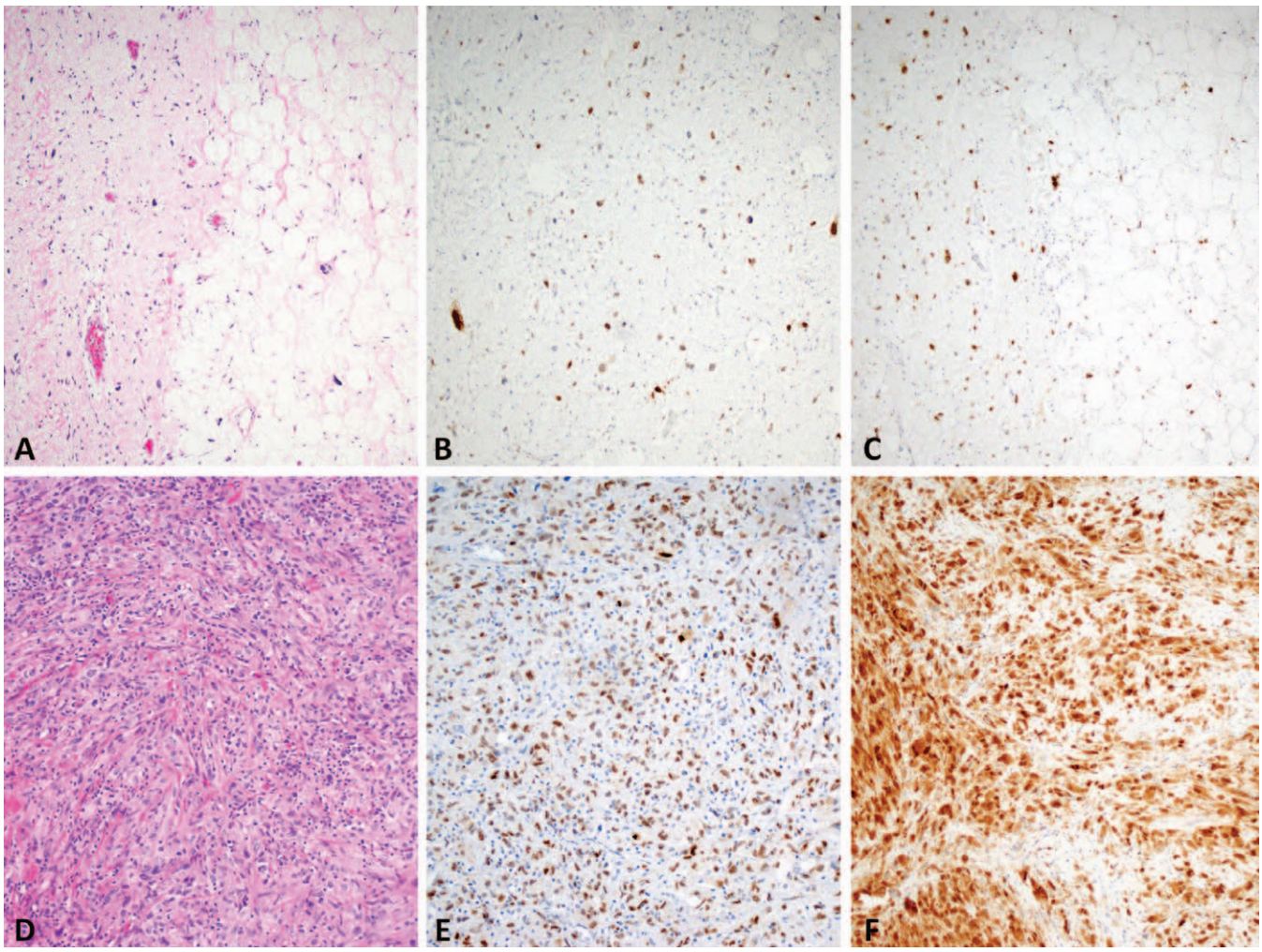

Figure 3. *Well-differentiated liposarcoma with scattered hyperchromatic atypical spindle cells (A). The tumor cells are positive for MDM2 (B) and CDK4 (C). Dedifferentiated liposarcoma with nondistinctive histologic features (D) shows strong diffuse positivity for both MDM2 (E) and CDK4 (F) (hematoxylin-eosin, original magnification ×200 [A and D]; original magnification ×200 [B, C, E, and F]).*

although a small number of MYC immunohistochemistry–negative cases also showed *MYC* gene amplification.[45]

MYC immunohistochemistry is therefore useful in differentiating atypical or benign vascular lesions occurring in irradiated skin from secondary postradiation angiosarcomas.

MDM2 (Murine Double-Minute-Type 2) and CDK4 (Cyclin-Dependent Kinase-4)

Amplification and overexpression of MDM2 is characteristic of well-differentiated and dedifferentiated liposarcoma, and both FISH and immunohistochemistry have become extremely useful tests for confirming the diagnosis of these tumor types, in conjunction with evaluation of CDK4 expression.[46] MDM2 protein is encoded by a gene at chromosome 12q14.3-q15 and acts as an inhibitor of the tumor suppressor effects of p53. Amplification of *MDM2* can be detected by FISH, and overexpression of MDM2 protein can be detected with mouse monoclonal antibodies (clones 2A10 and 1F2; Figure 3). CDK4 is encoded by a gene at chromosome 12q13 and functions in cell cycle progression—CDK4 inhibits the retinoblastoma-1 (*RB1*) gene, and similar to MDM2 is overexpressed in well-differentiated and dedifferentiated liposarcoma (Figure 3). This phenomenon reflects the common presence of ring or giant marker chromosomes that contain amplified material from the q13-15 region of chromosome 12, where both the *CDK4* and *MDM2* genes are located. For both MDM2 and CDK4, only nuclear staining is considered positive. Staining in well-differentiated liposarcoma is often limited in extent and may be present in scattered nuclei only, in contrast to dedifferentiated liposarcoma, where nuclear staining is usually more diffuse.[46,47] Benign lipomatous lesions are negative for both MDM2 and CDK4, as are pleomorphic liposarcoma and myxoid liposarcoma.

Perhaps the most useful role for these two markers is in confirming a diagnosis of dedifferentiated liposarcoma (Figure 3, D through F) when faced with an otherwise nondescript spindle cell or pleomorphic sarcoma in the retroperitoneum, and when a well-differentiated component is not seen. In addition, these markers are often helpful in the distinction between benign lipomas, particularly those with prominent fat necrosis, and atypical lipomatous tumor (well-differentiated liposarcoma), particularly when the latter shows very minimal atypical histologic features.[46–48] However, it must be noted that histiocytes, such as those present in areas of fat necrosis, are frequently MDM2 positive, emphasizing the need for concurrent CDK4

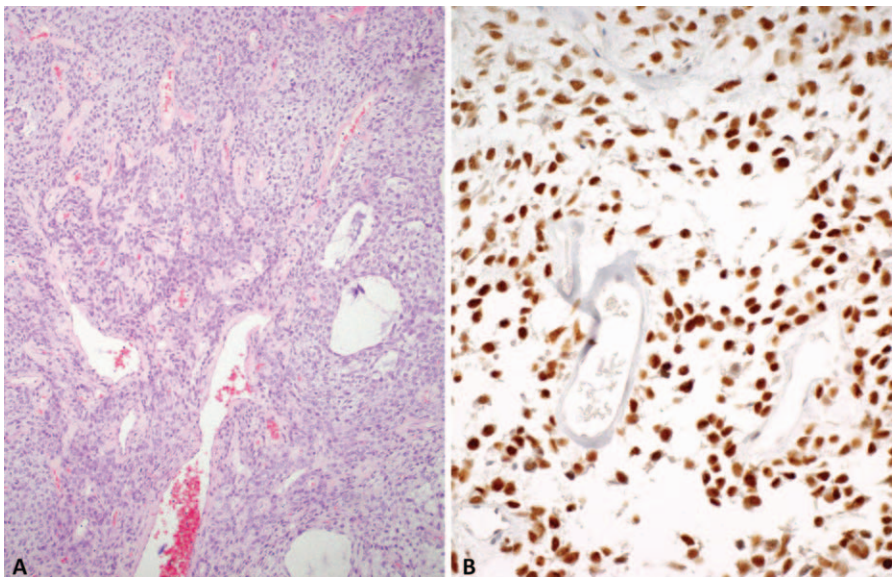

Figure 4. *Solitary fibrous tumor with characteristic hemangiopericytoma-like branching vessels and focally myxoid features (A). STAT6 is diffusely expressed in a nuclear pattern (B) (hematoxylin-eosin, original magnification ×200 [A]; original magnification ×400 [B]).*

evaluation. A small percentage of well-differentiated/dedifferentiated liposarcomas are negative for MDM2 and CDK4 by immunohistochemistry, with one study showing approximately 3% (3 of 105) and 10% (10 of 105) of cases of well-differentiated/dedifferentiated liposarcomas negative for MDM2 and CDK4, respectively, and in such cases where the histologic and clinical/radiologic findings are suggestive of this diagnosis, FISH for *MDM2* amplification should be considered.[46] It is also important to note that overexpression of MDM2 may also be seen in other spindle cell neoplasms—in up to 64% (21 of 33) of malignant peripheral nerve sheath tumors, 42% (10 of 24) of myxofibrosarcomas, and 29% (12 of 41) of embryonal rhabdomyosarcomas—but when combined with coexpression of CDK4, very few of these tumors will express both of these markers.[46] In addition, staining for MDM2 and CDK4 is usually less extensive in these tumor types than in dedifferentiated liposarcoma.

Several other tumor types show amplification of *MDM2* and *CDK4*, with corresponding overexpression at the protein level. Intimal sarcoma, a malignant neoplasm arising from large central vessels, most commonly the pulmonary artery, shows *MDM2* amplification and overexpression in 75% (6 of 8) of cases.[49,50] Similarly, cardiac sarcoma, previously considered as representing an otherwise unclassified spindle cell sarcoma, has also been found to show *MDM2* and *CDK4* amplification in most cases, suggesting that it is closely related to intimal sarcoma. This has allowed for improved classification of cardiac sarcomas, and it appears that intimal sarcoma represents the most common primary cardiac sarcoma.[51]

Recent studies have also shown that amplification of *MDM2* and *CDK4* occurs in 67% (10 of 15) of low-grade osteosarcomas (parosteal and central low-grade osteosarcomas) and only 12% (16 of 130) of high-grade osteosarcomas, with corresponding rates of immunohistochemical expression of these proteins.[52] This finding has been shown to be of diagnostic utility; in one study all low-grade osteosarcomas expressed MDM2 and/or CDK4, usually diffusely and with moderate or strong intensity, whereas expression of these markers in benign morphologic mimics of these tumors was extremely limited, with expression seen in only one case of bizarre parosteal osteochondromatous proliferation.[53] Because expression of MDM2 and CDK4 is generally limited to low-grade osteosarcomas and their dedifferentiated counterparts, expression of these two markers in an otherwise nondistinctive high-grade sarcoma of bone may therefore suggest evolution from (dedifferentiation of) a low-grade osteosarcoma.[54]

STAT6 (Signal Transducers and Activators of Transcription 6)

A recurrent *NAB2-STAT6* fusion gene has very recently been identified in the vast majority of solitary fibrous tumors (SFTs), both benign and malignant, using a variety of techniques, including whole-exome and transcriptome sequencing.[55–58] STAT6 is a member of the STAT family of cytoplasmic transcription factors, which regulate gene expression by transmitting signals to the nucleus and binding to specific DNA promoter sequences. STAT6 modulates signaling by interleukin 4 and interleukin 13 in the immune system.[59] STAT signaling is important for normal cellular processes, embryonic development, innate and adaptive immune function, and regulation of cell differentiation, growth, and apoptosis. NAB2 is a transcriptional corepressor, a regulator of the early growth response 1 (EGR1) transcription factor. The *NAB2-STAT6* gene fusion results in variable truncation of the repressor domain of NAB2 with replacement by the transcriptional activation domain of STAT6, and it is thought that the resulting fusion protein translocates to the nucleus, where it acts as a transcriptional activator, inducing expression of EGR target genes and resulting in increased proliferation.[57,60] In the early discovery studies, STAT6 was shown to be overexpressed at the protein level in SFT, whereas NAB2 showed less specific staining compared with other tumor types.[58] A large study of STAT6 expression in 231 tumors showed that nuclear expression of STAT6 is highly sensitive for SFT, with expression seen in more than 95% (59 of 60) of cases examined (Figure 4). Expression of STAT6 was limited in other tumors and was most commonly seen in a subset of dedifferentiated liposarcoma, as well as in 1 (of 10) deep fibrous histiocytoma, in which staining was weak and focal.[60] Other studies have since confirmed the high

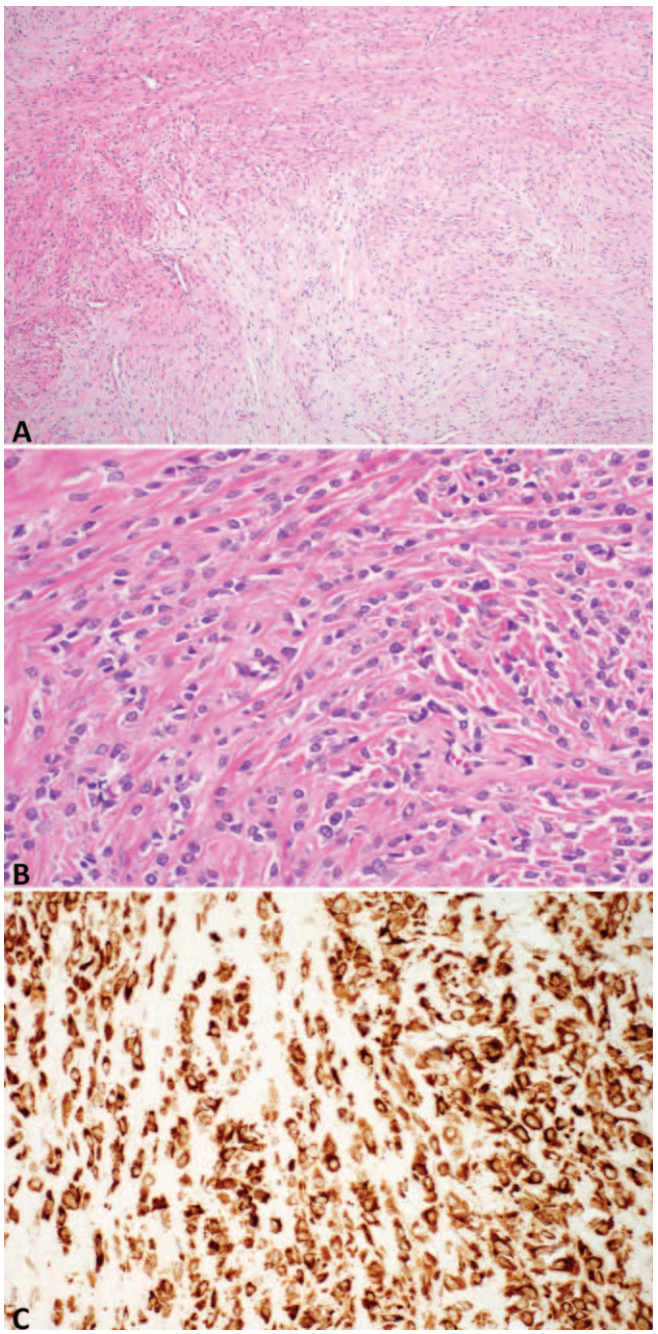

Figure 5. *Low-grade fibromyxoid sarcoma (LGFMS) showing characteristic alternating fibrous and myxoid areas (A), and the related sclerosing epithelioid fibrosarcoma (SEF) composed of cords of epithelioid cells with pale or clear cytoplasm embedded in a densely sclerotic stroma (B). More than 98% of LGFMS and 70% of SEF show diffuse cytoplasmic expression of MUC4 (C) (hematoxylin-eosin, original magnifications ×100 [A] and ×400 [B]; original magnification ×400 [C]).*

sensitivity and specificity of STAT6 for the diagnosis of SFT, with staining seen in a subset of dedifferentiated liposarcomas, and rarely in other tumor types.[60–63,193] The antibodies used differed in some of these studies, accounting for the additional cytoplasmic staining described by some authors; however, nuclear expression is expected in SFT.[61–63] Interestingly, a very recent study has shown a correlation between specific fusion types and morphology, with tumors with *NAB2ex4-STAT6ex2/3* fusion genes corresponding to classic SFTs that arise most often in the pleura of older patients, show diffuse fibrosis, and typically have a benign clinical course. Tumors with the less common NAB2ex6-STAT6ex16/17 fusion occur in younger patients, usually in deep soft tissue, are more cellular, and are associated with aggressive clinical behavior.[64]

Perhaps the greatest potential pitfall in the use of STAT6 for evaluating spindle cell lesions, particularly those in an intra-abdominal/retroperitoneal location, is the presence of STAT6 expression in approximately 7% to 15% of dedifferentiated liposarcomas, which likely represents amplification of the 12q13 region in this tumor type, where *STAT6* is located close to *MDM2* and *CDK4*.[60,62,193] The staining pattern in dedifferentiated liposarcoma is variable and may be focal or diffuse, weak or medium to strong in intensity, unlike the generally strong diffuse pattern seen in solitary fibrous tumor. In addition, unlike the predominantly nuclear pattern of staining seen in solitary fibrous tumor, both cytoplasmic and nuclear expression is common in dedifferentiated liposarcoma.[193] Diffuse expression of MDM2 and CDK4 helps favor a diagnosis of dedifferentiated liposarcoma, and if doubt persists, FISH for *MDM2* amplification may also be useful. Because *STAT6* and *NAB2* are located close together on the long arm of chromosome 12, FISH to demonstrate rearrangement of the genes is technically challenging and not diagnostically useful. The identification of the *NAB2-STAT6* fusion gene in meningeal hemangiopericytoma is further evidence that these lesions are in fact morphologic variants of solitary fibrous tumor.[58] STAT6 nuclear positivity helps distinguish meningeal hemangiopericytomas/solitary fibrous tumor from histologic mimics, because expression was not detected in any (n = 87) meningiomas and the vast majority of potential mimics in a large series of meningeal tumors evaluated for STAT6 expression.[58]

MUC4 (Mucin 4)

MUC4 is a useful marker for low-grade fibromyxoid sarcoma (LGFMS) and sclerosing epithelioid fibrosarcoma (SEF; Figure 5). The mucin 4 (*MUC4*) gene was found to be significantly upregulated in LGFMS compared with other tumor types through gene expression array analysis.[65] MUC4 is a transmembrane glycoprotein that is normally expressed on many epithelial surfaces, where it is thought to serve a protective role, and is also involved in cell proliferation and survival through interacting with the ErbB/HER2 family of growth factor receptors.[66–71] A large study evaluating MUC4 as an immunohistochemical marker for LGFMS evaluated whole-tissue sections of 309 soft tissue tumors, including 49 LGFMSs (all of which showed *FUS* gene rearrangement by FISH), and found expression of MUC4 in all cases of LGFMS, with a diffuse cytoplasmic pattern of staining in tumor cells (Figure 5).[72] However, it should be noted that very rare cases of LGFMS may be negative for MUC4,[73] and if strong suspicion for LGFMS persists, FISH for *FUS* or *EWSR1* rearrangement should be considered.

