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Diagnostic classification of soft tissue malignancies: A review and update from a surgical pathology perspective

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A B S T R A C T

Soft tissue sarcomas encompass a broad spectrum of histologically, clinically, and molecularly diverse neoplasms that present unique diagnostic and therapeutic challenges. Accurate classification is essential both for appropriate risk stratification and for guiding clinical management. Once classified almost exclusively based on the morphologic appearance of the tumor by light microscopy, many soft tissue sarcomas are now known to manifest recurrent patterns of genetic alterations. In addition to enabling molecular confirmation of histologic diagnoses, discovery of these recurrent genetic alterations has helped to refine existing morphologic definitions of sarcoma subtypes and even prompted the discovery of new subtypes. As therapy for sarcoma has become increasingly tailored to a specific entity, the integration of molecular data has assumed added importance in diagnostic decision making. In this article, we summarize principles of the histologic evaluation of soft tissue sarcomas, discuss specific diagnostic features of several of the most common sarcoma subtypes, and describe our vision for a future of soft tissue sarcoma diagnosis that merges morphologic, genetic, and epigenetic features to arrive at diagnoses that are aligned with tumor-specific, biologically targeted treatment approaches.

Introduction

Principles of classification

Soft tissue sarcomas encompass a clinically, histologically, and biologically diverse family of tumors that share a mesenchymal origin. Tumor classification systems play a critical role in pa-

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tient care by providing pathologists with reproducible guidelines to accurately diagnose malignancies. The WHO Classification of Tumors of Soft Tissue and Bone,[1] last updated in 2013, is the internationally-accepted classification system for soft tissue neoplasms and is based on the consensus opinion of expert pathologists. In general, soft tissue neoplasms are classified according to the non-neoplastic cell-type or -lineage they resemble or differentiate toward, such as adipocytes, fibroblasts, smooth muscle, etc. In soft tissue pathology, determining line of differentiation can often be achieved by routine histologic evaluation alone, but in difficult cases ancillary immunohistochemical and molecular studies are exploited. Even with modern molecular approaches, however, some cases defy definitive classification and a specific diagnosis cannot be made. In these circumstances, morphologic descriptors of the predominant neoplastic cell-type, like "spindle," "round," or "epithelioid" (Fig 1), are applied along with an assessment of expected clinical behavior (ie, benign, malignant, or uncertain), and a differential diagnosis is provided, accompanied by recommendations for clinical management.

Grading criteria

While the majority of soft tissue neoplasms are benign with a good prognosis, a subset of tumors (sarcomas) is highly malignant and associated with poor outcomes, highlighting the importance of accurate tumor classification. However, determination of tumor type is not always sufficient to predict a tumor's behavior, as some sarcomas, for example myxofibrosarcomas, exist along a spectrum from low malignant potential to high malignant potential. Grading criteria have been established to predict how aggressive a tumor will behave. The two grading systems used most commonly in soft tissue pathology are the United States National Cancer Institute system,[2] and the French Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) system.[3] In both systems, tumor grade is divided into 3 tiers: low (1), intermediate (2) and high grade (3), based purely on histologic features. The United States National Cancer Institute system defines tumor grade based on histologic type, tumor cellularity, pleomorphism, and mitotic rate. The FNCLCC system derives tumor grade from 3 factors: tumor differentiation, mitotic rate, and tumor necrosis (Fig 2). Tumor differentiation reflects how closely a sarcoma resembles normal non-neoplastic adult mesenchymal tissue; well-differentiated sarcomas receive a score of 1, whereas embryonal-appearing or undifferentiated tumors receive a score of 3. The overall sarcoma grade (1–3) is obtained from the summation of each factor's score. The FNCLCC system is the currently favored grading system for sarcomas, as studies have demonstrated a superior ability to predict overall and metastasis-free survival.[4]

Treatment effect

One caveat of tumor grading is that it applies only to untreated sarcomas. Adjuvant and neoadjuvant therapies can induce profound histologic changes to sarcomas, and tumors in general. For example, radiation therapy, chemotherapy, or embolization can induce tumor necrosis, cell proliferation arrest, tumor cell differentiation and degeneration, and stromal fibrosis and hyalinization.[5] The extent of post-treatment changes is variable and likely depends on a multitude of factors, including therapy type, duration of treatment, and tumor type, among others. Documenting these changes may be important because an extensive histologic response to treatment has been linked to improved survival in some studies.[6-8] However, other studies have failed to reproduce these findings,[5,9,10] and therefore the prognostic value of post-treatment histologic findings remains controversial in the realm of soft tissue sarcomas.

Review of major sarcoma entities

In this section, we outline the principle histologic, immunohistochemical, and genetic features of a variety of malignant, premalignant, or locally aggressive soft tissue neoplasms. This

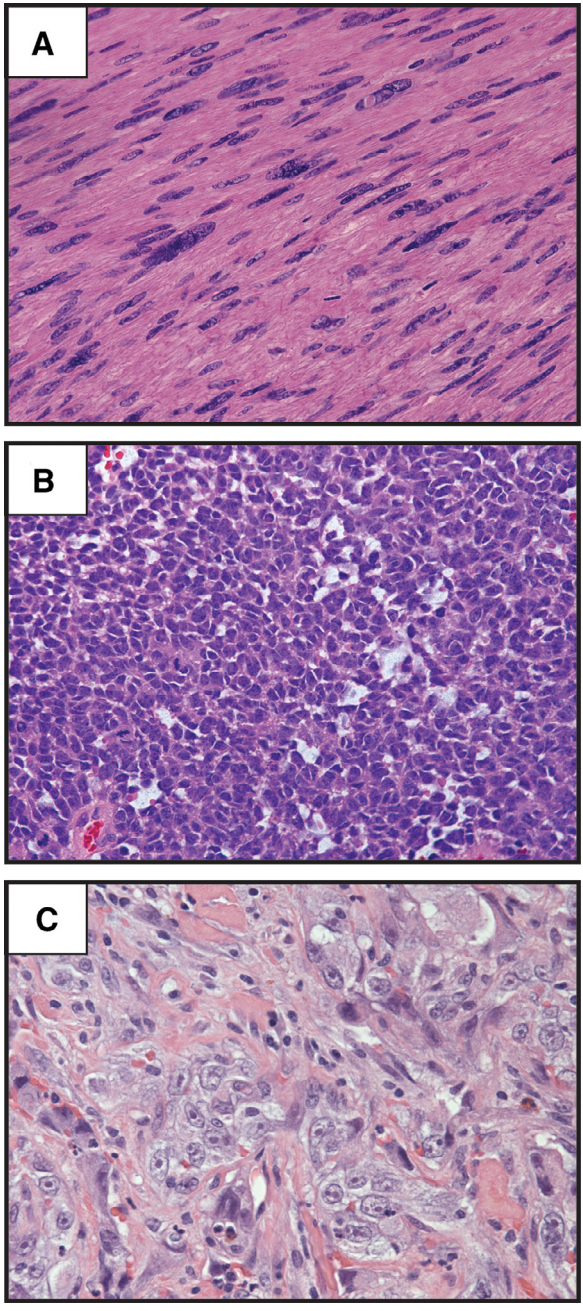

Fig. 1. Morphologic classification of soft tissue sarcoma. Primary morphologic subtypes of soft tissue malignancies include spindle-shaped (A; leiomyosarcoma), round (B; Ewing sarcoma), and epithelioid (C; epithelioid angiosarcoma) (400x, H&E).