Sclerosing epithelioid fibrosarcoma, another fibroblastic neoplasm, shows morphologic and molecular overlap with LGFMS, and hybrid tumors showing features of both exist. A subset of SEF has been shown to contain the *FUS-CREB3L2* gene fusion or *FUS* gene rearrangements, characteristic of LGFMS.[74–81] Similar to LGFMS, strong

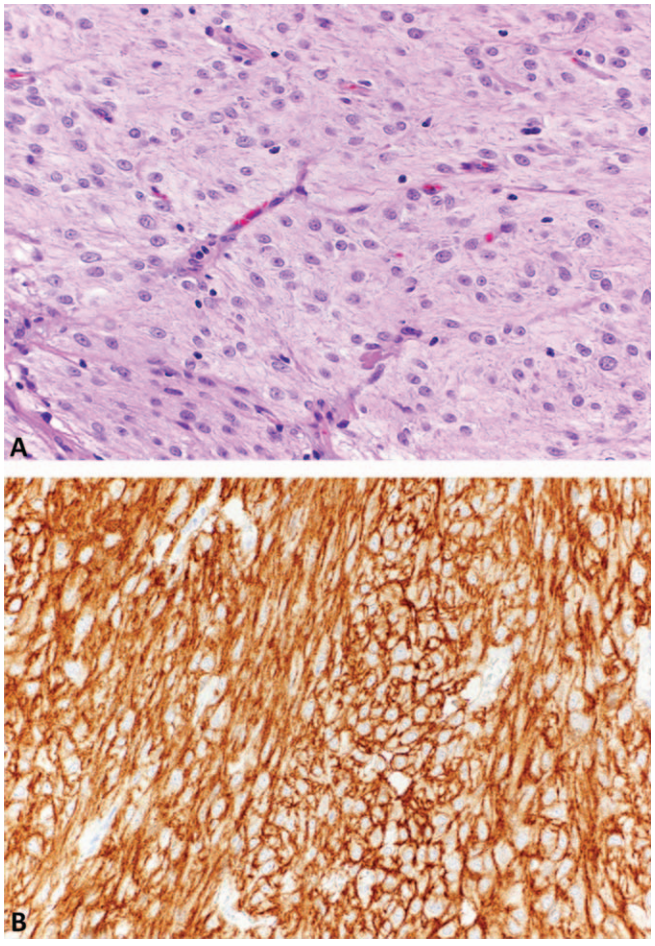

Figure 6. *Gastric gastrointestinal stromal tumor with* PDGFRA *mutation and epithelioid features (A) is frequently negative for KIT, but DOG1 is usually positive, with both cytoplasmic and membranous staining (B) (hematoxylin-eosin, original magnification ×400 [A]; original magnification ×400 [B]).*

diffuse cytoplasmic expression of MUC4 is seen in SEF, occurring in 69% (20 of 29) to up to 90% (9 of 10) of "pure" forms, and virtually all tumors (12 of 12) showing hybrid features of both LGFMS and SEF (Figure 5).[82,83] Recent studies have shown that *EWSR1-CREB3L1* is the predominant gene fusion in SEF; this fusion gene occurs uncommonly in LGFMS.[83,84]

MUC4 positivity is also seen in synovial sarcoma, predominantly in the glandular component of the biphasic subtype, with more limited expression in the spindle cell component of either biphasic or monophasic synovial sarcoma. Focal positivity has also been identified in ossifying fibromyxoid tumors (5 of 17; 29%), epithelioid GISTs (2 of 10; 20%), and some myoepithelial carcinomas (1 of 10; 10%).[82] However, unlike the diffuse strong pattern seen in LGFMS or SEF, staining in these tumor types is generally limited in extent, with either just scattered positive cells or focal staining. Importantly, regarding SEF, in which a poorly differentiated carcinoma may fall into the differential diagnosis, it should be remembered that MUC4 expression is seen in a variety of different carcinomas, such as pancreaticobiliary carcinomas, breast carcinoma, and colonic adenocarcinoma. Helpful features to support a diagnosis of SEF include the presence of characteristic dense sclerotic stroma, within which the tumor cells are embedded and may have pale or clear cytoplasm; the tumor cells of SEF are generally negative for cytokeratins, and in some cases FISH for *EWSR1* and *FUS* gene rearrangement may also help support a diagnosis of SEF.

DOG1 (Discovered on GIST 1)

Gastrointestinal stromal tumors typically harbor gain-of-function mutations in *KIT* or *PDGFRA*, and KIT immunoreactivity is important in the diagnosis of most GISTs; however, approximately 5% to 10% of GISTs are negative for KIT, usually those with *PDGFRA* mutations.[85–88] Gene expression profiling using cDNA microarrays identified overexpression of DOG1 in GIST relative to other tumor types, and immunoreactivity to DOG1 was shown in 98% (136 of 139) of GISTs, regardless of *KIT* or *PDGFRA* mutation status.[89] Expression of DOG1 is typically both cytoplasmic and membranous (Figure 6). Further studies have confirmed that DOG1 is a highly sensitive marker for GIST, being present in more than 87% of cases (986 of 1040 cases[90]; 370 of 425 cases[91]).[89–91] Importantly, DOG1 expression is found in up to 96% of KIT-negative GISTS (24 of 25 cases[92]; 10 of 28 cases[93]; 9 of 10 cases[94]), the most common of which are gastric tumors with *PDGFRA* mutations and epithelioid cytomorphology.[92–94] Approximately 3% (27 of 1040) of all GISTs are negative for both KIT and DOG1, and in cases where the diagnostic suspicion remains high for GIST, mutational analysis for *KIT* and *PDGFRA* mutations is indicated to confirm the diagnosis: 46% (11 of 24) of DOG1-negative GISTs have *KIT* or *PDGFRA* mutations.[90] The specificity of DOG1 for GIST is also relatively high, particularly among other mesenchymal neoplasms that may mimic GIST. Other mesenchymal tumors that show rare and/or focal DOG1 positivity include synovial sarcoma (2.5% to 16%, in 1 of 39 cases[91] and 6 of 37 cases,[90] respectively), uterine type retroperitoneal leiomyomas (5 of 42; 12%),[90] leiomyosarcoma (1 of 326; 0.3%),[91] and some perivascular epithelioid cell tumors (PEComas).[90,91]

DOG1 is also known as Anoctamin-1 (Ano-1), transmembrane protein 16A (TMEM16A), overexpressed in oral (squamous cell) carcinoma 2 (ORAOV2), and tumor-amplified and overexpressed sequence 1 (TAOS1).[95,96] DOG1 positivity is also common in esophageal squamous cell carcinomas and gastric adenocarcinomas, particularly intestinal type, and is less commonly seen in colorectal adenocarcinomas.[90] Infrequent positivity for DOG1 has been reported in a variety of other tumors, including endometrioid adenocarcinoma, acinic cell carcinoma, desmoplastic melanoma, malignant peripheral nerve sheath tumor, Ewing sarcoma, and glomus tumor.[97,98] DOG1 has also recently been found to be positive in the cellular areas of 100% (9 of 9) of chondroblastomas and may have utility in diagnosing this lesion.[99] The combination of both KIT and DOG1 reactivity is most useful in clinical practice in the diagnosis of GIST.[100]

SUCCINATE DEHYDROGENASE A AND B

Approximately 15% of GISTs in adults and more than 90% in children lack *KIT* and *PDGFRA* mutations, so-called wild-type GISTs.[101–103] Wild-type GISTs also include those tumors arising in the Carney triad, the Carney-Stratakis syndrome, and neurofibromatosis 1 (NF1).[104] Carney triad is a nonhereditary syndrome that typically occurs in young females and is characterized by gastric GIST, paraganglio-

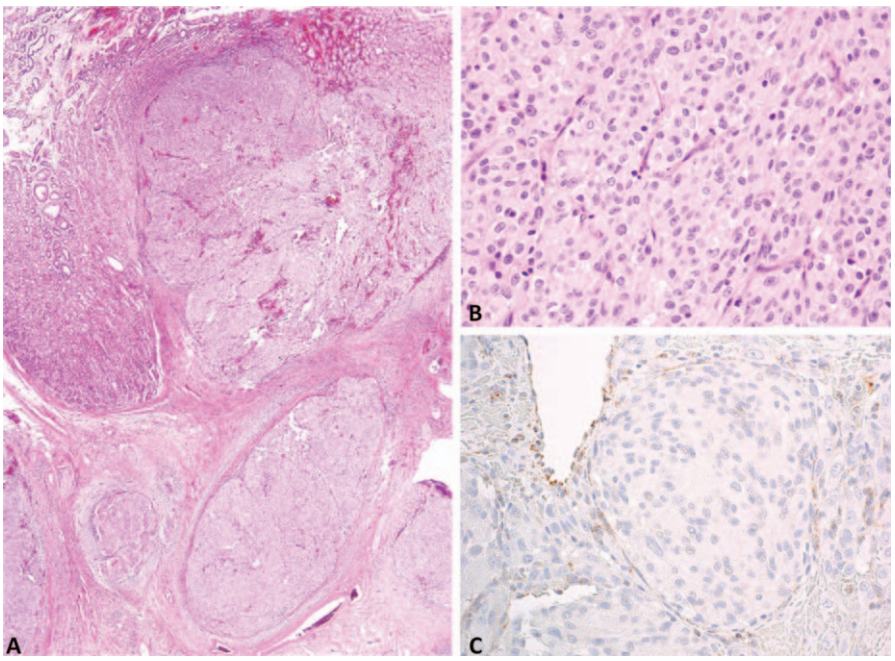

Figure 7. *Succinate dehydrogenase (SDH)–deficient gastrointestinal stromal tumor shows a characteristic multinodular or plexiform growth pattern (A), and tumor cells are usually epithelioid (B). This tumor type shows loss of expression of SDHB in tumor cells, in contrast to surrounding normal cells, such as endothelial cells and inflammatory cells, which show cytoplasmic expression of SDHB (C) (hematoxylin-eosin, original magnifications ×40 [A] and ×400 [B]; original magnification ×400 [C]).*

ma, and pulmonary chondroma; Carney-Stratakis syndrome is inherited in an autosomal dominant fashion and consists of the dyad of gastric GIST and paraganglioma.[105–108] Defects in the succinate dehydrogenase (SDH) metabolic pathway have been found to occur in most pediatric GISTs, a subset of adult "wild-type" GIST, and GISTs occurring in Carney-Stratakis syndrome and Carney triad.[109] These tumors form a clinicopathologically distinct subset of wild-type GISTs known as "SDH-deficient GIST." These tumors arise exclusively in the stomach, particularly the antrum, and show a characteristic multifocal and plexiform growth pattern, and usually a purely or predominantly epithelioid cytomorphology (Figure 7, A and B). Succinate dehydrogenase–deficient GIST is typically positive for KIT and DOG1. Unlike *KIT* mutant GIST, lymph node metastases are common in SDH-deficient GIST, and these tumors tend to be imatinib resistant, yet often show an indolent clinical course.[103,110–112] Succinate dehydrogenase is an enzyme complex composed of four subunit proteins (A, B, C, and D) that is localized in the inner mitochondrial membrane and is involved in the oxidation of succinate to fumarate in the citric acid cycle and the electron transport chain.[113,114] SDHB and SDHA are normally ubiquitously expressed in a granular cytoplasmic pattern. Germline mutations in *SDHA*, *SDHB*, *SDHC*, and *SDHD* were first identified in pheochromocytoma-paraganglioma syndrome, and they result in the destabilization of the SDH complex and loss of SDHB expression by immunohistochemistry (Figure 7, C).[115–117] However, somatic mutations in any of these genes result in loss of SDHB expression, and loss of expression is also seen in tumors where no identifiable mutations are detected, suggesting that dysfunction of the pathway may have arisen by other mechanisms or by other mutations that are not detectable by current methods. Loss of SDHB by immunohistochemistry defines the group of SDH-deficient GIST[118–121] and has been shown to occur with an estimated frequency of 7.5% among all gastric GISTs.[110] Although loss of SDHB expression is detected in 42% (22 of 53) of wild-type GISTs, GISTs with *KIT* or *PDGFRA* mutation consistently show intact SDHB expression.[121]

Germline mutations in *SDHA*, *SDHB*, *SDHC*, and *SDHD* occur in some, but not all, patients with SDH-deficient GIST.[109,120,122,123] Germline mutations in *SDHA* represent the most commonly mutated gene, and loss of expression of SDHA by immunohistochemistry has been shown to reliably predict the presence of *SDHA* mutations.[124] Interestingly, although germline mutations have been identified in association with Carney-Stratakis syndrome, mutations in *SDH* genes have not been found in patients with Carney triad, and the mechanism for deficient SDH function in Carney triad is uncertain at this time.[119,125–127] Wild-type GIST associated with NF1 do not show loss of SDHB expression.[119,121,128]

Given the significant prognostic and predictive implications with regards to clinical course, selection of appropriate therapy (SDH-deficient GIST responds better to second- or third-generation tyrosine kinase inhibitors), and implications for screening of other tumors in both the patient and family members, SDHB immunohistochemistry should be considered for gastric GISTs with an epithelioid cytomorphology and multinodular or plexiform growth pattern. If expression is lost in tumor cells, with normal endothelial cells and inflammatory cells acting as a positive internal control, the patient should be referred for genetic counseling. In addition, if SDHB expression is lost, additional loss of staining for SDHA suggests the presence of *SDHA* mutation.

INI1 (Integrase Interactor 1)

INI1 is the protein product of the gene *hSNF5/INI1/SMARCB1*, located on the long arm of chromosome 22 (22q11.2). INI1 is a core subunit of the SWI/SNF ATP-dependent chromatin remodeling complex, and is ubiquitously expressed in the nuclei of normal cells. INI1 is thought to function as a tumor suppressor. Loss of INI1 function (with corresponding loss of protein expression) can result from mutations or deletions of the *INI1* gene.[129] Loss of INI1 expression is observed in several tumor types. Abnormalities in *INI1* were first described in malignant

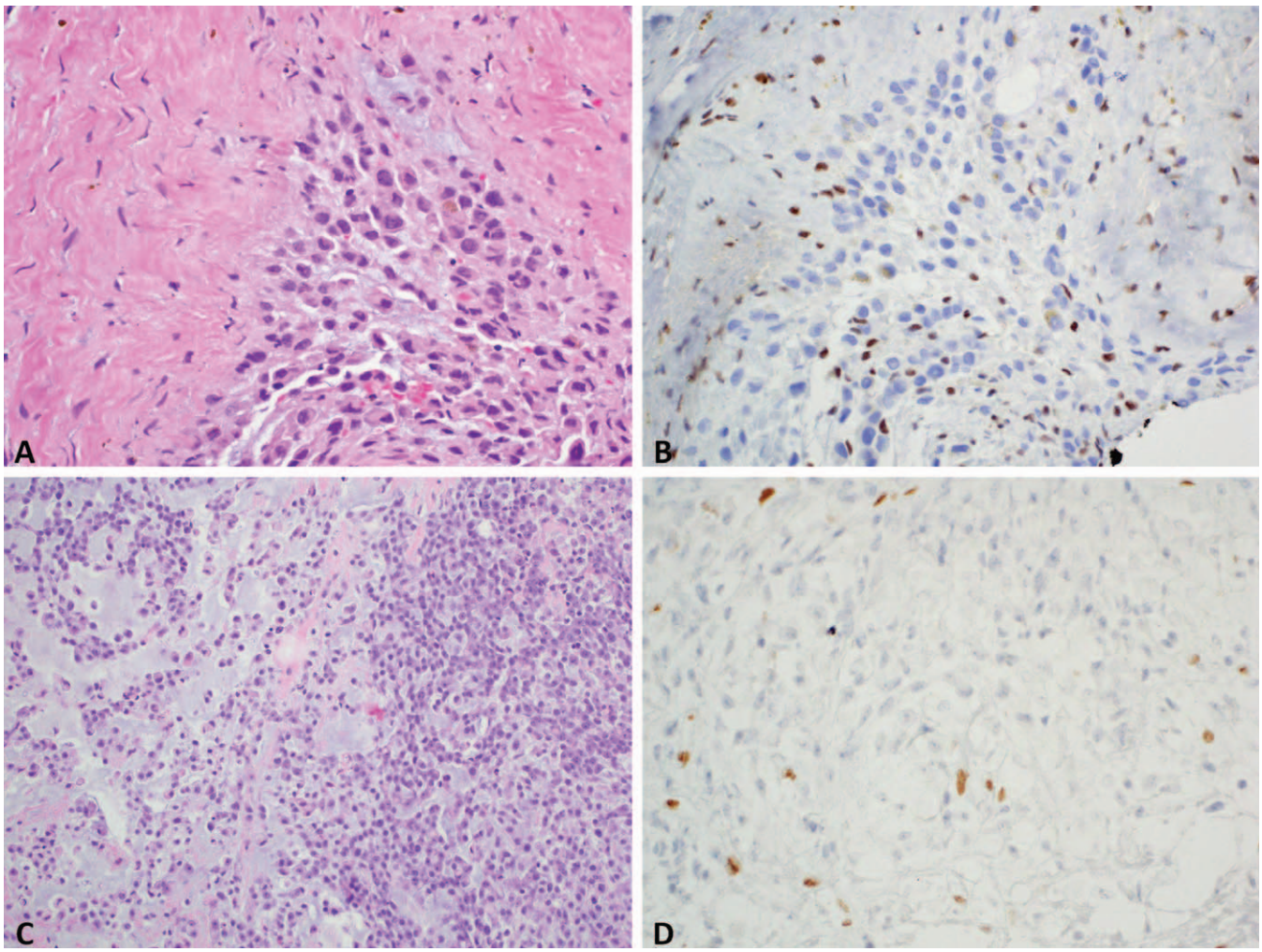

Figure 8. *Proximal-type epithelioid sarcoma, which may mimic carcinoma or melanoma, (A) shows diffuse loss of nuclear INI1, in contrast to surrounding normal cells (B). Approximately 40% of myoepithelial carcinomas (C) in children and 10% in adults show loss of nuclear INI1 expression (D) (hematoxylin-eosin, original magnifications ×400 [A] and ×200 [C]; original magnification ×400 [B and D]).*

rhabdoid tumors (renal, extrarenal, and atypical teratoid/rhabdoid tumor of the central nervous system), and loss of INI1 expression is seen in nearly all of these tumors. Although most are sporadic mutations, a small group of infants with malignant rhabdoid tumor have germline mutations in *INI1/SMARCB1*.[130] Loss of INI1 expression is also seen in approximately 93% (127 of 136) of epithelioid sarcomas, both conventional and proximal-type (Figure 8, A and B).[131] In contrast to malignant rhabdoid tumors, where mutations in *INI1* are the most common cause of loss of function, chromosomal deletions are usually identified in epithelioid sarcomas.[132] In addition to malignant rhabdoid tumors and epithelioid sarcoma, loss of INI1 expression is also seen in 50% (12 of 24) of epithelioid malignant peripheral nerve sheath tumors and in a subset of myoepithelial carcinomas (Figure 8, C and D)—particularly those arising in children (9 of 22; 40%; versus 2 of 22; approximately 10% in adults).[131,133]

Other tumor types reported to show variable loss of INI1 expression include extraskeletal myxoid chondrosarcomas (4 of 24; 17%),[134] poorly differentiated chordoma, undifferentiated hepatoblastoma, and renal medullary carcinoma.[135–138] Approximately 60% of patients with the rare hereditary syndrome of familial schwannomatosis have germline mutations in *INI1/SMARCB1*, which is associated with a "mosaic" pattern of protein loss by immunohistochemistry.[139] Reduced, but not complete loss of, INI1 expression has been reported in synovial sarcoma; the significance of this finding is uncertain.[140,141]

TLE1 (Transducin-Like Enhancer of split 1)

TLE1 is a relatively recently described marker of synovial sarcoma. Gene expression profiling studies have shown a major association of the Wnt signaling pathway with synovial sarcoma and overexpression of members of the *TLE* (*T*ransducin-*L*ike *E*nhancer of split) family of genes, particularly *TLE1*, which is one of four *TLE* genes that encode human transcriptional repressors homologous to the *Drosophila* corepressor *groucho*, and is an important component of the Wnt signaling pathway.[142–150] TLE proteins are involved in multiple developmental processes, including lateral inhibition, segmentation, sex determination, eye development, neuronal differentiation, and hematopoiesis.[149,151–154] An early large study using tissue microarrays showed strong positive nuclear staining for TLE1 in 91 of 94 molecularly confirmed synovial sarcomas (97%), including both the epithelial and spindle cell components of biphasic

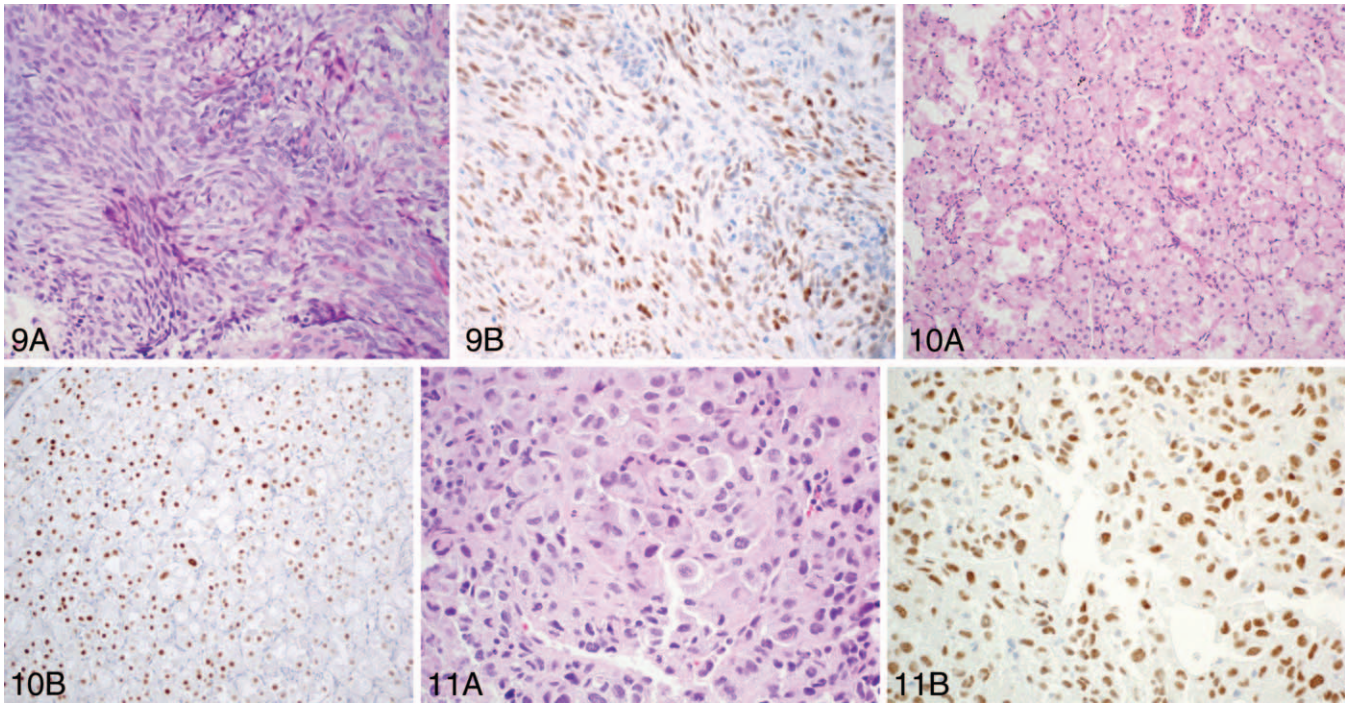

Figure 9. *Monophasic synovial sarcoma (A) shows nuclear expression of TLE1 in approximately 90% of cases (B) (hematoxylin-eosin, original magnification ×400 [A]; original magnification ×400 [B]).*

Figure 10. *Alveolar soft part sarcoma with the classic appearance of large cells with abundant granular palely eosinophilic cytoplasm arranged in a nested growth pattern (A). Nuclear expression of TFE3 is seen in virtually all cases (B) (hematoxylin-eosin, original magnification ×400 [A]; original magnification ×400 [B]).*

Figure 11. *Metastatic malignant melanoma with epithelioid cytomorphology (A) shows diffuse nuclear expression of SOX10 (B) (hematoxylin-eosin, original magnification ×400 [A]; original magnification ×400 [B]).*

synovial sarcoma as well as poorly differentiated variants (Figure 9).[150] Other studies have also shown high sensitivity and specificity of TLE1-positive immunohistochemical staining for molecularly confirmed synovial sarcoma.[155–157] However, one study evaluating 163 soft tissue and bone tumors using whole sections found TLE1 positivity in 18 of 20 synovial sarcomas (90%), but also in 53 of 143 nonsynovial sarcomas (37%).[158]

Regarding other tumors, TLE1 expression is most commonly seen in peripheral nerve sheath tumors and solitary fibrous tumors. Terry et al[150] reported 16 of 88 malignant peripheral nerve sheath tumors (MPNSTs; 18%) with any staining for TLE1, but only 4 tumors (5%) showed more than weak staining. Another study has shown a slightly lower rate of expression of TLE1 in synovial sarcoma (82%; 60 of 73).[159] In that study nuclear reactivity for TLE1 was also observed in 15% (7 of 47) of MPNSTs and 8% (4 of 49) of solitary fibrous tumors, but it was usually only weak in these tumor types. Occasional staining for TLE1 has also been described in clear cell sarcoma, high-grade chondrosarcoma, Ewing sarcoma, rhabdomyosarcoma, GIST, myxofibrosarcoma, and leiomyosarcoma.[150,158] TLE1 expression has also been reported in malignant mesothelioma.[160] However, in most of these tumors, staining is focal and weak to moderate in intensity, unlike the strong diffuse nuclear pattern characteristic of synovial sarcoma. Within nonneoplastic tissue, TLE1 expression has been reported to occur in basal keratinocytes, adipocytes, perineurial cells, endothelial cells, and mesothelial cells.[158]

TFE3 (Transcription Factor Binding to IGHM Enhancer 3)

TFE3 is a member of the microphthalmia (MiT) family of transcription factors, which includes MiTF, TFEB, TFEC, and TFE3, all of which share a common structure consisting of a helix-loop-helix leucine zipper dimerization motif, a transactivation domain, and a basic region involved in DNA binding.[161] The most well-studied MiT transcription factor is MiTF, which is important in the development of melanocytes (survival, growth, and migration), melanogenesis, and osteoclast development; the function of the other MiT family members is less well defined at this time.[161] The utility of MiTF in clinical practice is limited by its low specificity: nuclear expression of MiTF is seen in primary and metastatic melanomas, as well as in some PEComas, clear cell sarcomas, and histiocytic proliferations or neoplasms.[162,163]

Although TFE3 is ubiquitously expressed in humans, native TFE3 protein is usually not detected by routine immunohistochemical methods. Nuclear expression of TFE3 is seen in a variety of different tumors, most of which harbor *TFE3* gene fusions, including alveolar soft part sarcoma, Xp11 translocation renal cell carcinoma, "melanotic" Xp11 translocation renal cell carcinoma, and a subset of PEComas and epithelioid hemangioendotheliomas.[164–171] Although virtually all alveolar soft part sarcomas and Xp11 translocation renal cell carcinomas will show diffuse nuclear TFE3 expression (Figure 10), only a subset of PEComas will show nuclear immunoreactivity for TFE3, with a subset of those cases also harboring a *TFE3* gene rearrangement.[170,172] Moreover, PEComas with a *TFE3* gene fusion have been

found not to harbor the tuberous sclerosis complex (TSC2) alterations characteristic of conventional PEComas, and expression of TFE3 in PEComa is mutually exclusive to expression of MiTF.[173] Although limited by small numbers, cutaneous PEComas do not appear to show reactivity for TFE3 by immunohistochemistry or *TFE3* rearrangement by FISH.[174]

Very recently, a *YAP1-TFE3* fusion gene has been detected in a subset of epithelioid hemangioendothelioma, with corresponding detectable TFE3 nuclear reactivity by immunohistochemistry.[171] Interestingly, epithelioid hemangioendotheliomas with this fusion gene show distinct morphologic appearances, in that they typically have voluminous eosinophilic cytoplasm, show well-formed vascular channels and focally solid growth, and usually arise in young adults.[171]

TFE3 immunohistochemistry is therefore useful in confirming a diagnosis of alveolar soft part sarcoma and Xp11 translocation renal cell carcinoma, and is also expressed in epithelioid hemangioendotheliomas with a distinctive fusion gene and clinicopathologic features, as well as a small subset of PEComas.

SOX10 (Sex-determining Region Y-related HMG-box 10)

SOX10 is a nuclear transcription factor normally expressed in neural crest cells that is crucial for differentiation of Schwann cells and melanocytes.[175–177] Nuclear staining is seen in normal melanocytes, Schwann cells, secretory cells of the eccrine coil, myoepithelial cells, and acinar cells of salivary gland tissue.[178–180] Expression of SOX10 is correspondingly seen in tumors showing neural crest differentiation (ie, melanocytic and nerve sheath tumors), as well as a subset of myoepithelial and salivary gland–type tumors (acinic cell carcinomas, adenoid cystic carcinomas, epithelial-myoepithelial carcinomas, myoepitheliomas/myoepithelial carcinomas, and mixed tumors/pleomorphic adenomas).[179–182] In addition to staining most benign nerve sheath tumors (ie, neurofibroma and schwannoma), recent studies have shown that SOX10 is expressed in 27% to 50% of MPNSTs (21 of 78 cases[179]; 38 of 77 cases[182]), a sensitivity similar to that of S100 protein for this tumor type.[179,182] SOX10 expression is also detected in a large majority of melanocytic neoplasms, including benign nevi (blue, neurotized, dysplastic, Spitz, and nodal capsular) and malignant melanoma (conventional, spindled, desmoplastic, and metastatic), with one study showing SOX10 positivity in 97% of melanomas (76 of 78)[182] and another study showing SOX10 positivity particularly in desmoplastic melanoma (7 of 9; 78%),[179] as well as clear cell sarcoma (4 of 7; 57%[179]; malignant melanoma of soft parts).[179,182,183] Expression of this marker should therefore be interpreted in context, and in general it is not useful in distinguishing melanocytic from neural tumors. However, it can be useful to confirm a diagnosis of melanoma when other melanocytic markers are negative, in the right histologic and clinical context (Figure 11). Karamchandani et al[179] reported expression of SOX10 in 26 of 26 granular cell tumors (100%). The specificity of SOX10 for tumors showing neural crest differentiation (ie, melanocytic and nerve sheath tumors) is greater than that for S100 protein because expression of SOX10 in other mesenchymal and epithelial tumors is limited; expression is found in a subset of myoepithelial and salivary gland tumors, diffuse astrocytomas, and some ductal breast carcinomas.[181–183] Sustentacular cells of paraganglioma/pheochromocytoma and a subset of carcinoid tumors also express SOX10.[182]

NY-ESO-1 (New York Esophageal Squamous Cell Carcinoma 1)

Cancer-testis antigens are a family of antigens normally expressed in adult testicular germ cells, but which have been found to be aberrantly expressed in a variety of tumors. These antigens elicit both humoral and cell-mediated immune responses and are becoming attractive targets for immune-based cancer therapies.[184,185] NY-ESO-1 is encoded by the *CTAG 1B* gene and was initially discovered by serologic analysis of a cDNA expression library of a patient's esophageal carcinoma and autologous serum, and it has since been found to be expressed in melanoma, sarcomas, and various other carcinomas.[186,187]

NY-ESO-1 is expressed in up to 80% (20 of 25 cases[188]; 38 of 50 cases[189]) of synovial sarcomas (both monophasic and biphasic types) by immunohistochemistry, with strong reactivity seen in both the spindle cell and glandular components, and with a predominantly cytoplasmic pattern of staining.[188,189] Expression appears to be limited among other spindle cell tumors, with 10% (2 of 20) of dermatofibrosarcoma protuberans, 3% (1 of 34) of MPNSTs, and 1% (2 of 155) of GISTs showing diffuse positivity. NY-ESO-1 may therefore be useful in the distinction of synovial sarcoma from other spindle cell tumors.

NY-ESO-1 has also been found to be frequently expressed in myxoid liposarcoma,[190,191] with a lower frequency seen in other liposarcoma subtypes.[191] In a study of 138 tumors, including myxoid liposarcoma and other myxoid neoplasms, some of which may fall into the differential diagnosis, such as extraskeletal myxoid chondrosarcoma, 36 of 38 cases of myxoid liposarcoma (95%) were positive for NY-ESO-1, whereas all other tumor types were negative.[192] NY-ESO-1 may therefore also be useful in the distinction of myxoid/round cell liposarcomas from other myxoid neoplasms.

SUMMARY

In summary, many new diagnostic immunohistochemical markers for soft tissue tumors have been described in the last 10 years. This has resulted in improved classification of tumors, identification of clinically and pathologically distinct groups of tumors with prognostic and predictive implications, most notably SDH-deficient GIST, and has also likely reduced the need for additional ancillary molecular studies in routine surgical pathology practice. However, the use of all of these markers requires careful clinical correlation and knowledge of the spectrum of staining in other tumor types, as no one marker is 100% sensitive or specific for a given diagnosis. This article has reviewed the main clinical uses of several newly described immunohistochemical markers that have proved to be of significant clinical utility, along with potential pitfalls and indications where additional molecular studies may be warranted.

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Soft Tissue Tumor Immunohistochemistry Update

Illustrative Examples of Diagnostic Pearls to Avoid Pitfalls

Shi Wei, MD, PhD; Evita Henderson-Jackson, MD; Xiaohua Qian, MD, PhD; Marilyn M. Bui, MD, PhD

• ***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 histiogenesis 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 malignant. 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, desmoplatic 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)	*Mod Pathol*. 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)	*Histopathology*. 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, AP1β, TLE1, ALK, ROS1, NR4A3, BCL2, WT1, MYC, NUT, BCL6, TFE3, ZAP70, MUC4	*Anal Chem Insights*. 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)	*Arch Pathol Lab Med*. 2015;139(1):106–121

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

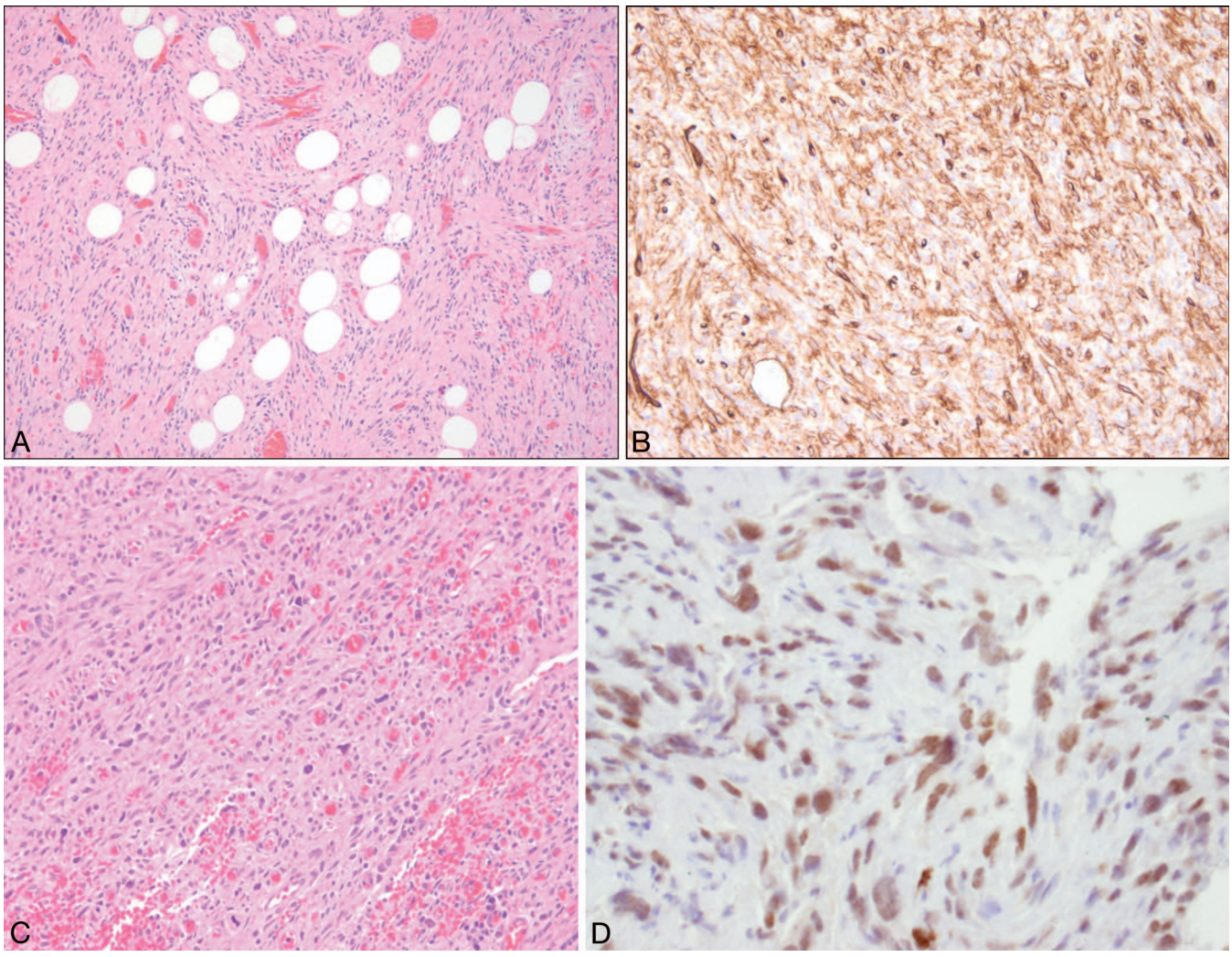

Figure 1. *Dedifferentiated liposarcoma misdiagnosed as dermatofibrosarcoma protuberans. A, The tumor shows adipocytic and spindle cell morphology mimicking dermatofibrosarcoma protuberans. B, The spindle cells are immunoreactive to cluster of differentiation 34 (CD34). C, The spindle cells exhibit moderate to severe cytologic atypia and frequent mitotic activity. D, Immunohistochemical study is positive for mouse double minute 2 homolog (MDM2) (hematoxylin-eosin, original magnifications ×10 [A] and ×20 [C]; original magnifications ×10 [B] and ×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 judicial 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 *COLIA1-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 *COLIA1-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

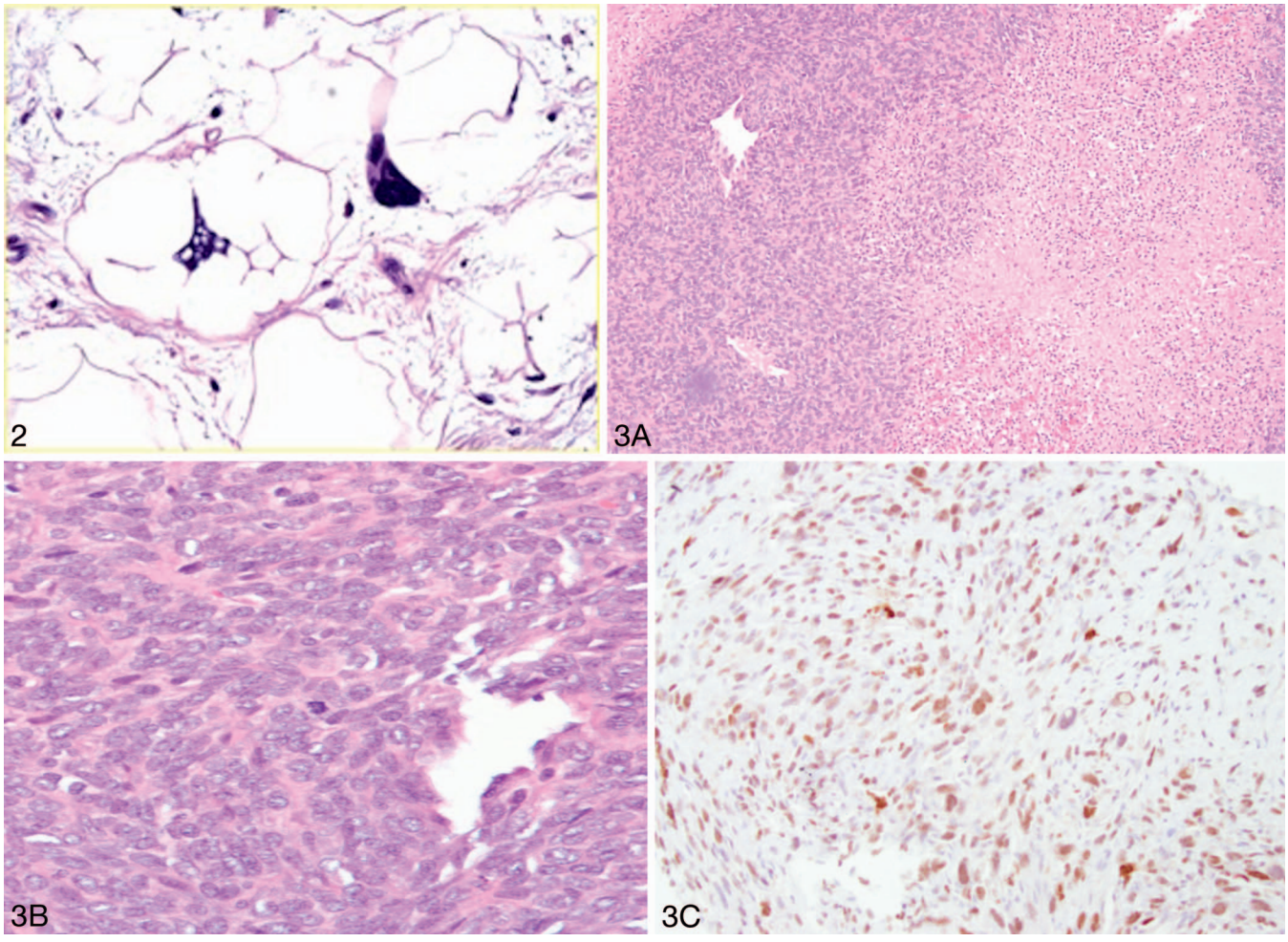

Figure 2. *Atypical lipomatous tumor. There is a highly atypical large stromal cell with hyperchromatic nuclei that is also multinucleated. There is a lipoblast that has sharply marginated cytoplasmic vacuoles scalloping the large and hyperchromatic nucleus (hematoxylin-eosin, original magnification ×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 ×10 [A] and ×40 [B]; original magnification ×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 cytomorphology, 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 judicial 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, makers 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-a-like" 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