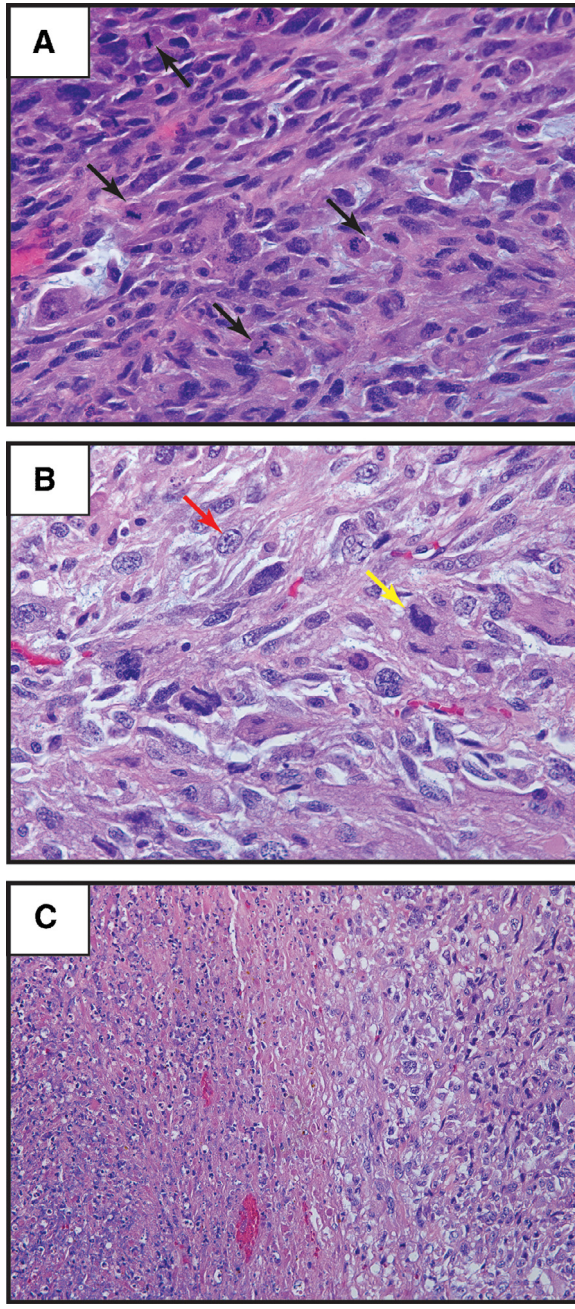

Fig. 2. Histologic features of malignancy in sarcoma. (A) Mitotic activity is a morphologic surrogate of cell proliferation, which can be recognized by the appearance of condensed chromosomes in distinct stages of mitosis (eg, arrows). Increased mitotic activity is generally associated with clinically aggressive/malignant behavior. Quantification of the number of mitotic figures in a defined area of tumor enables comparisons of the rate of proliferation between tumors (400x, H&E). (B) Variation in the size, shape, and chromatin texture of tumor nuclei often, but not always, correlates with higher-grade malignancy. Note that in this example the chromatin in some nuclei is opaque (eg, yellow arrow), while in others it is optically clear with occasional nucleoli (eg, red arrow). When seen in neoplastic cells, the presence of multiple nuclei in a single cell (multinucleation) is also suggestive of malignancy (400x, H&E). (C) Necrosis (left) of neoplastic cells is also a feature of more aggressive behavior, indicating that a tumor is proliferating rapidly enough to outgrow its blood supply. In some tumors, this feature manifests distinctly with viable cells only in the vicinity of blood vessels. (200x, H&E) (Color version of figure is available online).

list is not meant to be exhaustive, but rather serves as a primer for a subset of the most common and biologically interesting mesenchymal tumors.

Tumors of adipocytic differentiation

Well-differentiated liposarcoma

Well-differentiated liposarcoma (WDLS) is a low-grade malignancy of adipocytic differentiation that tends to arise in the deep soft tissue of the extremities and retroperitoneum. The term atypical lipomatous tumor is considered synonymous with WDLS. Historically, atypical lipomatous tumor was used to describe superficial lesions, while WDLS was used for more deep seated retroperitoneal lesions that have a propensity to recur and eventually transform into high-grade dedifferentiated liposarcomas.[11] Histologically, WDLS is characterized by variably sized adipocytes and strands of fibrosis that harbor large atypical stromal cells with hyperchromatic nuclei (Fig 3 A). There are also cells exhibiting irregular intracytoplasmic fat droplets that distort an ordinarily enlarged and pleomorphic nucleus. These so-called lipoblasts, along with the atypical stromal cells, generally distinguish WDLS from benign adipocytic tumors. From a genetic standpoint, WDLS demonstrate characteristic supernumerary "ring" chromosomes and/or giant rod-shaped marker chromosomes that result in amplification of the 12q13-15 chromosomal region.[12] This amplified region harbors the *MDM2* and *CDK4* genes, overexpression of which leads to increased cell proliferation by inhibiting p53 and RB1, respectively. Gene amplification, which is regularly assessed by fluorescence in situ hybridization (FISH) for diagnostic purposes, leads to overexpression of MDM2 and CDK4 proteins, which can be detected in tissue specimens by immunohistochemistry.[13]

Dedifferentiated liposarcoma

Dedifferentiated liposarcoma (DDLS) is a high-grade malignancy of the deep soft tissue and retroperitoneum that can arise *de novo* or by transformation of WDLS. Compared to its well-differentiated counterpart, DDLS is a highly cellular tumor composed of at least mildly pleomorphic cells that bear no resemblance to mature adipocytes (Fig 3B). This neoplasm can exhibit an impressive variety of cytomorphologic features and architectural arrangements, including spindled, epithelioid, fascicular, storiform, and myxoid patterns. In keeping with its high-grade nature, there can be numerous mitoses and areas of necrosis. Often, a component of classic WDLS coexists with the high-grade dedifferentiated component; identification of this WDLS component is an important cue to the classification of otherwise histologically undifferentiated DDLS (Fig 3C). DDLS can also undergo "heterologous differentiation," transformation into a high-grade sarcoma of another cell lineage, such as leiomyosarcoma, rhabdomyosarcoma, osteosarcoma, or chondrosarcoma.[14] While the varied histologic manifestations of DDLS can create diagnostic challenges, detection of *MDM2* and *CDK4* amplification (a feature shared by WDLS and DDLS) can aid in classification.[13]