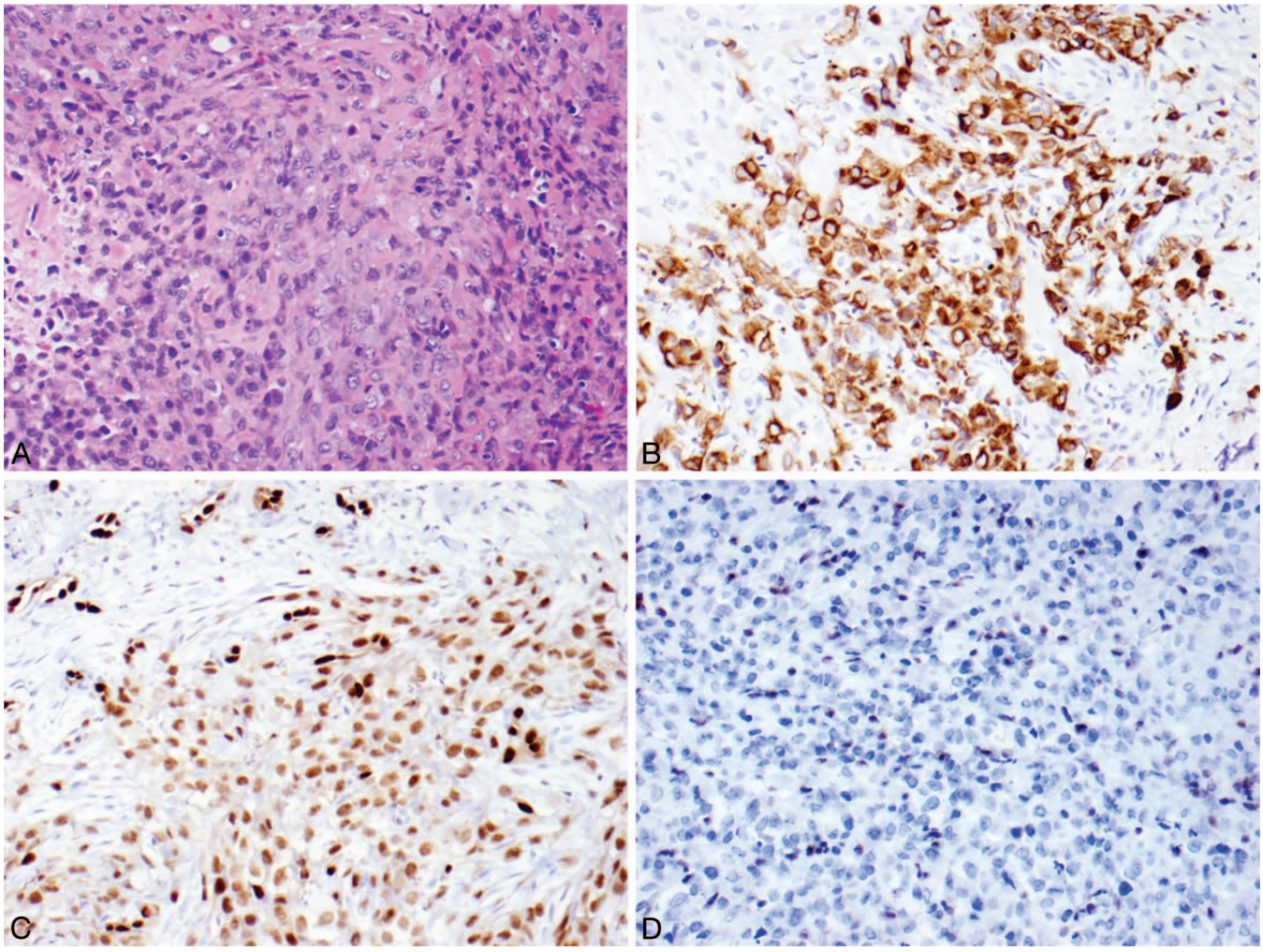

Figure 4. *Epithelioid sarcoma. A, The tumor shows epithelioid morphology and moderate to severe cytologic atypia. The lesional cells are positive for cytokeratin (B) and erythroblast transformation-specific transcription factor (ERG) (C) and demonstrate loss of nuclear integrase interactor 1 (INI1)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

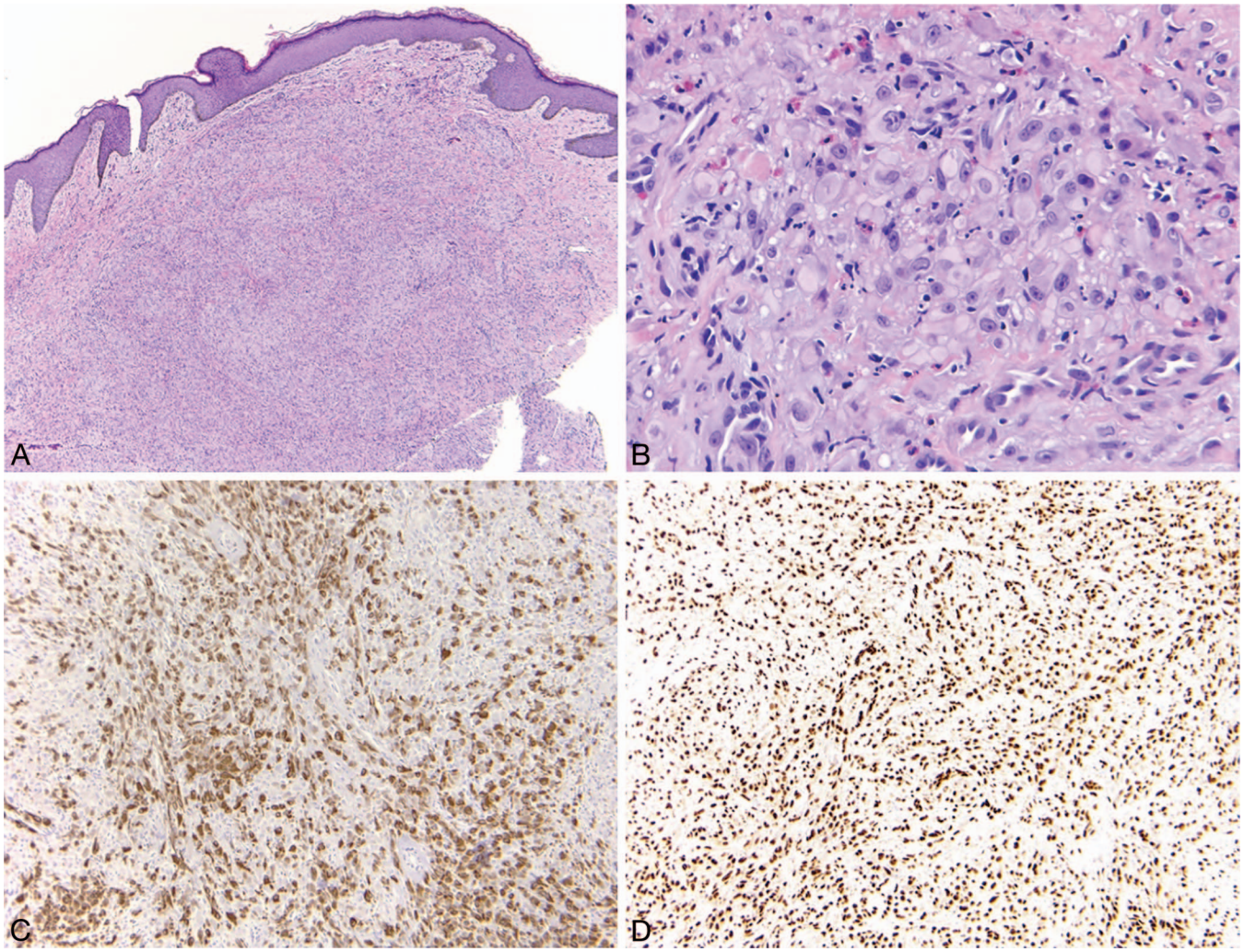

Figure 5. *Epithelioid hemangioma of the penis. A, Sections show a multinodular lesion in the dermis. B, A high-power view demonstrates pleomorphic epithelioid cells with eosinophils. C, The lesional cells are focally positive for pancytokeratin (not shown), and they are diffusely immunoreactive for 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 ×20 [A] and ×200 [B]; original magnification ×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-

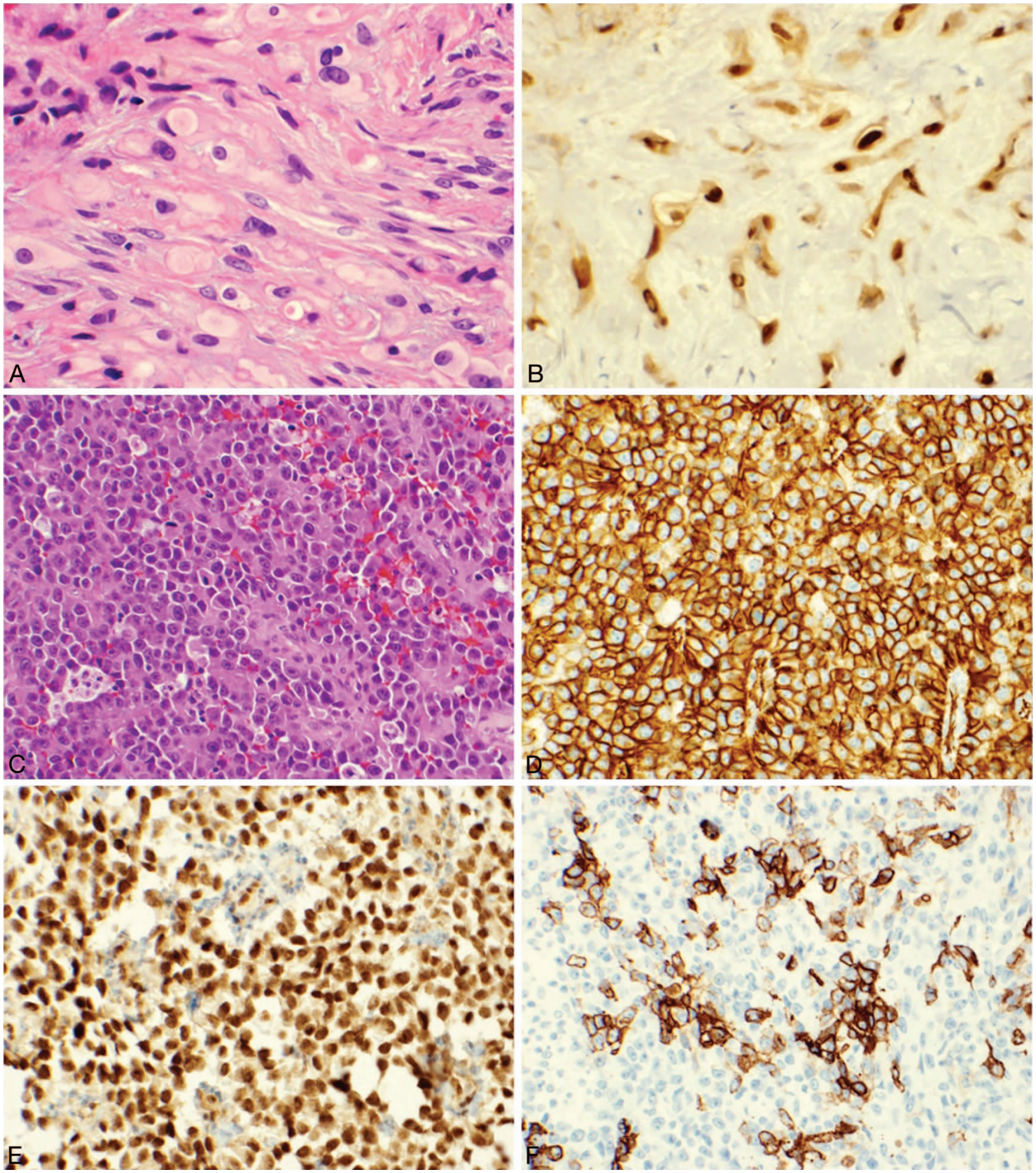

Figure 6. *Epithelioid vascular tumors. A, Epithelioid hemangioendoethelioma is composed of epithelioid cells with abundant eosinophilic cytoplasm that often contains vacuoles. B, The endothelial nature of the cells is confirmed by erythroblast transformation-specific transcription factor (ERG) staining. 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 ×200 [A] and ×100 [C]; original magnification ×200 [B]; original magnification ×20 [D through F]).*

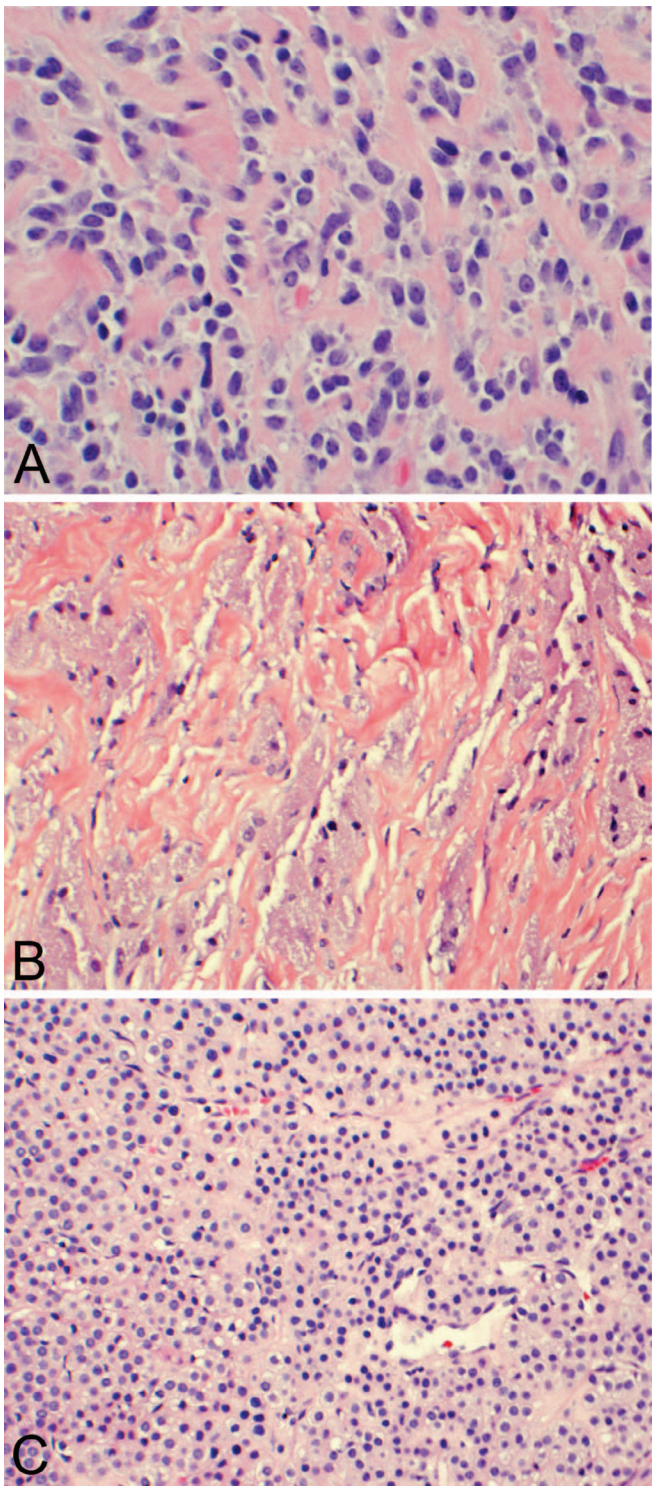

Figure 7. *Selected epithelioid tumors. A, Sclerosing epithelioid fibrosarcoma shows cytologically atypical cells in the background of collagenized stroma. B, Granular cell tumor demonstrates large cells with small nuclei and abundant granular cytoplasm. C, Glomus tumor shows small, uniform cells (hematoxylin-eosin,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

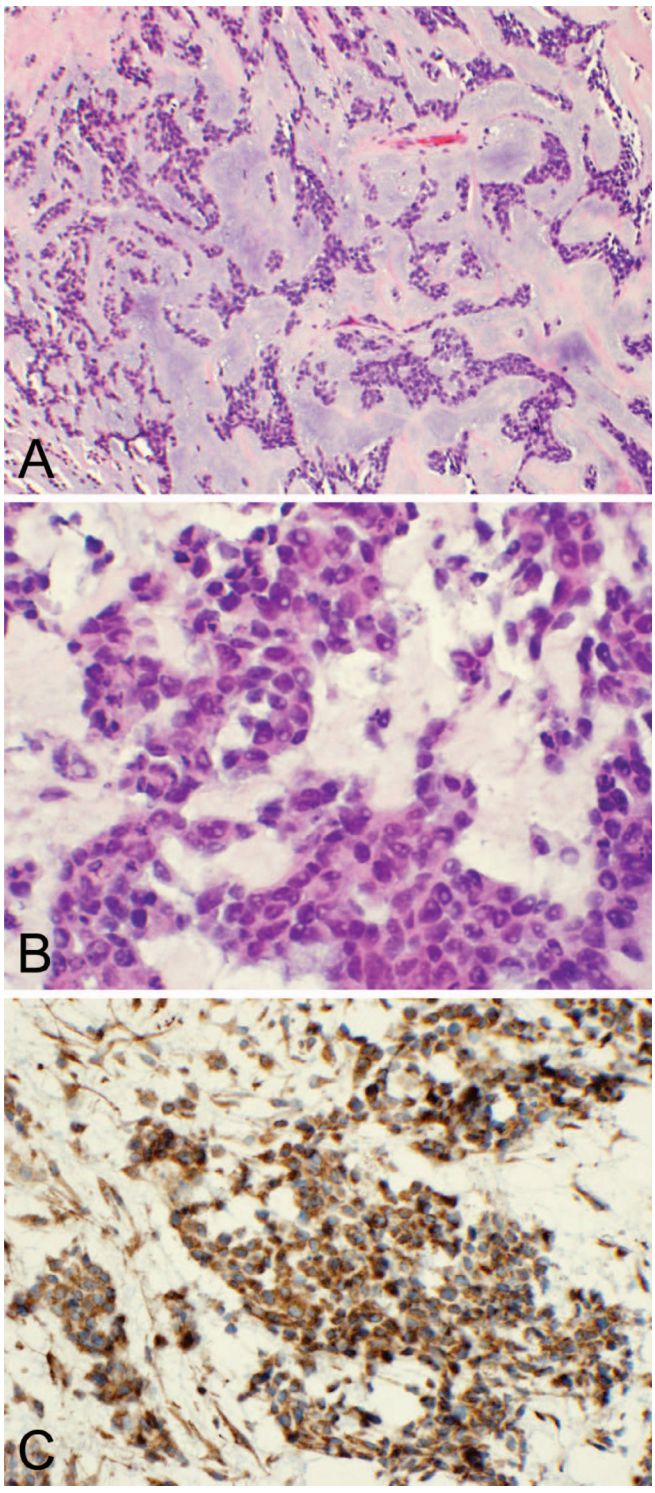

Figure 8. *Myoepithelial tumors. A, Myoepithelioma exhibits nests of myoepithelial cells in a chondromyxoid stroma. B, Myoepithelial carcinoma shows significant cytologic atypia, frequent mitoses, and calponin expression (C) (hematoxylin-eosin, original magnifications ×40 [A] and ×200 [B]; original magnification ×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 Immunophenotyes 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)	• Keratin • Vascular marks • INI1 loss
Epithelioid hemangioma	Head and neck, distal extremities, penis	Well-formed vascular channels with a prominent inflammatory infiltrate including eosinophils	• Vascular markers • Keratin (focal)
Epithelioid hemangioendothelioma	Any anatomic site	Angiocentric, cords or chains of cells with a myxoid or hyalinized stroma	• Vascular markers • Keratin (less common) • TFE3 (small subset) • t(1;3)(p36;q23-25) *WWTR1-CAMTA1* fusion
Epithelioid angiosarcoma	Highly aggressive	At least focally vasoformative	• Vascular markers • Keratin (35%)
Sclerosing epithelioid fibrosarcoma		Small epithelioid cells with a sclerotic collagenized stroma	• MUC4 • t(7;16)(q33;p11) *FUS-CREB3L2* fusion
Glomus tumor	Mostly distal extremities	Small, uniform cells	• SMA, h-caldesmon • Type IV collagen
Granular cell tumor	More common in head and neck	Abundant granular cytoplasm	• 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	• 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	• TFE3 (C-terminal) • t(X;17)(p11;q25) *ASPSCR1-TFE3* fusion
Myoepithelioma/ myoepithelial carcinoma	Cutaneous	Epithelioid, histiocytoid, plasmacytoid, or spindled cells, with little matrix or a chondromyxoid or collagenous stroma	• 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	• 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	• Melanocytic markers and SOX10 • t(12;22)(q13;q12) *EWSR1-ATF1* fusion
Epithelioid RMS	Wide age range	Large cells with or without rhabdomyoblastic differentiation	• Skeletal muscle markers (ie, desmin and myogenin)
SDH-deficient GIST	Stomach	Multinodular	• 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