Myxoid liposarcoma

Myxoid liposarcoma is a translocation-associated neoplasm that accounts for roughly one-third of liposarcomas. Classically, it occurs within the deep soft tissue of the extremities and, unlike WDLS and DDLS, rarely arises in the retroperitoneum. This tumor forms a lobulated mass and has distinctive light blue "myxoid" stroma and a network of delicate branching capillaries that has been compared to chicken wire or poultry netting (Fig 4). Two predominant cytomorphologic types of tumor cells are found: small, bland, spindle-shaped mesenchymal cells, and a variable number of uni- or multivacuolated lipoblasts. Mitotic activity and nuclear pleomorphism are generally inconspicuous. A more uncommon high-grade form of myxoid liposarcoma, descriptively named "round cell liposarcoma," is defined by primitive cytomorphology, absence of

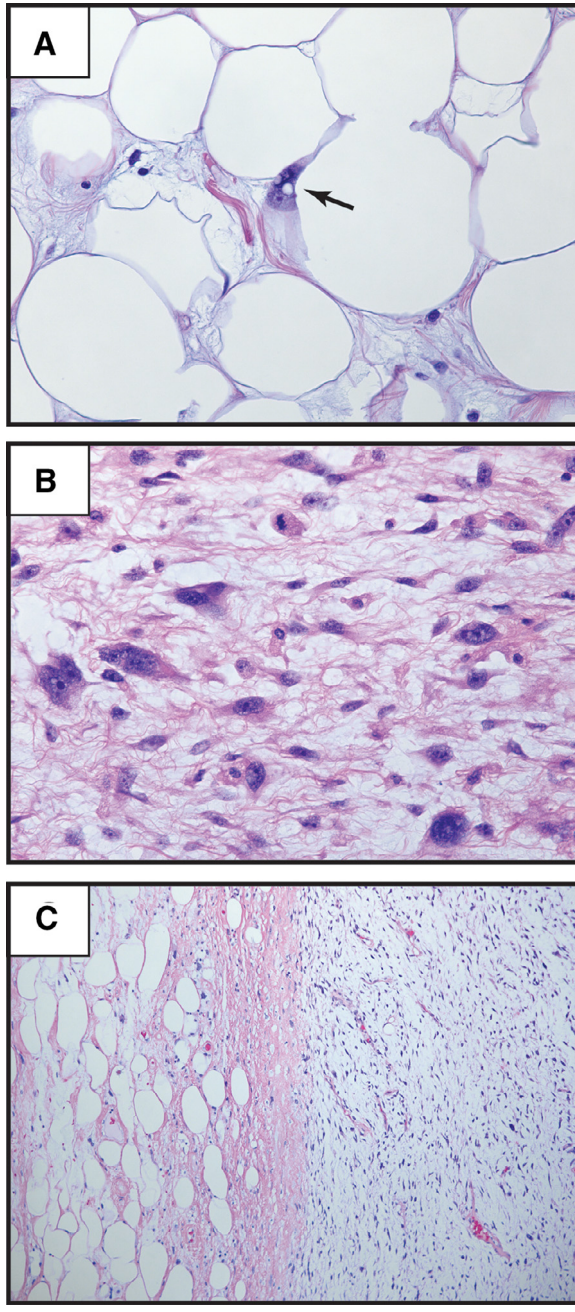

Fig. 3. Well-differentiated and dedifferentiated liposarcoma. (A) Well-differentiated liposarcoma is characterized by predominantly mature-appearing white adipose tissue consisting of fully differentiated adipocytes punctuated by atypical stromal cells (arrow), also known as "smudge" cells, exhibiting enlarged, irregularly shaped, and darkly stained (hyperchromatic) nuclei (400x, H&E). (B) Dedifferentiated liposarcoma is a morphologically high-grade sarcoma lacking mature adipocytes and thus resembling an undifferentiated pleomorphic sarcoma. The lesional cells are often spindle-shaped, mitotically active, and pleomorphic. (400x, H&E). (C) Although not absolutely required for the diagnosis of dedifferentiated liposarcoma, the juxtaposition of well-differentiated liposarcoma (left) with high-grade sarcoma (right), if identified, is essentially diagnostic (200x, H&E).

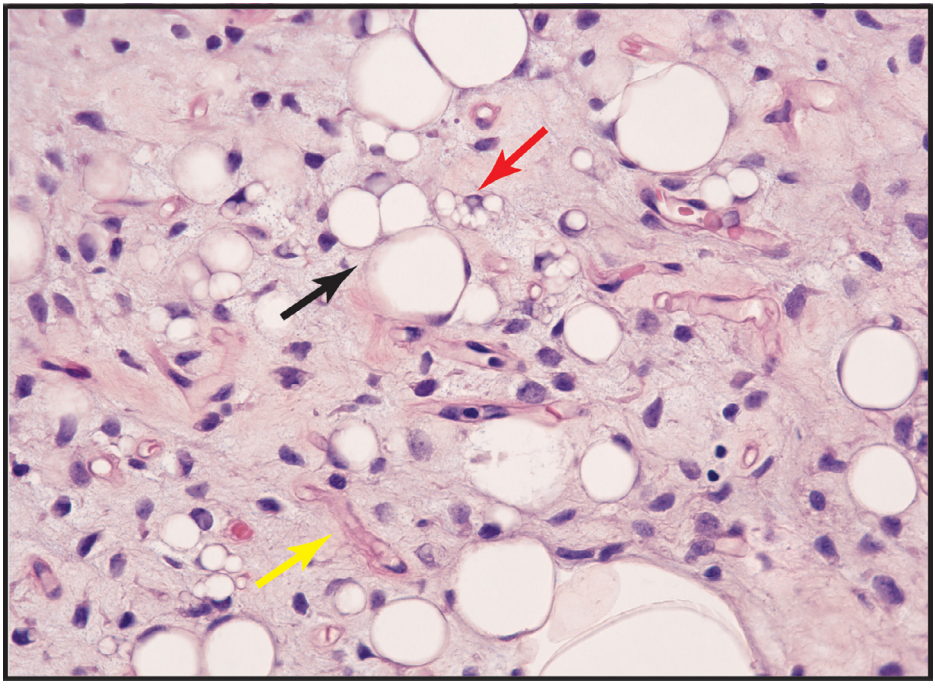

Fig. 4. Myxoid liposarcoma. Myxoid liposarcoma exhibits atypical multivacuolated lipoblasts (red arrow), mature-appearing univacuolated adipocytes (black arrow), and a distinctive network of small caliber branching capillaries in a background of blue-grey appearing myxoid stroma (400x, H&E) (Color version of figure is available online).

lipogenic differentiation, and hypercellularity that obscures the characteristic tumor vasculature. Round cell liposarcoma is thought to represent evolution of myxoid liposarcoma, often presenting as a component of the latter. The histologic identification of a round cell component that comprises more than 5% of an otherwise typical myxoid liposarcoma represents an important threshold for distinguishing a tumor that may carry particular risk for progressive, metastatic disease. Classically, myxoid liposarcomas are associated with a recurrent fusion of *DDIT3* (CHOP) on chromosome 12p13 most often paired with the *FUS* gene on chromosome 16p11.[15] Evidence of this translocation is often used to support the diagnosis of myxoid liposarcoma or its round cell counterpart.

Tumors of myogenic differentiation

Leiomyosarcoma

Leiomyosarcoma is a malignant tumor of smooth muscle that classically arises in the retroperitoneum and proximal extremities with a generally high rate of local recurrence and metastasis. Microscopically, leiomyosarcoma is ordinarily a highly cellular spindle cell neoplasm in which groups of tumor cells are arranged in intersecting bundles, or fascicles. By H&E stain, the cells display dark, cigar-shaped nuclei and bright pink, or eosinophilic, cytoplasm (Fig 1A). Nuclear pleomorphism (even if focal), mitotic activity, and necrosis are common findings that are used to discriminate leiomyosarcoma from its benign counterpart leiomyoma. Occasionally, areas of myxoid degeneration are seen. Immunohistochemical studies can be used to demonstrate expression of smooth muscle differentiation markers, such as desmin, heavy caldesmon, and

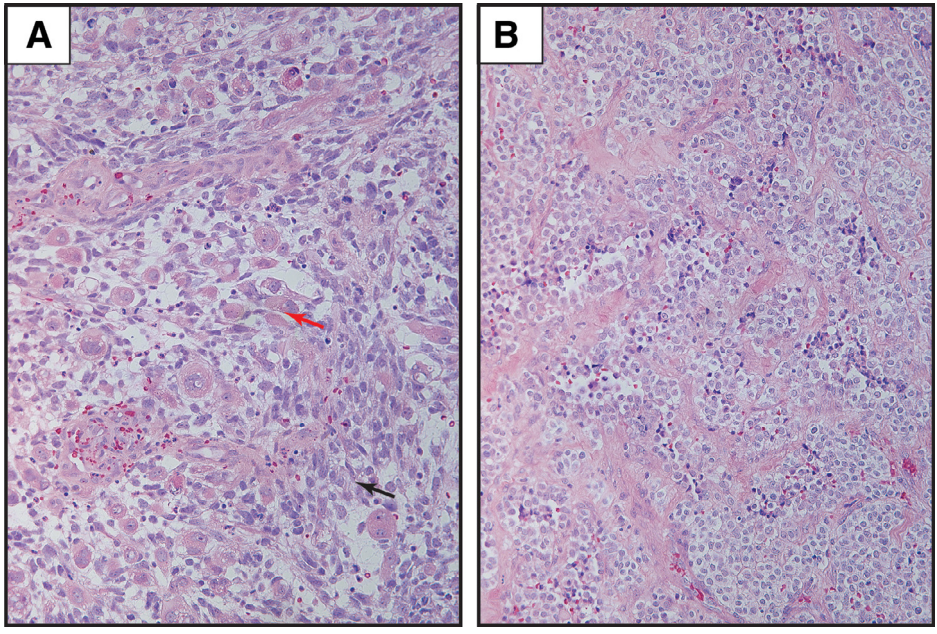

Fig. 5. Rhabdomyosarcoma. (A) Representative example of embryonal rhabdomyosarcoma, which is characterized by a morphologically primitive-appearing neoplastic spindle cell population (eg, black arrow) that recapitulates phases of developmental myogenesis to varying degrees. In this example, there is a prominent component of rhabdomyoblasts (eg, red arrow), neoplastic cells exhibiting features of the skeletal muscle contractile apparatus as a form of aberrant terminal differentiation that reflects the myogenic nature of this malignancy (200x, H&E). (B) Representative example of alveolar rhabdomyosarcoma, which consists of morphologically undifferentiated and discohesive round cells arranged in somewhat ill-defined nests bounded by fibrovascular septa, mimicking the microscopic architecture of alveolated lung parenchyma (200x, H&E). (Color version of figure is available online).

smooth muscle actin.[16] Leiomyosarcomas are genetically complex and lack a consistent structural rearrangement or gene fusion, though *TP53* and *RB1* gene mutations characterize a subset of cases.[17]

Rhabdomyosarcoma

An aggressive malignancy defined by skeletal muscle differentiation, *rhabdomyosarcoma (RMS)* is the most common soft tissue sarcoma in children, but can also occur in adults. RMS arises at any anatomical site, although it is most frequently seen in the head and neck, genitourinary tract, and subfascial tissues of the extremities. Several histologic subtypes of RMS exist with distinct clinical behaviors and prognoses.[18] The current WHO classification recognizes embryonal, alveolar, spindle cell/sclerosing, and pleomorphic subtypes of rhabdomyosarcoma. The following discussion focuses on the embryonal and alveolar variants, more commonly seen in the pediatric population.