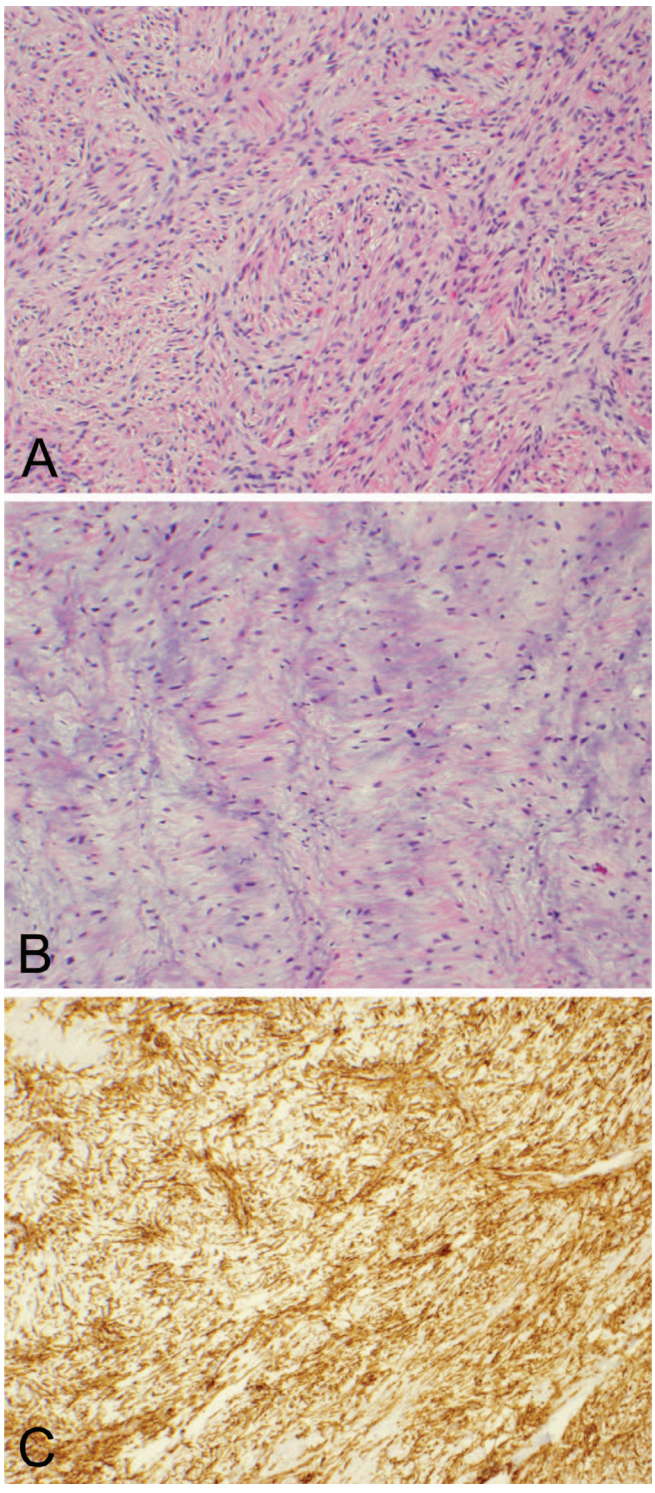

Figure 9. *Low-grade fibromyxoid sarcoma (LGFMS). A and B, LGFMS exhibits alternating fibrous and myxoid areas and mucin 4 (MUC4) expression (C) (hematoxylin-eosin, original magnification ×100 [A and B]; original magnification ×100 [C]).*

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	*GNAS* 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) *MYH9-USP6* fusion	Nodular fasciitis
	Spindle	Alternating fibrous and myxoid areas	MUC4 t(7;16)(q33;p11) *FUS-CREB3L2* 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) *FUS-DDITS* fusion t(12;22)(q13;q12) *EWSR1-DDITS* fusion	Myxoid liposarcoma
	Round	Cells arranged in cords, chains, or small clusters	S100 (~20%) CD117 (30%) *EWSR1-NR4A3* 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) *TGFBR3-MGEA5* 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,

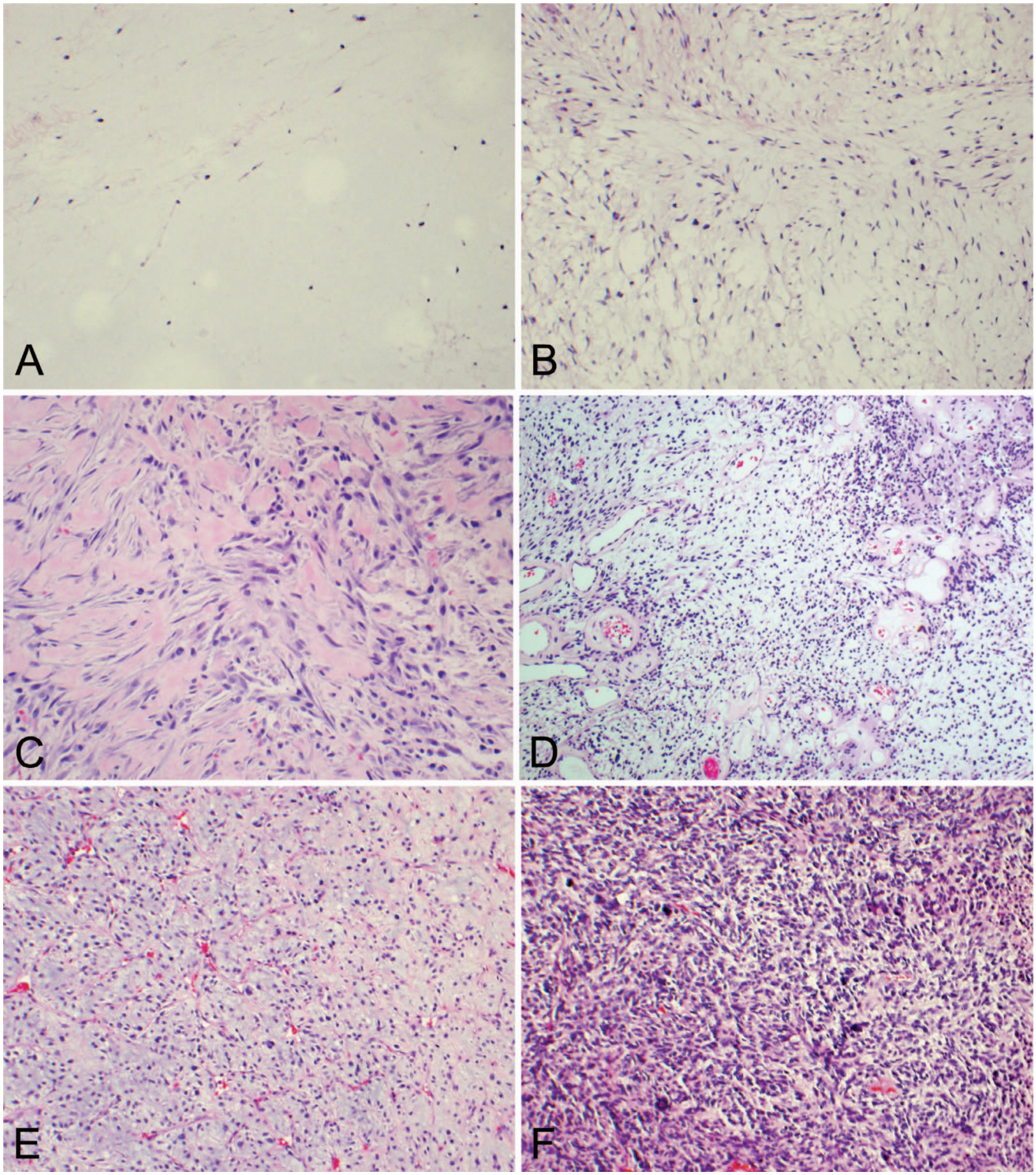

Figure 10. *Selected myxoid tumors: myxoma (A), cellular myxoma (B), nodular fasciitis (C), schwannoma (D), low-grade myxofibrosarcoma (E), and high-grade myxofibrosarcoma (F) (hematoxylin-eosin, original magnification ×100 [A through F]).*

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

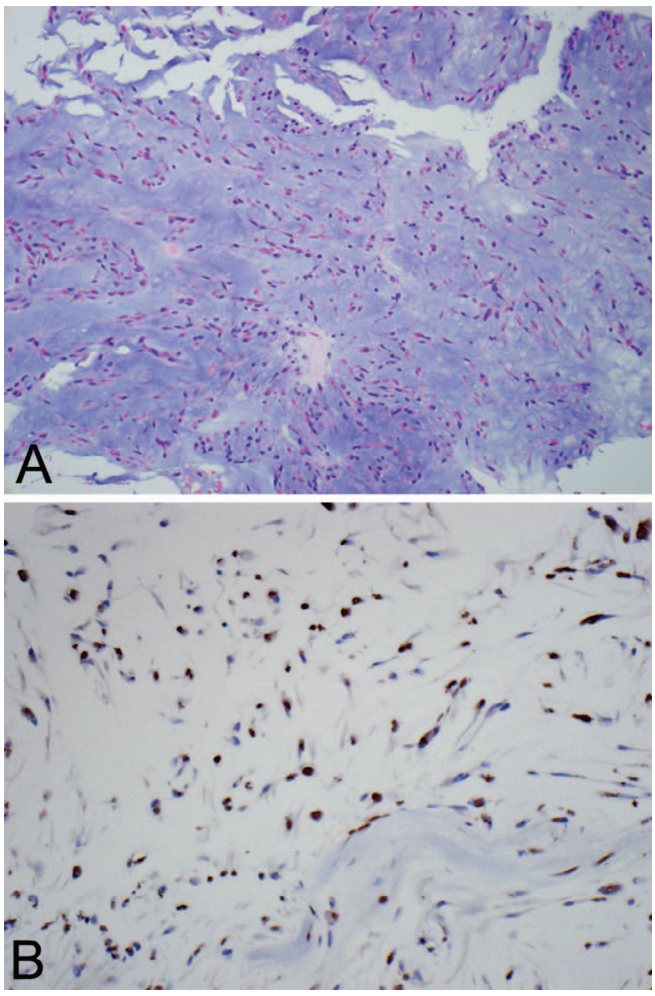

Figure 11. *Extraskeletal myxoid chondrosarcoma. A, Tumor shows cords and chains of small, uniform cells with round to oval nuclei and abundant chondromyxoid matrix. B, The lesional cells may occasionally express cytokeratin (hematoxylin-eosin, original magnification ×200 [A]; original magnification ×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 study[73] has demonstrated that NKX2.2, a homeodomain-containing transcription factor that plays a critical role in neuroendocrine/glial 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 cytomorphology (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

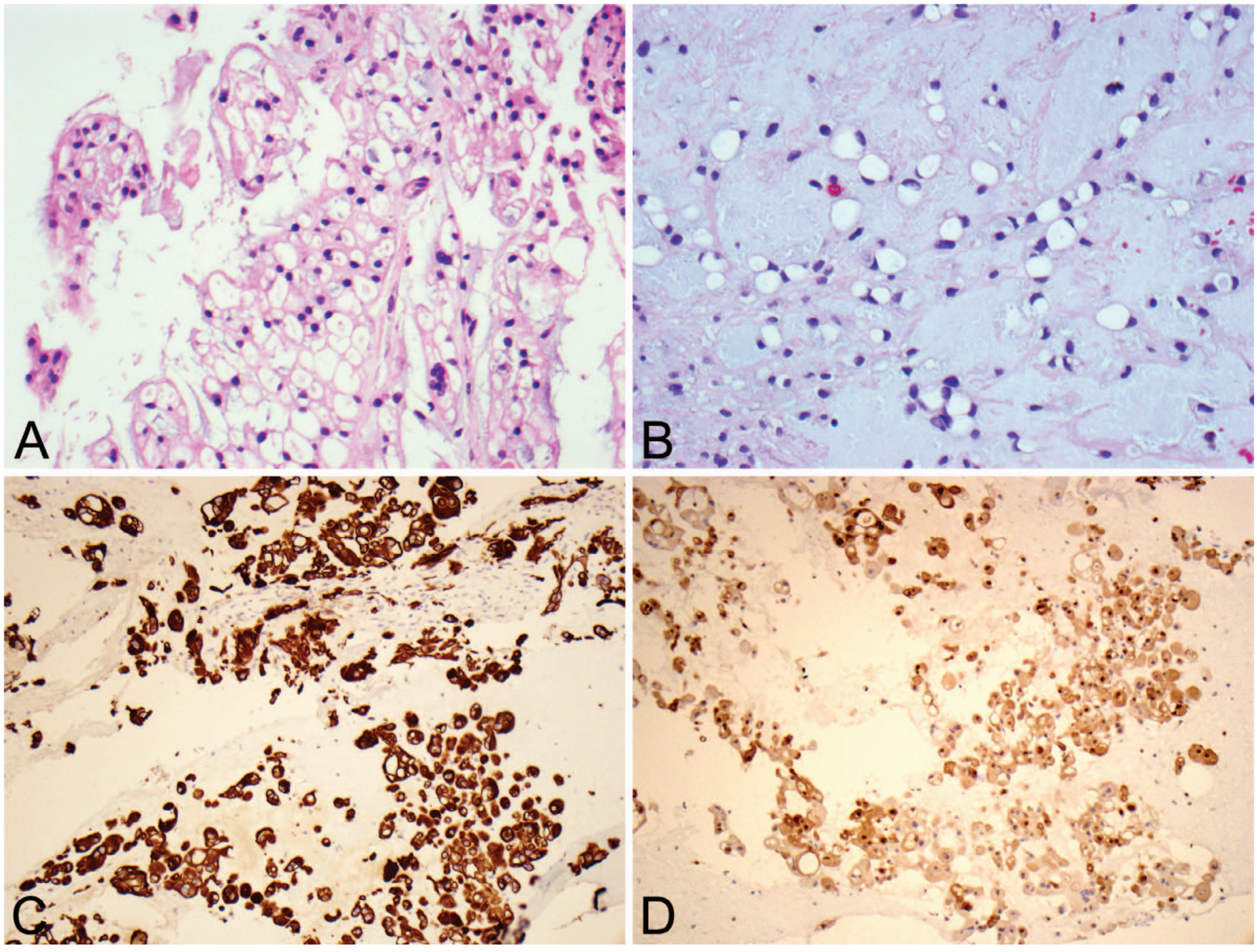

Figure 12. *Chordoma. A, This tumor shows characteristic "physaliferous cells." The stroma may less commonly have a chondromyxoid appearance (chondroid chordoma) (B). The tumor cells are typically immunoreactive for cytokeratin (C) and S100 protein (D) (hematoxylin-eosin, original magnification ×200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

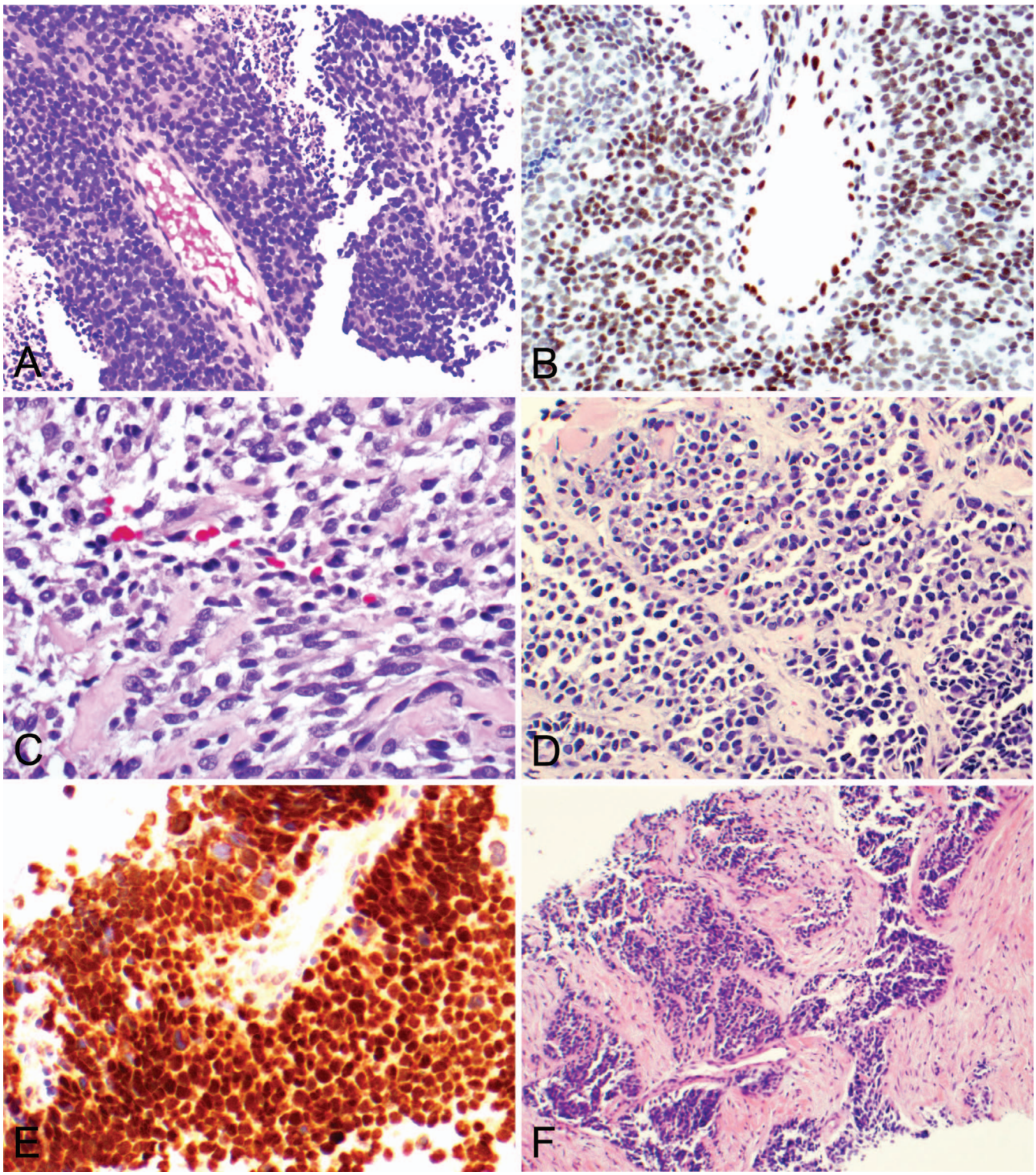

Figure 13. *Selected round cell tumors. A, Ewing sarcoma consists of solid sheets of small, blue, round cells with geographic necrosis and nuclear expression of Friend leukemia integration 1 transcription factor (FLI1) (B). C, Embryonal rhabdomyosarcoma demonstrates primitive mesenchymal cells with round 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 ×200 [A and D], ×400 [C], and ×100 [F]; original magnifications ×200 [B] and ×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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Application of Immunohistochemistry to Soft Tissue Neoplasms

Josefine Heim-Hall, MD; Sophia L. Yohe, MD

• ***Context.*—Soft tissue tumors are composed of numerous and complex diagnostic entities. Because of this complexity and the recognition of an intermediate malignancy category including some tumors with a deceptively bland histologic appearance, soft tissue tumors may represent a major diagnostic challenge to the general practicing pathologist.**

***Objective.*—To correctly diagnose soft tissue tumors with the ancillary use of immunohistochemistry.**

***Data Sources.*—Review of the current literature with emphasis on those tumors for which immunohistochemistry has proven to be particularly useful.**

***Conclusions.*—Immunohistochemistry plays an important role in the diagnosis of soft tissue tumors. One of its major utilities is to correctly identify a tumor as being of mesenchymal or nonmesenchymal origin. Once mesenchymal origin has been established, histologic subtyping according to specific cell lineage may be achieved with the use of lineage-specific markers. Tumors of uncertain cell lineage and tumors with primitive small round cell morphology are often characterized by a unique immunohistochemical phenotype. In this group of tumors, immunohistochemistry is most widely applied and is of greatest value. Despite the rapid development of molecular genetic techniques, immunohistochemistry still remains the most important diagnostic tool in the diagnosis of soft tissue tumors aside from recognition of morphologic features and clinical correlation.**

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Soft tissue tumors (STTs) are composed of a complex group of diagnostic entities. Most STTs are of connective tissue (mesenchymal) origin. Nonmesenchymal STTs, that is, those of hematolymphoid origin, are not covered in this article.

Given the overall rarity of many of these lesions and an ever-growing list of new diagnostic entities, the general pathologist may be faced with a major diagnostic challenge when dealing with these lesions. The usual approach to soft tumor classification is by presumed cell lineage. The World Health Organization classification, for example, divides tumors into adipocytic, fibroblastic/myofibroblastic, so-called fibrohistiocytic, smooth muscle, pericytic, skeletal muscle, vascular, chondro-osseous, and lastly ''of uncertain differentiation'' category.[1]

Immunohistochemistry (IHC) plays an important role in STT diagnosis. The first approach consists in ruling out a nonmesenchymal tumor, followed by trying to define mesenchymal cell lineage. This approach, achieved with a panel of commonly used antibodies, helps narrow down the differential to a more manageable level. In addition, there are specific tumor types requiring a more refined set of immunohistochemical antibodies. Unfortunately, there is also a substantial number of diagnostic entities in which IHC is of limited or no use. It is important to mention that molecular genetics of STTs has developed at a rapid pace in recent years and has helped in gaining insight into pathogenesis and histogenesis. The more current textbooks including the current World Health Organization edition on tumors of soft tissue and bone reserve specific paragraphs to include the most recent cytogenetic and molecular data. However, many of these tumors have been detected in a small number of cases and confirmatory tests are not yet commercially available. Their diagnostic utility is therefore limited with the exception of several fluorescence in situ hybridization probes, which are being used with increasing frequency, in particular for the diagnosis of synovial sarcoma, Ewing/primitive neuroectodermal tumor (PNET), and alveolar rhabdomyosarcoma (ARMS). It is hoped that detection of tumor-specific alterations and validation through genetic analysis on larger samples will lead to development of new IHC antibodies. These new markers can detect tumor-specific fusion proteins that are either overexpressed or aberrantly expressed as a result of a translocation. Examples of such antibodies are ALK-1, WT-1, and FLI-1.

Given the bewildering number of STTs and likewise continuously growing list of IHC antibodies used in STT diagnosis, this article concentrates on pathologic entities as broad categories and discusses the applicability of IHC (or lack thereof) instead of providing a detailed discussion of individual antibodies. Emphasis is placed on specific tumor entities for which IHC has proven to be particularly useful.

ADIPOCYTIC TUMORS

Hematoxylin-eosin (H&E) morphology and clinical correlation are still the most helpful aids in the diagnosis of

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Table 1. Immunoprofile of Adipocytic Tumors*			
	CD34	MDM2	CDK4
Spindle cell/pleomorphic lipoma	+++	?	?
Well-differentiated liposarcoma	Spindle cells focally +; lipoblasts −	+	+
Dedifferentiated liposarcoma	+/−	+	+

* +++ indicates strongly positive in more than 95% of tumors; ?, unknown (insufficient data); +, positive in more than 70%; −, negative in more than 70%; and +/−, variable.

fatty neoplasms, whereas IHC plays a relatively minor role in their diagnosis. Normal fat cells are generally positive for S100,[2] but this marker may be lost in neoplastic adipocytes making it unsuitable for determining adipocytic lineage.[3]

Spindle Cell Lipoma/Pleomorphic Lipoma

Spindle cell and pleomorphic lipoma are 2 closely related benign entities with overlapping histologic features. The spindle cell component in these tumors stains strongly with CD34.[4] Well-differentiated liposarcoma (WDLS) on the other hand exhibits only focal positivity with CD34 in spindle cells, whereas lipoblasts are negative. When nonadipocytic spindle cell lesions resembling spindle cell lipoma are a consideration, a negative CD34 result is helpful in ruling out spindle cell lipoma.[5]

WDLS and Dedifferentiated Liposarcoma

Some authors have recommended the use of a panel of 2 immunohistochemical stains, MDM2 and CDK4, to be used in the differential diagnosis of WDLS and dedifferentiated liposarcoma.[6,7] In their studies, most WDLSs expressed both markers, whereas benign lipomatous lesions were generally negative for both. For increased specificity and sensitivity, the authors recommend using these 2 markers in conjunction. They also found that when WDLS dedifferentiates, the high-grade component usually retains MDM2 and CDK4 positivity.[7] However, the specificity of this finding was limited by the fact that up to 19% of nonlipogenic sarcomas also expressed 1 or both of these 2 markers.[6]

Myxoid Liposarcoma

Myxoid liposarcoma/round cell liposarcoma is a genetically distinct subset of liposarcoma having a characteristic translocation involving the *CHOP* gene on chromosome 12. Variant translocations include t(12;16) and t(12;22) and result in production of a TLS-CHOP or EWS-CHOP fusion protein, respectively. Although the corresponding fusion genes can be detected by molecular techniques, a novel IHC antibody to the TLS/EWS-CHOP chimeric oncoproteins has shown promise in being able to detect most genetic variants of myxoid liposarcoma/round cell liposarcoma. This antibody appears to have good sensitivity and can be used on formalin-fixed paraffin-embedded tissues.[8] Further studies will be needed to validate the utility of this antibody (Table 1).

FIBROBLASTIC/MYOFIBROBLASTIC TUMORS

Fibrous STTs are a heterogeneous group of spindle cell proliferations composed of a mixture of fibrocytes, fibroblasts, and myofibroblasts. Some of these tumors have been termed fibrohistiocytic because of a morphologic and functional resemblance of cultured tumor cells to histiocytes and positive staining for CD68. However, CD68 is not specific for histiocytes, and immunophenotypic studies with more specific markers did not confirm monocyte/macrophage derivation. In general, the group of fibrous STTs stains positively with vimentin and variably with muscle markers (smooth muscle actin [SMA], less commonly muscle-specific actin and desmin). Two distinct subsets of fibroblasts can be identified by IHC: myofibroblasts and CD34-positive fibroblasts.[9]

Myofibroblasts are present in reactive lesions and in most fibroblastic neoplasms and display a profile that is intermediate between smooth muscle and fibroblasts. As such, they have some, although less than smooth muscle, actin positivity with variable coexpression of desmin.[10] The subset of CD34-positive fibroblasts is found around blood vessels, in skin adnexa, and in connective tissue septa throughout the body.[11]

Most fibrous tumors are distinguished by their morphology taken in conjunction with clinical features. Immunohistochemistry is not helpful in differentiating benign from malignant fibrous lesions. The main use of IHC in fibrous tumors is to rule out nonfibrous tumors, although in some entities a characteristic staining profile will help in the distinction from other fibrous lesions.

Solitary Fibrous Tumor and Hemangiopericytoma

Originally considered a tumor of pericytic origin, hemangiopericytoma (HPC) of soft tissue is now grouped with solitary fibrous tumor (SFT), a tumor of likely fibroblastic origin. The unifying concept has not been universally accepted, especially for tumors in the central nervous system/meninges[12,13] and the term HPC is still retained in the World Health Organization classification of central nervous system tumors. Arguments in favor of abandoning the term HPC, at least in the soft tissue, are the lack of specificity of HPC-defining histologic features that are shared by many other tumors and the striking overlap of morphologic and immunohistochemical features with SFT.[14] For this reason, the term HPC is now used with decreasing frequency and is often replaced by the term *cellular variant of SFT* or other specific tumor entities with an HPC-like pattern. Although the IHC staining profiles of tumors previously classified as HPC and the more recently recognized SFT differ slightly in the literature, they show considerable overlap and are listed here together for practical purposes. Markers frequently expressed include CD34[15–19] (positive in 44%–95%) and CD99 (positive in 64%–91%).[20,21] Smooth muscle actin is only rarely positive (<15%),[21] and desmin is usually negative.[17,22,23] Endothelial marker CD31 is uniformly negative.[24]

Dermatofibrosarcoma Protuberans

Much work has been done using immunohistochemical stains to differentiate dermatofibroma (DF) and dermatofibrosarcoma protuberans (DFSP) because these entities have overlapping morphologic features. Dermatofibrosarcoma protuberans is generally factor XIIIa negative and CD34 positive, whereas DF shows the opposite staining[25,26]

Table 2. Comparison of Dermatofibroma (DF), Dermatofibrosarcoma Protuberans (DFSP), and Fibrosarcoma Arising in DFSP*

	CD34	Factor XIIIa
DF	−/+	+
DFSP	+	−
Fibrosarcoma in DFSP	+/−	−

* −/+ indicates mostly negative but may show focal staining, especially at the periphery; +, positive in more than 70% of tumors; −, negative in more than 70%; and +/−, variable.

(Table 2; Figure 1, A through F). Occasionally, DF may stain for CD34,[27,28] especially at the periphery of the lesion.[29] CD34 positivity is also helpful in differentiating recurrent DFSP from scar tissue, which is generally CD34 negative.[30] Occasionally DFSP can show dedifferentiation with fibrosarcomatous areas. Several studies have shown partial or complete loss of CD34 staining in these areas.[25,31–33] Other potentially useful markers in the differential diagnosis of DF and DFSP are HMGA1 and HMGA2. Both markers are positive in nearly all cases of DF cases and negative in most DFSPs.[29] A smaller study showed that DF stains with CD44 but not with hyaluronate binding protein, whereas DFSP stains in the reverse manner.[34]

Aggressive Angiomyxoma Versus Angiomyofibroblastoma

Angiomyofibroblastoma and aggressive angiomyxoma are both tumors that occur predominantly in the vulva in women of childbearing age. Differentiating these two is important for prognosis because aggressive angiomyxoma, as the name implies, frequently recurs. Both of these tumors stain for vimentin and estrogen receptor and may stain for progesterone receptor and actin as well.[35,36] Angiomyofibroblastoma is generally strongly desmin positive,[36–38] and aggressive angiomyxomas may be positive although the staining is more variable.[39,40]

Inflammatory Myofibroblastic Tumor

Inflammatory myofibroblastic tumor (IMT) has been known in the literature under a number of designations, including inflammatory pseudotumor, plasma cell granuloma, and inflammatory fibrosarcoma. Although the lungs are overall the most common site, this tumor also occurs in the soft tissues. Inflammatory myofibroblastic tumor is composed of fibroblastic/myofibroblastic spindle cells set against a background of a plasma cell–rich inflammatory infiltrate. The neoplastic nature of IMT has been confirmed by the detection of recurrent clonal aberrations involving chromosome 2p23 similar to the ones found in anaplastic large cell lymphoma. The abnormally expressed protein product of the *ALK* gene can be detected with immunohistochemical stain ALK-1 (or p80) in approximately 50% of IMTs (36%–60%).[41–43] The staining pattern in IMT is usually cytoplasmic, whereas it can be both nuclear and cytoplasmic in anaplastic large cell lymphoma. ALK staining among mesenchymal tumors is not specific for IMT and has been described in malignant peripheral nerve sheath tumor, rhabdomyosarcoma (RMS), leiomyosarcoma (LMS), and malignant fibrous histiocytoma (MFH). On the other hand, benign fibrous lesions entering the differential diagnosis such as nodular fasciitis, des-

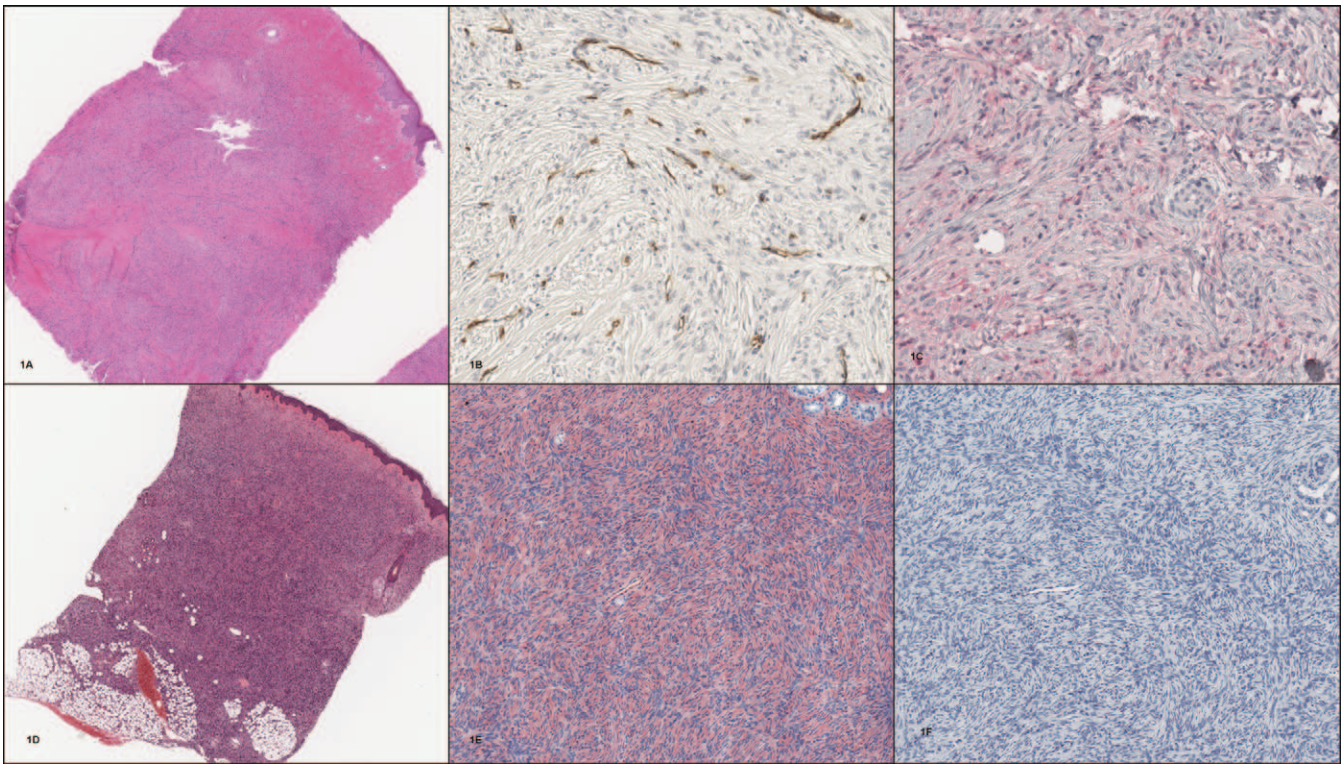

Figure 1. *A comparison of dermatofibroma (DF) and dermatofibrosarcoma protuberans (DFSP) by hematoxylin-eosin, CD34, and factor XIIIa. Dermatofibroma stains for factor XIIIa but not for CD34, whereas DFSP has the opposite pattern. A, Dermatofibroma by hematoxylin-eosin (original magnification ×2). B, CD34 stain of DF (original magnification ×20). C, Factor XIIIa stain of DF (original magnification ×20). D, DFSP by hematoxylin-eosin (original magnification ×2). E, CD34 stain of DFSP (original magnification ×10). F, Factor XIIIa stain of DFSP (original magnification ×10). Courtesy of Thomas L. Davis, MD, South Texas Dermatopathology.*

moid, myofibroma, and leiomyoma are ALK negative.[44] The spindle cells in IMT also stain with vimentin, HHF35, and SMA.[45–47] Less consistent positivity is seen with desmin (~50%)[45,46] and epithelial markers AE1/AE3 and CAM 5.2 (approximately one third).[45,48,49] Skeletal muscle markers MyoD1/myogenin, c-Kit,[46,47,50] and S100[50] are negative.

Calcifying Fibrous Tumor Versus IMT

Calcifying fibrous tumor is a rare tumor of children and young adults composed of fibroblasts, psammomatous dystrophic calcifications, and lymphoplasmacytic infiltrates that can be confused with IMT. Both of these tumors will stain with vimentin; however, in contrast to IMT, calcifying fibrous tumor is both SMA and ALK negative.[51,52]

Mammary-Type Fibroblastoma

This tumor, similar to its counterpart in the breast, has a fairly unique coexpression of CD34 and desmin.[53,54] These stains are therefore useful in differentiating this tumor from SFT (desmin negative) and desmoid fibromatosis (CD34 negative).

Reactive Fibrous Lesions (Nodular Fasciitis, Proliferative Fasciitis/Myositis, Myositis Ossificans, Ischemic Fasciitis)

Immunohistochemistry is of limited value in the diagnosis of these reactive fibroblastic/myofibroblastic proliferations. In addition to uniform vimentin expression, they are frequently and sometimes strongly positive for SMA and HHF35, whereas desmin is usually negative.[55–57] When desmoid tumor is in the differential diagnosis, desmin positivity would favor desmoid over a reactive process. The IHC profile of these reactive lesions is not helpful in ruling out low-grade sarcomas of fibroblastic/myofibroblastic origin, in particular low-grade fibromyxoid sarcoma, fibrosarcoma, and myofibroblastic sarcoma, all of which have a similar staining pattern.

Fibrous Tumors of Infancy (Fibrous Hamartoma, Myofibroma, Inclusion Body Fibromatosis, Infantile Fibrosarcoma)

Fibrous hamartoma of infancy is a benign pediatric tumor with a characteristic triphasic composition including intersecting trabeculae of fibrocollagenous tissue, areas of primitive-appearing mesenchymal cells, and mature fat. All components of the tumor stain with vimentin. Actin and desmin are negative except for focal staining in spindle cells in the fibrous trabeculae.[58–60] S100 is negative except in the adipocytic areas.[61]

Myofibroma/myofibromatosis is a solitary or multicentric benign tumor that occurs predominantly but not exclusively in childhood. It contains spindled myofibroblastic cells arranged around blood vessels and more immature-appearing round cells in a zoned distribution. Both the myofibroblastic and more primitive component of myofibroma stain for vimentin and SMA, whereas only the myofibroblastic component will stain with HHF35. Both components are negative for S100, epithelial membrane antigen (EMA), and keratins.[62,63]

Inclusion body fibromatosis is a rare type of fibromatosis occurring on the digits of infants. It is composed of fascicles of spindle cells containing characteristic intracytoplasmic eosinophilic inclusions. The spindle cells are positive for vimentin and muscle actins. The inclusions stain positive with actins, although results may vary with tissue preparation.[64–67]

Infantile fibrosarcoma is histologically similar to adult fibrosarcoma but carries a much better prognosis. The IHC profile is rather nonspecific with vimentin positivity and variable expression of a number of markers including SMA, HHF35, neuron-specific enolase, desmin, S100, CD34, and cytokeratin (CK). However, staining with MyoD1 and myogenin has not been reported and can be used in the exclusion of RMS.[68–72]

Other Fibromas (Nuchal Fibroma, Gardner Fibroma, Collagenous Fibroma, Calcifying Aponeurotic Fibroma, Fibroma of Tendon Sheath)

Nuchal fibroma, also called nuchal-type fibroma, is a paucicellular lesion composed of thick collagen fibers and typically occurs in the posterior neck. Gardner fibroma is histologically identical to nuchal fibroma and can occur in various superficial or deep soft tissue sites. It occurs in association with Gardner syndrome and may be the first manifestation of the syndrome. Both nuchal and Gardner fibroma stain with CD34, which may be helpful in the differential diagnosis with fibromatoses and reactive fibrous lesions.[73–77] Other specific types of fibromas are collagenous fibroma (desmoplastic fibroblastoma), calcifying aponeurotic fibroma, and fibroma of tendon sheath. They have a nonspecific staining pattern, and IHC is not useful in the differential diagnosis of these entities.[78–80]

Fibromatoses (Desmoid Tumor and Superficial Fibromatosis)

Desmoid tumor frequently stains with SMA and sometimes, but usually only focally, with desmin.[81,82] It is negative for CD34, S100, c-Kit, and epithelial markers. In the differential diagnosis with SFT, CD34 positivity favors SFT over desmoid. Nuclear staining of both superficial and deep fibromatoses with β-catenin has been demonstrated in a limited number of studies and appears useful to differentiate fibromatoses from most sarcomas and other benign fibrous lesions.[83,84] However, it should not be used in the differential diagnosis of fibromatosis with synovial sarcoma or SFTs, as a significant number of these will also stain with β-catenin.[84,85] Superficial fibromatoses (plantar and palmar) have a staining profile similar to desmoid tumor. Immunohistochemistry is usually not needed in their diagnosis.