Embryonal rhabdomyosarcoma (ERMS) is the most common subtype in young patients, accounting for up to two-thirds of pediatric cases.[19] Histologically, ERMS shows alternating hypercellular and hypocellular, myxoid areas. The majority of tumor cells are primitive-appearing, with round, stellate, or spindle cell morphology and hyperchromatic nuclei. More well-differentiated cells with abundant eosinophilic cytoplasm, called rhabdomyoblasts, are found in varying numbers. In their most differentiated form, rhabdomyoblasts produce intracellular myofibrils with cross-striations, so-called “strap cells,” which are seen in about 50% of cases (Fig 5A).[18] Botryoid embryonal rhabdomyosarcoma is a rare variant of ERMS occurring at mucosal sites, especially the bladder, vagina, and nasopharynx, with a particularly favorable prog-

nosis.[1] In addition to its archetypal location, the cells in botryoid ERMS characteristically form a dense "cambium" layer just under the epithelium. Although the genetics of ERMS is complex and no recurrent fusions have been identified to date, loss of heterozygosity of chromosomal region 11p15.5 is common.[20]

The second most frequent subtype of RMS in children, alveolar rhabdomyosarcoma (ARMS), bears a worse prognosis compared to ERMS. Named for its architectural resemblance to lung alveoli, ARMS is composed of nests or sheets of primitive round tumor cells that disperse into single poorly cohesive cells within the center of pseudo-alveoli (Fig 5B). Variably thick and interconnecting bands of fibrovascular tissue separate tumor cells. Scattered rhabdomyoblasts are often seen, and mitotic activity is abundant. Unlike ERMS, the majority of ARMS are associated with a characteristic reciprocal translocation that fuses *FOXO1* of chromosome 13 with either *PAX3* of chromosome 2 or *PAX7* of chromosome 1.[21,22] Detection of myogenin or MYOD1 expression by immunohistochemistry is critical to establishing skeletal muscle-type differentiation in RMS, but distinguishing ERMS from ARMS can sometimes be histologically challenging. Identifying a *FOXO1* translocation is a reliable means of making this distinction, one which has important prognostic and treatment implications.

RMS in adults most often takes the form of the pleomorphic or spindle cell variants.[23] These present as at least intermediate-grade malignancies located in the extremities or trunk wall. Immunohistochemical evidence of expression of master transcriptional regulators of skeletal muscle differentiation (MYOD1 and myogenin) is again essential in establishing the diagnosis of adult-type RMS.

Tumors of fibroblastic differentiation

Myxofibrosarcoma

Myxofibrosarcoma is a malignant tumor of fibroblastic differentiation that tends to arise in the superficial soft tissue of the extremities, particularly the lower extremities, of older adults.[24] The histologic features of this tumor are variable, but commonly include a multinodular architecture, prominent myxoid stroma, and curvilinear vessels. The tumor comes in 3 grades. In low-grade myxofibrosarcoma, overall cellularity and mitotic activity are low. The nuclei are large and hyperchromatic with cells often contain cytoplasmic vacuoles filled with myxoid material. By comparison, the intermediate-grade tumor is more cellular and has some nuclear pleomorphism. The high-grade form has sheets or fascicles of markedly pleomorphic cells, including bizarre-appearing multinucleated cells, with abundant mitotic activity and areas of necrosis. In line with their pleomorphic nature, myxofibrosarcomas have a complex karyotype and do not harbor any recurrent mutations or gene fusions.[1] Immunohistochemistry is generally not required to establish the diagnosis, but is ordinarily used in cases of myxofibrosarcoma to rule out other high-grade spindle cell malignancies.

Low-grade fibromyxoid sarcoma

Low-grade fibromyxoid sarcoma (LGFMS) is a rare tumor of young-middle age adults that usually arises in deep soft tissue of the proximal extremities and trunk. Classically, it is a rather hypocellular tumor composed of homogeneous-appearing, hyperchromatic fibroblasts within a collagenized and myxoid matrix (Fig 6). High-grade features, such as nuclear pleomorphism, conspicuous mitotic activity, and necrosis, are not seen. Occasionally, tumor cells organize into a rosette-like structure with a central collagen core. Cases in which these rosettes are particularly prominent have been previously classified as "hyalinizing spindle cell tumor with giant rosettes", although this tumor is fundamentally a variant of LGFMS with a prognosis similar to that of histologically ordinary cases.[25] LGFMS is positive for expression of MUC4, which can be detected immunohistochemically.[26] MUC4 is a downstream target of the translocation protein *FUS-CREB3L2* that results from a t(7;16) translocation typical of LGFMS.[27]

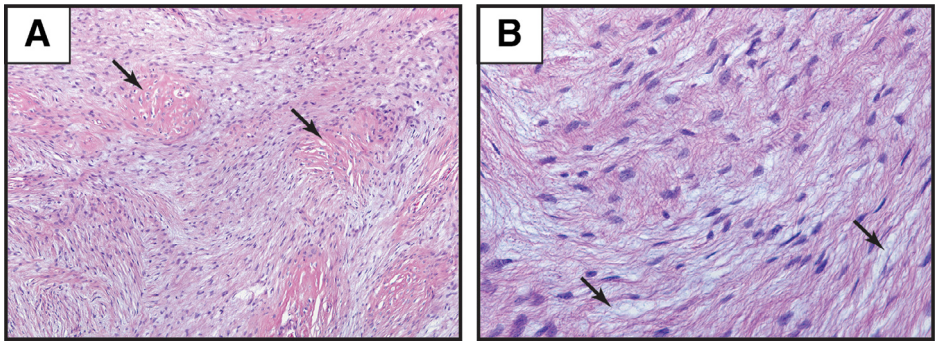

Fig. 6. Low-grade fibromyxoid sarcoma. (A) Vague fascicles of neoplastic spindle cells in a background of bluish myxoid stroma merge with areas of more densely collagenized, pink-appearing stroma that are relatively hypocellular (eg, arrows) (200x, H&E). (B) The tumor cells are characterized by small, round-to-oval nuclei, indistinct cell borders, and rare mitotic activity. The blue-grey hue of the intercellular spaces (eg, arrows) is indicative of the tumor's myxoid quality (400x, H&E). (Color version of figure is available online)

Dermatofibrosarcoma protuberans

Dermatofibrosarcoma protuberans (DFSP) is low-grade fibroblastic sarcoma that presents as a dermal or subcutaneous nodule in young adults, usually on the trunk.[28] As with other translocation-associated sarcomas, DFSP is composed of generally uniform spindle cells. Despite its bland cytology, it infiltrates the dermis and subcutaneous adipose tissue, but does not invade the epidermis. Mitotic figures are not abundant and necrosis is not seen, although DFSP can exhibit evolution to a fibrosarcoma with features of higher grade malignancy. By immunohistochemistry, the tumor is diffusely positive for the nonspecific marker CD34, reflecting its fibroblastic differentiation. DFSP has a recurrent fusion of *collagen type I alpha 1* on chromosome 17 with *platelet-derived growth factor subunit beta* on chromosome 22,[29] accounting for the susceptibility of fibrosarcomatous DFSP to tyrosine kinase inhibitors targeting PDGF receptor beta.[30]

Inflammatory myofibroblastic tumor

Inflammatory myofibroblastic tumor (IMT) is a spindle cell neoplasm of intermediate malignant potential that presents in the lungs, mesentery, and omentum of children and young adults, with a slight female predominance.[31] Histologically, IMT is composed of spindled myofibroblasts, fibroblasts, and a characteristic inflammatory infiltrate of plasma cells, lymphocytes, and eosinophils (Fig 7). The tumor assumes 3 main histologic appearances: loosely arranged spindle cells in an edematous and myxoid stroma, spindle cells in compact fascicles with aggregates of plasma cells, and sparse spindled cells and inflammatory cells in a sclerotic matrix resembling a scar.[31] Mitotic activity and necrosis are unusual. The rare and more aggressive epithelioid variant of IMT has plump, round cells with cherry-red nucleoli and abundant inflammation including neutrophils.[32] IMT can express both smooth muscle actin and desmin, with particularly exuberant expression in the epithelioid variant. More than half of IMTs have an *ALK* gene rearrangement that correlates with ALK protein overexpression by immunohistochemistry.[33] Detection of an *ALK* translocation has therapeutic implications since ALK is targetable by crizotinib in surgically intractable/recurrent cases.[34] The non-ALK rearranged IMTs frequently have fusions involving alternative kinase receptors such as *ROS1, PDGFRB, NTRK1,* and *RET*.[35]

Solitary fibrous tumor

Solitary fibrous tumor (SFT) is a fibroblastic neoplasm of adults with a wide anatomical distribution and a benign clinical course in the majority of cases, although up to a quarter metasta-

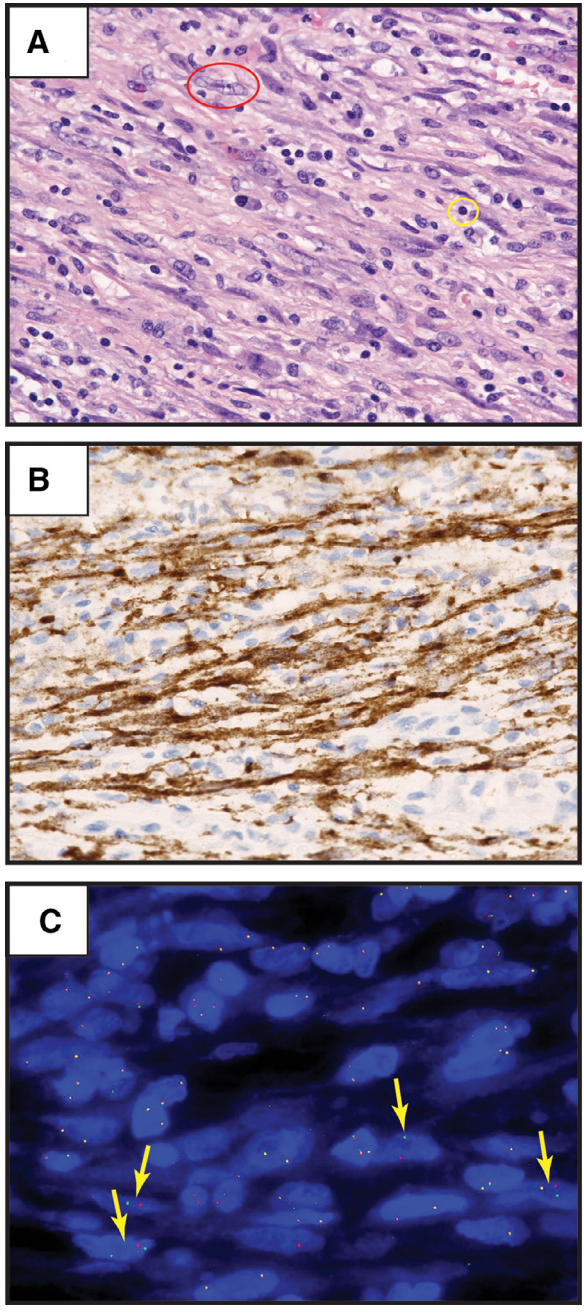

Fig. 7. Inflammatory myofibroblastic tumor. (A) Neoplastic spindle cells with enlarged ovoid nuclei exhibiting irregular nuclear borders (eg, red circle) are obscured by an inflammatory infiltrate consisting predominantly of lymphocytes (eg, yellow circle) (400x, H&E). (B) Anti-ALK immunohistochemistry reveals strong and diffuse expression of ALK in neoplastic spindle cells (400x). (C) Rearrangement of the *ALK* locus is revealed by break-apart FISH probes showing separation of red and green fluorescent signals (arrows). Note that majority of cells in the tumor does not show *ALK* rearrangement, consistent with an abundance of non-neoplastic inflammatory cell infiltrate (600x, DAPI counterstain for nuclei). (Color version of figure is available online).

size. These hypercellular tumors have been described as having a “pattern-less pattern” of bland spindle-shaped fibroblasts with hypocellular and hypercellular zones, set within a collagen-rich stroma (Fig 8). Dilated, branching blood vessels with the appearance of ramifying antlers or horns are a distinctive feature. SFTs can rarely transition into a high-grade de-differentiated sarcoma with anaplastic and heterologous elements.[36,37] No single histologic feature correlates with aggressive/malignant behavior, and even histologically benign SFTs can recur and metastasize. A risk assessment model incorporating patient age, tumor size, mitotic activity, and tumor necrosis provides a reasonably powerful prediction of tumor behavior.[38,39] SFT is characterized by an intrachromosomal inversion of chromosome 12 resulting in fusion of *NAB2* and *STAT6*,[40] which manifests with STAT6 over-expression by immunohistochemistry.[41]

Tumors of vascular differentiation

Kaposi sarcoma

Kaposi sarcoma (KS) is a low-grade vascular neoplasm associated with human herpes virus 8 infection, and is a tumor of the immunocompromised, especially patients with acquired immunodeficiency syndrome (AIDS) or iatrogenic immunosuppression secondary to transplantation. In its cutaneous form, KS has 3 distinct clinical stages: patch, plaque, and tumor. In the patch stage, only subtle dermal changes are appreciated, with increased cellularity around preexisting vessels and adnexal structures. Proliferating tumor cells occasionally protrude into native vessel lumina, a finding termed the “promontory sign.” Plasma cells are often seen in the background. In the plaque stage, the vascular proliferation becomes much more apparent, forming a raised lesion clinically. Proliferative endothelial cells form jagged vessels and at least focally take on a spindle cell morphology. By the tumor stage, a clinical nodule has formed and the tumor is comprised almost exclusively of monomorphic spindle cells. Compared to fully malignant angiosarcoma, the neoplastic cells in KS lack pleomorphism and mitotic figures. Several other histologic variants of KS have been described.[42] This tumor generally expresses markers of vascular endothelial differentiation (ie, CD31, CD34, and ERG); the diagnosis is confirmed by detection of human herpes virus 8 virus latent nuclear antigen