Low-Grade Fibroblastic/Myofibroblastic Sarcomas (Low-Grade Fibromyxoid, Myxoinflammatory Fibroblastic, and Low-Grade Myofibroblastic Sarcoma)

This group of sarcomas may pose a significant diagnostic challenge because of their relatively bland cytologic features. Unfortunately, their IHC staining profiles are not distinctive and overlap with those benign fibrous lesions.[86,87] The only potential role of IHC is to exclude tumor of distinct nonfibrous cell lineage, that is, a peripheral nerve sheath tumor.

Sclerosing Epithelioid Fibrosarcoma

Sclerosing epithelioid fibrosarcoma is an unusual variant of fibrosarcoma with a distinctive arrangement of epithelioid tumor cells in nests and cords. Despite their epithelioid appearance, tumors cells are only rarely positive for keratins and EMA and are diffusely positive with vi-

Table 3. Initial Immunohistochemical Panel for the Evaluation of Pleomorphic Spindle Cell Neoplasm	
Immunohistochemistry Stain	**Pleomorphic Neoplasm**
Pankeratin positive	Pleomorphic carcinoma
S100 positive	Melanoma
Lymphoid marker positive*	Lymphoma/follicular dendritic tumor
Vimentin positive, keratin negative,† S100 negative,‡ lymphoid negative	Sarcoma

* Leukocyte common antigen, CD20, CD30, CD43, CD21/23.
† Spotty keratin expression may be seen in some sarcomas as aberrant expression.
‡ Focal S100 positivity is seen in some sarcomas including synovial sarcoma and malignant peripheral nerve sheath tumor.

mentin.[88] S100 expression is likewise rare, and CD34, desmin, CD68, and glial fibrillary acidic protein are negative.[89]

Tenosynovial Giant Cell Tumor and Other Giant Cell Tumor of Soft Tissue

Tenosynovial giant cell tumors may be localized (giant cell tumor of tendon sheath and localized intra-articular tumors) or diffuse lesions (intra-articular type, the so-called pigmented villonodular synovitis, and the diffuse extra-articular type). The osteoclast-like giant cells in these lesions coexpress CD68 and CD45, whereas the mononuclear cells are CD68 positive and CD45 negative.[90,91] Desmin-positive dendritic cells may be found in the background in up to 50%.[92]

Giant cell tumor of soft tissue is the soft tissue counterpart of giant cell tumor of bone and is not related to tenosynovial giant cell tumor. The mononuclear cells stain are positive for vimentin and CD68 and focally also for SMA. More rarely, focal S100 and keratin positivity may be found. The giant cells are CD68 positive.[93–95]

Plexiform Fibrohistiocytic Tumor

Plexiform fibrohistiocytic tumor is a rare subcutaneous tumor occurring on the extremities of children or young adults. It is composed of interconnected nodules containing mononuclear spindled fibroblast-like or plump histiocyte-like cells and interspersed multinucleated cells. The cells are positive for vimentin, CD68 (in the multinucleated giant cells and mononuclear histiocyte-like cells),[96,97] and SMA (in the fibroblast-like cells).[97–100]

Pleomorphic Sarcoma Versus MFH

Malignant fibrous histiocytoma as a diagnostic entity has become increasingly disputed. Although MFH was reported to be the most common soft tissue sarcoma in adults older than 40 years and is still listed as such in the current World Health Organization edition of *Pathology and Genetics of Tumours of Soft Tissue and Bone,* it is also said to be, in the same textbook, a ''term which may disappear completely.'' Critics consider MFH a heterogeneous group of pleomorphic tumors for which a better, more specific designation should be sought.[101–104] In addition to careful search of lineage-typical features by H&E morphology, a panel of IHC stains is recommended to help define cell lineage. The following question arises: How far should one go with ancillary studies in trying to determine specific lineage and what are the clinical/prognostic implications? There is little disagreement over the importance of separating pleomorphic sarcoma from nonmesenchymal malignancies because treatment modalities and prognosis will be entirely different. The main considerations are metastatic sarcomatoid carcinoma, metastatic melanoma, and, in some instances, an unusual hematolymphoid tumor with spindle cell features. The initial battery of IHC markers should always include a broad keratin (pankeratin), S100, and vimentin (Table 3). The use of vimentin is mainly to confirm immunoreactivity of the tissue, and positivity is not specific for mesenchymal differentiation. A positive keratin result should open the door to further clinical workup to detect a primary carcinoma elsewhere. Additional IHC stains, including keratin subtypes, may be used to help narrow this search. A positive S100 stain should be followed by additional melanoma markers and the search for a suspicious skin lesion. Some hematolymphoid neoplasms with predominant spindle cell and pleomorphic features may also be confused with a pleomorphic sarcoma, in particular some Hodgkin lymphomas, some large cell lymphomas including anaplastic large cell lymphoma, and follicular dendritic cell tumor. It is important to remember that some of these tumors may be negative for leukocyte common antigen, and the addition to the screening panel of this marker alone may not be sufficient. When a hematolymphoid neoplasm is suspected, a panel including leukocyte common antigen, CD30, CD2, CD43, and CD21 (or CD23) is appropriate to exclude all of these entities.

Once a nonmesenchymal neoplasm has been excluded, the more difficult step follows, which is subcategorizing a pleomorphic sarcoma (Table 4). Recent publications have stressed a significantly worse prognosis of sarcomas with myogenic differentiation, in particular pleomorphic rhabdomyosarcoma (PRMS) and LMS.[104] Dedifferentiated liposarcoma on the other hand has been shown to behave

Table 4. Immunohistochemical Panel to Subtype Pleomorphic Sarcoma*				
	PLS	**PLMS**	**PRMS**	**PS-NOS**
Vimentin	+	+	+	+
MDM2/CDK4	+	−	−	−
SMA	−	++ (diffuse)	− (10% +)	− (may be focally +)
Desmin	−	+ (focal)	++	− (rarely focally +)
Myogenin	−	−	+	−

* PLS indicates pleomorphic liposarcoma; PLMS, pleomorphic leiomyosarcoma; PRMS, pleomorphic rhabdomyosarcoma; PS-NOS, pleomorphic sarcoma not otherwise specified; +, positive in more than 70% of tumors; −, negative in more than 70%; SMA, smooth muscle actin; and ++, positive in more than 95%.

less aggressively than most other pleomorphic sarcomas.[105] In addition to a careful search for a component of WDLS by morphology and thorough sampling, IHC for MDM2 and CDK4 may be helpful in separating dedifferentiated liposarcoma from pleomorphic sarcoma not otherwise specified.[6] The interpretation of positive IHC muscle markers is problematic because diagnostic criteria to define a sarcoma as LMS, RMS, or ''myogenic sarcoma'' are not uniform. If one relaxes criteria too much, any pleomorphic sarcoma with focal SMA positivity becomes a myogenic sarcoma. Focal SMA positivity has long been recognized and allowed in the diagnosis of MFH and should not be used alone as evidence of specific lineage differentiation (Figure 2, A through D). Recommended criteria for the diagnosis of LMS are diffuse SMA positivity, at least focal desmin positivity, and smooth muscle features on H&E.[106] Criteria required for skeletal muscle differentiation are myogenin (or MyoD1) in addition to desmin positivity, supporting H&E morphology, and, in myogenin-negative cases, ultrastructural evidence of skeletal muscle differentiation.[105,107,108]

The decision whether to attempt a more precise subcategorization of a pleomorphic sarcoma will ultimately be driven by clinical relevance. At this time, adult sarcoma protocols do not discriminate between histologic subtypes with the possible exception of PRMS, which may be treated according to an RMS protocol, and LMS for which oncologists often use a specific chemotherapeutic agent. Prognostic data reported in the literature are still being based on differing and somewhat subjective criteria, and prospective studies using unified histologic criteria will be needed to correlate prognosis with histologic subtype. It is expected that prognostically significant molecular genetic differences will eventually resolve this controversy and further guide the use of IHC.

Myxofibrosarcoma

Formerly included under the category of MFH (myxoid MFH), this tumor is now considered a separate entity with more defined histologic and clinical characteristics. It encompasses a range from low- to high-grade tumors. The low-grade variant may be difficult to distinguish from benign myxomatous lesions, whereas high-grade variants show considerable overlap with pleomorphic sarcoma not otherwise specified. The IHC profile is not unique, and focal SMA or mucle-specific actin positivity can be seen just like in pleomorphic sarcoma or so-called MFH. Desmin, S100, keratins, CD68, and factor XIIIa[109] are generally negative.

Gastrointestinal Stromal Tumor

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the gastrointestinal tract. Although not strictly speaking a tumor of soft tissue in the anatomical sense, it is discussed here because of its unique place among mesenchymal tumors and the importance of IHC in diagnosis, treatment, and prognosis. Before the discovery of the *KIT* receptor kinase gene mutations and associated expression of Kit protein detected by IHC, many GISTs were classified as smooth muscle tumors.

Kit (CD117) positivity is seen in the great majority of GISTs (>95%). Given the effectiveness of receptor tyrosine kinase inhibitor imatinib mesylate (Gleevec, Novartis, East Hannover, NJ) in the treatment of GIST, Kit positivity has traditionally been required as a diagnosis-defining feature for enrollment in imatinib-based protocols.

Kit positivity is usually strong, diffuse cytoplasmic with frequent dotlike accentuation of the Golgi zone. Mesenchymal spindle cell tumors in the differential diagnosis with GIST include leiomyoma, LMS, SFT, desmoid tumor, and schwannoma, all of which are Kit negative.[110] Additional tumors with rare, usually just focal Kit positivity include angiosarcoma, Ewing sarcoma, metastatic melanoma, and a few others.[111] CD34 positivity is seen in 60% to 70% of GISTs with relatively higher frequency in GISTs of esophageal and rectal origin and in benign gastric GISTs.[110] CD34 positivity is also observed in 10% to 15% of smooth muscle tumors and occasionally in schwannoma making it a less specific marker. Desmin is useful in distinguishing GIST from true smooth muscle tumor with only rare GISTs expressing focal desmin (<5%).[112,113] S100 is focally positive in 5% to 10% of GISTs, whereas schwannoma is diffusely positive. Smooth muscle actin is positive in 30% of GISTs[113] and is therefore not helpful in distinguishing GISTs from smooth muscle tumors or other tumors with possible SMA expression (desmoid tumor, SFT). β-Catenin has been used by some investigators to differentiate GIST from desmoid tumor with the former being consistently negative and the latter positive in most cases.[114]

Not all tumors fulfilling the clinical and morphologic criteria of GIST are Kit positive and whether these tumors can be diagnosed as GIST is a matter of debate. This small subset of Kit-negative GISTs makes up about 4%[115,116] and occurs relatively more frequently in the stomach and omentum. Despite Kit negativity, these tumors may respond to imatinib treatment and should therefore not be denied kinase inhibitor therapy.[117] There are several explanations for this apparent discrepancy, which are briefly addressed in the following.

Mutational Analysis of GIST and Response to Treatment

After the first discovery of *KIT*-activating mutations in GIST,[118] numerous studies have further explored the genetic profile of these tumors. *KIT*-activating mutations are found in 85% to 90% of GISTs and may involve different sites within the *KIT* gene (most frequently exon 11). An additional subset, making up about 5% of GISTs, harbors an alternate mutation of a related receptor tyrosinase kinase gene, platelet-derived growth factor receptor α (*PDGFRA*). Lastly, there is a subset of GISTs (5%–10%) lacking mutations in either kinase (so-called wild type). It is important to emphasize that the extent and pattern of Kit staining by IHC does not correlate with the type of *KIT* mutation and does not predict response to treatment. In GISTs lacking demonstrable *KIT* mutations, *KIT* may be nevertheless strongly activated, possibly because of *KIT* mutations that are not readily detected by conventional screening methods or, alternately, because *KIT* is activated by nonmutational mechanisms.[119] It has been shown, however, that imatinib response correlates with the location of mutations on the *KIT* and *PDGFRA* genes (best response with exon 11 mutations), whereas GISTs lacking either mutation are less likely to respond.[120,121] Whether to perform mutational analysis as part of the standard diagnostic workup for patients with GIST is a matter of controversy but at this time does not appear practical. Immunohistochemistry for detection of *PDGFRA* in Kit-negative tumors has been successfully used in several research

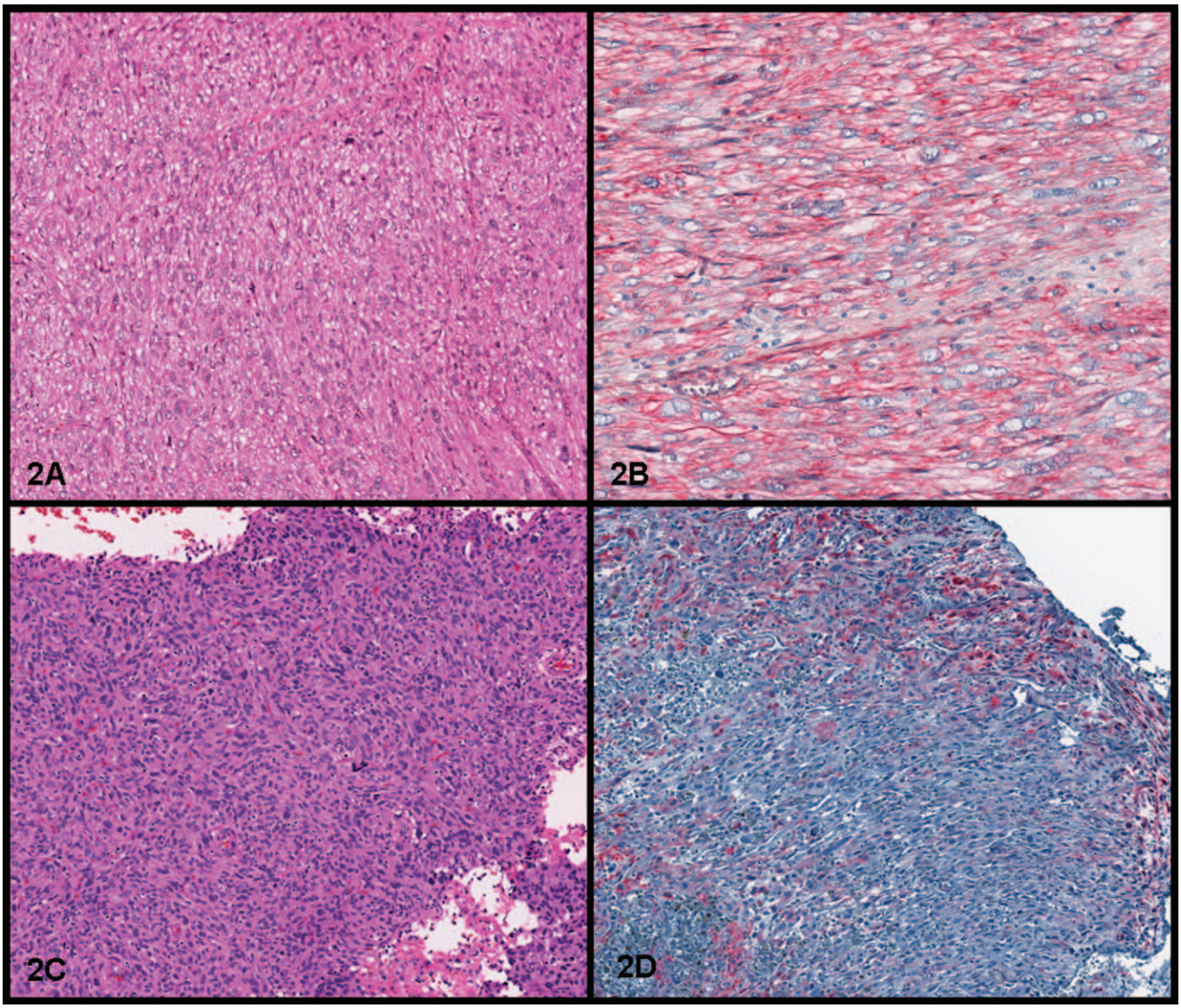

Figure 2. *Although malignant fibrous histiocytomas (MFHs) may show patchy staining with smooth muscle markers, such as smooth muscle actin, leiomyosarcomas will show diffuse staining. A, Leiomyosarcoma by hematoxylin-eosin (original magnification ×10). B, Smooth muscle actin stain of leiomyosarcoma (original magnification ×20). C, MFH by hematoxylin-eosin (original magnification ×10). D, Smooth muscle actin stain of MFH (original magnification ×10).*

studies, but commercially available *PDGFRA* antibody does not yield reproducible results for clinical use at this time.[122]

SMOOTH MUSCLE TUMORS

Leiomyoma and LMS

Leiomyoma and LMS are generally strongly and uniformly positive for SMA and HHF35 (Figure 2, A and B). Smooth muscle actin is more specific for smooth muscle than panactin HHF35. It is usually negative in skeletal muscle tumors in contrast to HHF35, which is frequently positive in these tumors. Desmin positivity varies with leiomyoma being usually at least focally positive, whereas LMS is positive in only 70% to 80% of cases, with less staining in the poorly differentiated examples.[123,124] Calponin, a cytoskeleton-associated actin-binding protein, is frequently used as a myoepithelial marker but is also very useful in detecting smooth muscle differentiation in STTs.[125] In addition to smooth muscle and myoepithelial cells, it is also expressed in myofibroblasts. It is consistently positive in leiomyoma, and it is positive in a high percentage of LMSs (90% in conventional LMS, 70% in pleomorphic LMS).[126] To define a poorly differentiated spindle cell sarcoma as LMS, at least 2 of 3 muscle markers (using for example SMA/desmin/HHF35 or SMA/desmin/calponin) should be positive, and the H&E appearance should be supportive as well. Markers specific for skeletal muscle differentiation (myoglobin, myogenin) are consistently negative in LMS. Keratins and EMA are positive in 10% to 30% of LMSs.[127,128]

Tumors with myofibroblastic differentiation also stain with SMA and HHF35, at least focally, whereas desmin staining is less commonly observed. This may lead to potential confusion of myofibroblast-rich lesions, that is, desmoid fibromatosis with smooth muscle tumors. A less uniform staining pattern and recognition of appropriate mor-