Epithelioid hemangioendothelioma

Hemangioendotheliomas encompass a family of vascular neoplasms with borderline malignant potential, intermediate between benign hemangiomas and clinically aggressive angiosarcomas. *Epithelioid hemangioendothelioma (EHE)* is the most aggressive lesion within this family. EHE can occur almost anywhere, including visceral organs, skin, superficial and deep soft tissue, and bone.[43] About half of cases arise from a large vessel, typically a vein. Histologically, EHE is characterized by small nests, cords, and single infiltrating epithelioid endothelial cells set in a background of light blue, chondromyxoid stroma. Classically, scattered tumor cells display intracytoplasmic vacuoles or lumina, some of which harbor degenerated erythrocytes. These so-called “blister cells” are pathognomonic of EHE and may represent primitive attempts of vessel formation. EHE variably expresses vascular endothelial markers and cytokeratins. The diagnosis can be confirmed either by identifying the *WWTR1-CAMTA1* fusion that is characteristic of EHE or by demonstrating nuclear expression of CAMTA1 protein as a surrogate of the fusion by immunohistochemistry.[44]

Angiosarcoma

Angiosarcoma is a rare vascular tumor with an aggressive clinical course. It classically occurs in the skin and subcutis of patients with a history of radiation or chronic lymphedema, although

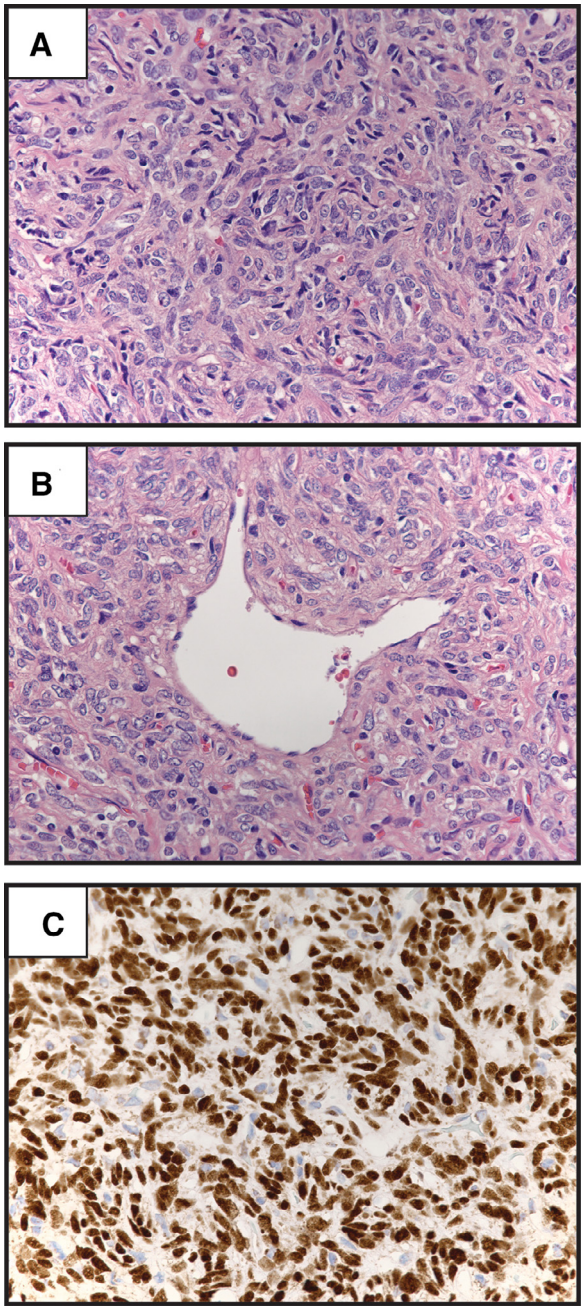

Fig. 8. Solitary fibrous tumor. (A) Solitary fibrous tumor exhibits a haphazard arrangement of neoplastic spindle cells with no discernible architectural pattern (400x, H&E). (B) Diagnosis is aided by identification of thin-walled, dilated blood vessels with antler-like branching (400x, H&E). (C) The *NAB2-STAT6* translocation of solitary fibrous tumor results in an abundance of nuclear-localized fusion protein that can be detected immunohistochemically with anti-STAT6 antibody (400x, anti-STAT6 immunohistochemistry).

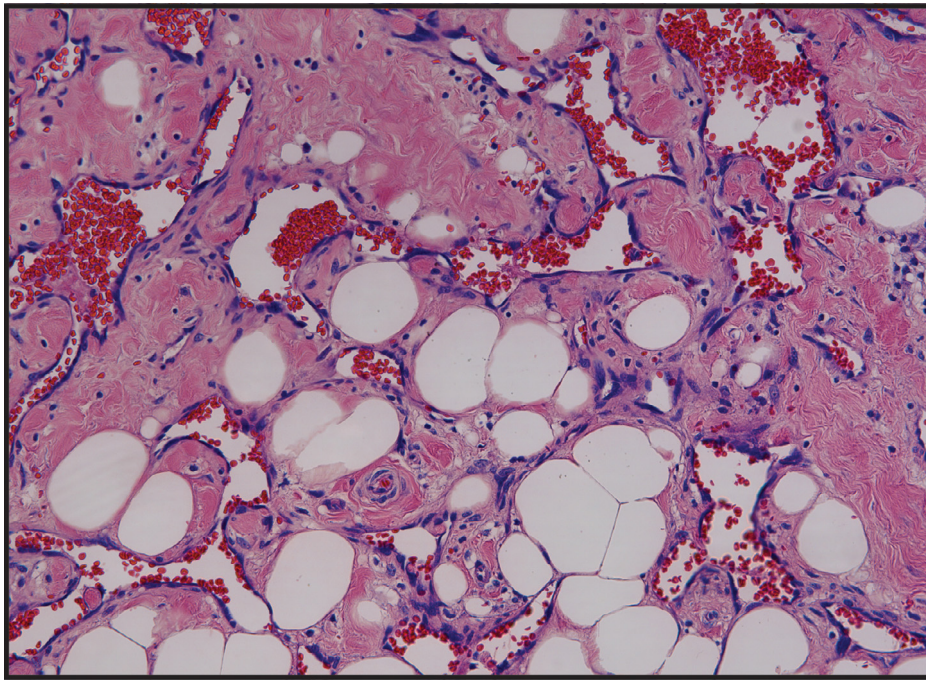

Fig. 9. Well-differentiated angiosarcoma. In this example, irregularly shaped, thin-walled, inter-anastomosing neoplastic blood vessels exhibit infiltration of mature adipocytic tissue toward the bottom of the image. The malignant endothelial cells lining the blood vessels are characterized by enlarged, hyperchromatic nuclei (400x, H&E).