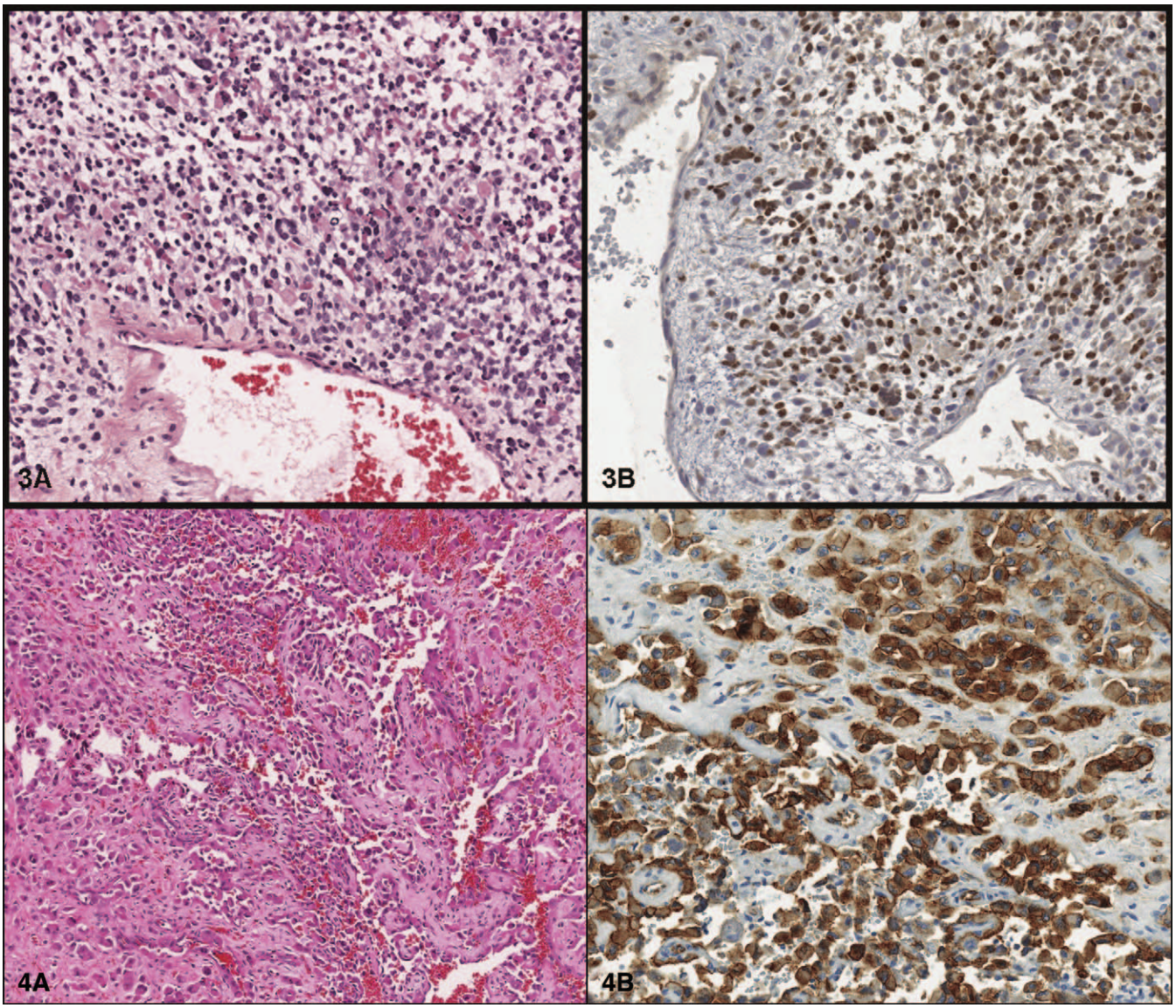

Figure 3. *Rhabdomyosarcoma (RMS) shows strong nuclear staining for muscle markers, such as MyoD1. A, Embryonal RMS by hematoxylin-eosin (original magnification ×20). B, MyoD1 staining of embryonal RMS (original magnification ×20). Courtesy of Victor A. Saldivar, MD, Christus Santa Rosa Children's Hospital, San Antonio, Tex.*

Figure 4. *Tumors of vascular origin show membranous staining with CD31. A, Angiosarcoma by hematoxylin-eosin (original magnification ×10). B, CD31 staining of angiosarcoma (original magnification ×20).*

phologic and clinical features will be helpful in making this distinction.

SKELETAL MUSCLE TUMORS

Rhabdomyosarcoma

Rhabdomyosarcoma comprises a group of tumors with skeletal muscle differentiation. These tumors are divided into 3 main biologically distinct categories: embryonal rhabdomyosarcoma (ERMS), ARMS, and PRMS. The role of IHC in these tumors is mainly to confirm their skeletal muscle lineage, which may not be apparent on H&E morphology, especially in the more aggressive alveolar and pleomorphic types. The two overall most useful markers in RMS diagnosis are desmin and myogenin.[129]

Desmin is highly sensitive for all tumors with skeletal differentiation[130] but somewhat nonspecific because it may also stain smooth muscle cells and occasionally even myofibroblasts. It is also positive in desmoplastic small round cell tumor, IMT, and alveolar soft part sarcoma and should therefore never be used as the sole marker to diagnose RMS.[131–133]

MyoD1 and myogenin are very specific for skeletal muscle differentiation with only rare reports of nuclear staining in nonrhabdomyosarcomatous tumors.[134] These antibodies react with nuclear transcription factors expressed early in skeletal muscle differentiation. Only nuclear staining should be considered as a positive result in both MyoD1 and myogenin (Figure 3, A and B). Myogenin is easier to use and more reliable than MyoD1 because of frequent cytoplasmic staining[134] and a tendency by the latter to fade.[135] Neither MyoD1 nor myogenin should be used on B5-fixed sections.[135]

Although all types of RMS express these nuclear markers, there are relative differences in the amount and inten-

sity of staining. The most uniform and strongest staining pattern is seen in the alveolar type, including its solid variant. Staining is more patchy in ERMS, with islands of intensely positive cells alternating with fascicles of totally negative cells.[135,136] Immunohistochemistry staining pattern is not reliable in subtyping RMS. Genetic markers are required for this distinction, in particular of ERMS from ARMS, given that these 2 types may have overlapping or mixed morphology.

Smooth muscle actin positivity is seen in a minority (approximately 10%) of RMSs.[132,137] Aberrant marker expression for CK, S100, and neurofilament is rare.[138,139]

Pleomorphic rhabdomyosarcoma is positive for desmin[140] but less consistently for MyoD1 and myogenin (~50%). In MyoD1/myogenin–negative tumors, other features of skeletal muscle differentiation (H&E morphology, ultrastructure) are needed to classify a pleomorphic sarcoma as PRMS.[107,108]

VASCULAR TUMORS

Immunohistochemistry is very useful in determining vascular lineage, especially in poorly differentiated angiosarcoma and vascular tumors mimicking epithelial neoplasms (epithelioid hemangioendothelioma and epithelioid angiosarcoma). Commonly used vascular markers are factor VIII, CD34, CD31, and FLI-1 (Figure 4, A and B). Although factor VIII is highly specific for endothelium, it is less sensitive than CD31 and more difficult to interpret because of its presence in serum, which can result in high background staining in necrotic and hemorrhagic tissues.[9] CD34, the hematopoietic progenitor cell antigen, is only positive in 50% of angiosarcoma.[141–144] The most sensitive marker for angiosarcoma is CD31, which is seen as a membranous staining pattern in more than 90% of cases.[141,142] Specificity is excellent with only rare reports of CD31-positive carcinomas.[145] FLI-1 is a more recently introduced endothelial marker, which is also used in the diagnosis of Ewing/PNET (see later). Its specificity and sensitivity approach 100% when used in the differential diagnosis of vascular tumors.[146] Other FLI-1–positive tumors (Ewing/PNET and lymphomas) are morphologically quite distinct and are generally not confused with these vascular tumors.

TUMORS OF UNCERTAIN DIFFERENTIATION

These tumors comprise a heterogeneous group for which no definite histogenesis has been elucidated. Nevertheless, IHC is helpful in the diagnosis of many of these tumors, the most important ones being discussed in the following. Special emphasis is given to the immunohistochemical workup of Ewing sarcoma/PNET versus other primitive-appearing small round cell tumors.

Synovial Sarcoma

Synovial sarcoma is a unique soft tissue sarcoma showing both mesenchymal and epithelial differentiation. Despite its name, it is neither related to nor arising from synovial cells. Immunohistochemistry is particularly useful in the diagnosis of the monophasic spindle cell variant, which is more difficult to distinguish from other spindle cell sarcomas on morphologic grounds alone (Table 5). Vimentin is positive in both the epithelial and mesenchymal component.

Epithelial membrane antigen is the most sensitive marker to detect the epithelial component.[147,148] A number of keratins including AE1/AE3 cocktail, CK7, CK8, CK18, and CK19[149] stain the epithelial component in the biphasic tumors. Monophasic tumors stain only focally and not as consistently with these keratins. The use of both EMA and CK7 appears to yield the best chance of detecting epithelial differentiation in synovial sarcoma.[150] Other commonly positive epithelial markers are BerEp4 and E-cadherin. Calretinin staining is found in about 70%.[151]

S100 positivity is seen in 30% of synovial sarcomas.[152] It is therefore not helpful in distinguishing it from malignant peripheral nerve sheath tumor. CD34 is only rarely positive, whereas SMA and desmin are usually negative. The spindle cells in synovial sarcoma are usually positive for Bcl-2, a marker of limited usefulness given its lack of specificity.[153] Another frequently positive stain is CD99, either in a strong membranous or cytoplasmic staining pattern.[154]

Poorly differentiated synovial sarcomas are less consistently positive for CKs (40%–50%), whereas they still retain EMA expression in 90%. In such cases, cytogenetic or molecular techniques to detect the characteristic *SYT-SSX* fusion are needed to arrive at the correct diagnosis.

Table 5. Differential Diagnosis of Nonpleomorphic Spindle Cell Sarcoma*

	S100	Desmin	Actins†	EMA	HMB-45
SS	−/+	−	−	+	−
MPNST	+/(−)	−	−	+	−
LMS	−	+	+	−/(+)	−
FS	−	−	−/(+)	−	−
CCS	+	−	−	−	+

* EMA indicates epithelial membrane antigen; SS, synovial sarcoma; −/+, positive in 30% of tumors; +, positive in more than 70%; −, negative in more than 70%; MPNST, malignant peripheral nerve sheath tumor; +/(−), between 50% and 70% positive, often weakly; LMS, leiomyosarcoma; −/(+), positive in up to 30%; FS, fibrosarcoma; and CCS, clear cell sarcoma.

† Various actin stains include smooth muscle actin, muscle-specific actin, and HHF35.

Clear Cell Sarcoma of Soft Tissue

Despite their similar IHC staining profiles, clear cell sarcoma of soft tissue is clinically and genetically distinct from cutaneous melanoma.[155] Like the latter, it is positive for melanocytic markers S100, HMB-45, and less consistently for melan (Mart-1).[156–158] Other positive markers include neuron-specific enolase, CD57, and vimentin. Variable results are seen with c-Kit (CD117).[159] Negative markers include keratins, EMA, SMA, and desmin. Although melanocytic stains are helpful in separating clear cell sarcoma from other soft tissue sarcomas, they cannot distinguish clear cell sarcoma from cutaneous melanoma. This distinction is based on tumor morphology, location, or genetic confirmation of the *EWSR1-ATF* fusion gene, which has been found in clear cell sarcoma but never in cutaneous melanoma.[160]

Epithelioid Sarcoma

Epithelioid sarcoma is a rare soft tissue sarcoma showing epithelial differentiation (Figure 5, A and B). Cytokeratins and EMA are strongly positive in almost all cases, mostly coexpressed with vimentin.[161,162] CD34 is positive in about 50% with a strong membranous staining pattern.[163] When positive, this stain is helpful in excluding carcinoma because carcinomas are almost always CD34

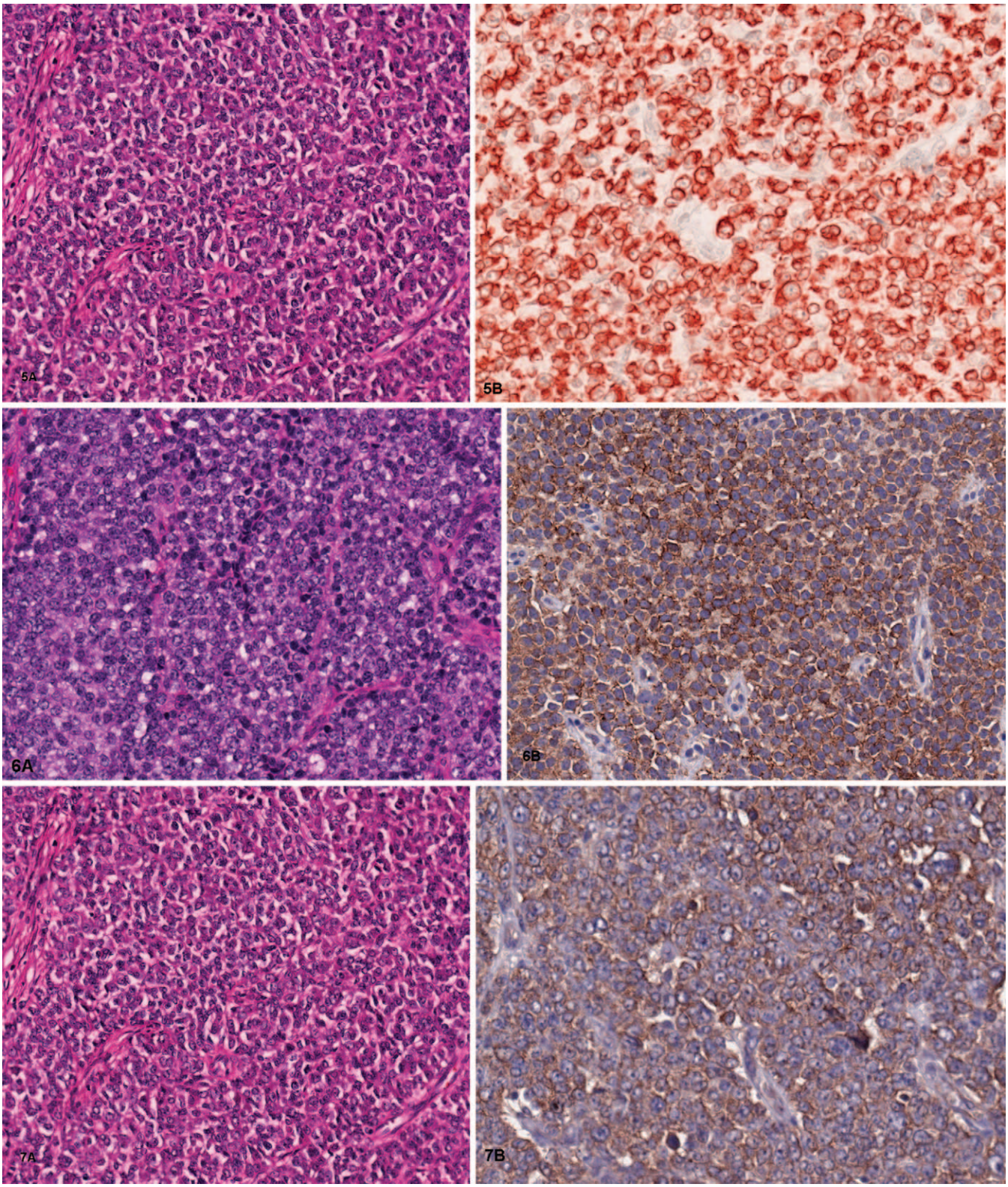

Figure 5. *Epithelioid sarcoma shows epithelial differentiation by immunohistochemistry. A, Epithelioid sarcoma by hematoxylin-eosin (original magnification ×20). B, Pankeratin (AE1/AE3) staining of epithelioid sarcoma (original magnification ×20).*

Figure 6. *Ewing sarcoma shows membranous staining with CD99. A, Ewing sarcoma by hematoxylin-eosin (original magnification ×20). B, Membranous CD99 staining of Ewing sarcoma (original magnification ×40).*

Figure 7. *CD99 staining is not specific for Ewing sarcoma as shown by this case of epithelioid sarcoma. A, Epithelioid sarcoma by hematoxylin-eosin (original magnification ×20). B, Membranous CD99 staining of epithelioid sarcoma (original magnification ×40).*

Table 6. Immunoprofile of Small Round Cell Tumors of Soft Tissue*					
	EWS/PNET	RMS	SS, Round Cell Type	Epithelioid Sarcoma	Lymphoma/Leukemia
Lymphoid markers†	−	−	−	−	+
CD99	+	−	+	−	+/−
FLI-1	+	−	−	−	+/−
CK/EMA	−/(+)	−	+/(−)	++	−
Desmin	−	+	−	−	−
Myogenin	−	+	−	−	−
S100	−/(+)	−	−/+	−	−
CD34	−	−	−	+/−	−

* EWS/PNET indicates Ewing sarcoma/primitive neuroectodermal tumor; RMS, rhabdomyosarcoma; SS, synovial sarcoma; −, negative in more than 70% of tumors; +, positive in more than 70%; +/−, variable; CK/EMA, cytokeratin/epithelial membrane antigen; −/(+), mostly negative but may be positive in up to 30%; +/(−), keratin positive in 40% to 50%, EMA in 90%; ++, strongly positive in more than 70%; and −/+, variable but more often negative.

† Lymphoid markers: terminal deoxyribonucleotide transferase, leukocyte common antigen, CD79a, CD19, and CD3.

negative.[161] S100 and desmin are only rarely positive.[164] E-cadherin is negative in contrast to many carcinomas.[165]

Alveolar Soft Part Sarcoma

This rare tumor containing periodic acid-Schiff–positive crystalline inclusions was thought to be of myogenic origin. Subsequent studies did not confirm this theory and histogenesis remains unclear to this day. A relationship to muscle was theorized because of frequent desmin positivity in alveolar soft part sarcoma. Percentages of positivity vary between studies (~50% on average).[166,167] Smooth muscle actin staining is sometimes also seen, whereas nuclear staining for MyoD1 and myogenin is consistently absent.[133,168] A unique feature of this sarcoma is its weak or even negative staining with vimentin. S100 staining is sometimes present, whereas other neural markers and keratins are negative.[168] In recent years, a characteristic translocation (X;17) resulting in a *ASPL-TPE3* fusion gene has been found in alveolar soft part sarcoma.[169,170] This fusion gene is not exclusive to alveolar soft part sarcoma but is also present in a pediatric variant of renal cell carcinoma.[171,172] A new immunohistochemical stain for TFE3 protein has been developed, which detects the overexpressed TFE3 protein in alveolar soft part sarcoma and in other *ASPL-TFE3* fusion gene–positive tumors.[173]

Ewing Sarcoma/PNET and "Small Round Cell Tumors"

Ewing sarcoma and PNET are now considered members of the Ewing family of tumors. They are highly aggressive primitive round cell tumor of uncertain histogenesis with variable degrees of neural differentiation.

Immunohistochemical stains are negative with most mesenchymal markers with the exception of vimentin.[174] Neural markers S100, CD56, chromogranin, and synaptophysin may be positive but are often only focally or weakly.[175] Cytokeratin positivity is not uncommon (up to 20%) and may be focal or diffuse.[176] The most helpful marker is CD99 (O13, HBA71), which is positive in more than 90% of PNET with a characteristic membranous staining pattern[177,178] (Figure 6, A and B). After the discovery of the Ewing-specific tumor translocations t(11; 22)(q24;q12) and respective *EWS/ETS* fusion oncogene, the immunohistochemical stain FLI-1 has proven to be more specific for Ewing/PNET than CD99 with somewhat inferior sensitivity.[179–181] Specificity is limited by cross reactivity with acute lymphoblastic leukemia, non-Hodgkin lymphoma, and endothelial cells[182] (Figure 7, A and B). Sensitivity is limited by the occurrence of variant translocations that do not involve the *FLI* gene or by low expression of FLI-1. Given the lack of specificity of CD99 and more limited sensitivity of FLI-1, these stains have to be interpreted in the context of a comprehensive panel of immunohistochemical stains aimed at ruling out other tumors with small round cell phenotype (Table 6). Examples of such tumors occurring predominantly in young individuals are leukemia/lymphoma, solid variant of ARMS, mesenchymal chondrosarcoma, desmoplastic round cell tumor, poorly differentiated synovial sarcoma, metastatic neuroblastoma and metastatic Wilms tumor, mesenchymal chondrosarcoma, extrarenal rhabdoid tumor, and proximal type epithelioid sarcoma. In adults, metastatic melanoma and metastatic carcinomas (poorly differentiated, ie, small cell carcinoma) have to be considered as well. A comprehensive IHC panel should include hematolymphoid markers able to identify cells at various stages of lymphocyte differentiation (terminal deoxyribonucleotide transferase, CD79a, CD19, CD3, and leukocyte common antigen), vimentin, muscle markers desmin and myogenin, pankeratin, and S100 with the possible addition of neural markers chromogranin, synaptophysin, and CD57. When metastatic melanoma is a consideration, additional melanocytic markers should be added. Suspicion of metastatic carcinoma is supported by positive CK that can then be followed by the appropriate IHC panel to define the specific type and most likely primary site.

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