cases certainly can arise spontaneously. This lesion exists on a wide histologic spectrum and, as a result, can be especially challenging to diagnose. On one end, angiosarcomas can mimic a benign hemangioma with only subtle atypical features of nuclear hyperchromasia and infiltrative, asymmetric architecture (Fig 9). On the other end of the spectrum, angiosarcomas can be overtly malignant with marked nuclear atypia, abundant mitotic figures, and sheets of undifferentiated spindle cells or large epithelioid cells (Fig 1C), mimicking the morphology of other poorly differentiated neoplasms and thus requiring immunohistochemistry to prove endothelial differentiation. *MYC* gene amplifications are seen in angiosarcomas associated with radiation and chronic lymphedema, and positive staining for MYC support the diagnosis in this context.[45]

Malignant soft tissue tumors of uncertain histogenesis

Synovial sarcoma

Originally named for its vague morphologic resemblance to synovium, *synovial sarcoma (SS)* is a high-grade malignancy that most commonly arises in the extremities of young adults, but can affect patients of any age at essentially any anatomical site.[1] Despite its name, this tumor is not derived from synovium and its precise cell of origin remains unclear.

Histologically, the tumor is highly cellular and shows 1 of 2 major patterns. In the “monophasic” form (Fig 10A), the cells are uniformly spindled (oval to cigar shaped), or less commonly, epithelioid (polygonal cells with distinct cytoplasm). In the “biphasic” form (Fig 10B), both spindled and epithelioid elements co-exist, the latter of which can form glandular structures reminiscent of epithelial neoplasms. Large gaping blood vessels are seen in the background. By immunohis-

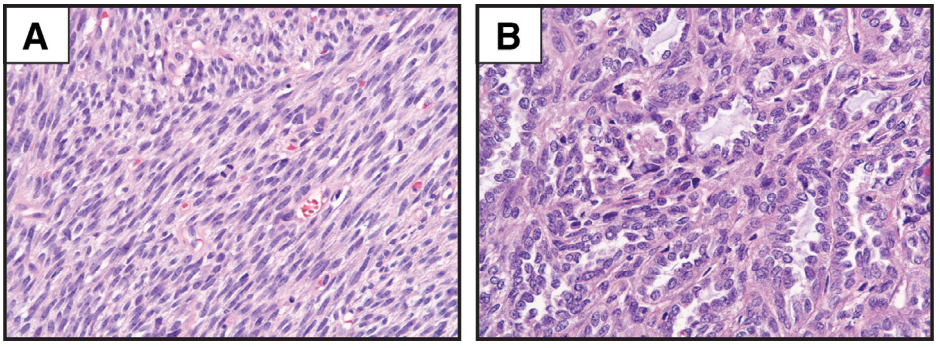

Fig. 10. Synovial sarcoma. (A) Monophasic synovial sarcoma is composed of homogeneous-appearing, mitotically active spindle cells with crowded plump oval nuclei that often overlap (400x, H&E). (B) In addition to the neoplastic spindle cells seen in the monophasic variant, biphasic synovial sarcoma exhibits a subpopulation of morphologically epithelioid cells. In this example, the epithelioid cells line small tubules reminiscent of glandular differentiation as seen in adenocarcinoma (400x, H&E).

tochemistry, the tumor cells usually express cytokeratins (especially in epithelioid areas) and, more specifically, the transcriptional co-repressor TLE1.[46] SS is associated with a translocation between chromosomes X and 18, which fuses portions of *SS18* and *SSX* genes.[47] Identification of the t(X;18) translocation remains the "gold standard" for confirming the diagnosis.

Ewing sarcoma

Ewing sarcoma (ES) is the quintessential "small round blue cell tumor", which aptly describes its histology. This high-grade malignancy is the second most common bone and soft tissue sarcoma in children.[1] As the name suggests, the tumor is comprised of sheets of undifferentiated tumor cells with dark, round nuclei, and scarce cytoplasm (Fig 1B). Two entities previously thought to be biologically distinct and now considered variants of ES are peripheral primitive neuroectodermal tumors and Askin tumor. Peripheral primitive neuroectodermal tumor differs from classic ES only in that it has peculiar wreath-like arrangements of cells termed Homer-Wright rosettes that may represent some degree of neural differentiation. The Askin tumor is simply a Ewing sarcoma that distinctively arises in the chest wall of adolescents.[48]

ES is associated with a recurrent translocation most often between *EWSR1* on chromosome 22 and members of the ETS family of transcription factors, most commonly *FLI1* on chromosome 11.[49] Other less common fusion variants have been identified, involving *FUS, ERG, ETV1*, and *ETV4,* among others.[50] Owing to their undifferentiated histology, Ewing sarcomas are only diagnosed after a battery of ancillary tests are performed. While immunohistochemistry can be helpful to rule out other entities, studies to detect characteristic ES-associated gene rearrangements are often required for definitive classification.

Ewing-like sarcomas

The *Ewing-like sarcomas* are a heterogeneous family of tumors that share morphologic similarities to classic Ewing sarcoma, but lack the defining *EWSR1/FUS-ETS* translocation. Like Ewing sarcomas, these are undifferentiated round cell tumors driven by specific chromosomal translocations. Recent molecular analyses of these tumors have identified recurrent gene translocations involving *CIC* and *BCOR* in many, as well as fusions of *EWSR1*/*FUS* with non-ETS partners.[50]

The majority of Ewing-like sarcomas harbor a fusion between the transcriptional repressor *CIC* and the transcription factor *DUX4.*[51,52] On microscopic examination, *CIC-DUX4* sarcomas have

moderate nuclear pleomorphism, areas of myxoid change, increased mitoses, and necrosis.[53] While detection of the defining rearrangement is necessary for confirmation, immunohistochemical staining for the transcription factors PAX7 and WT1 can support a diagnosis of *CIC-DUX4* sarcoma (PAX7 negative, WT1 positive) vs Ewing sarcoma (PAX7 positive, WT1 negative).[54,55] Recent data suggests a more aggressive clinical course and treatment resistance in patients with *CIC-DUX4* sarcoma compared to classic ES.[56,57]

The second most common group of Ewing-like sarcomas is characterized by *BCOR1* gene rearrangements. Fusions of *BCOR1* with the cyclin dependent kinase *CCNB3* make up the majority of these sarcomas,[58] although several other fusions have been reported.[59] Unlike classic ES, these sarcomas can have cytologically spindle-shaped areas, as well as features more often seen in ES such as myxoid change, necrosis, and high mitotic counts. Immunohistochemistry for CCNB3 supports the diagnosis.[60]

Extraskeletal myxoid chondrosarcoma

Extraskeletal myxoid chondrosarcoma (ESMC) is a rare sarcoma typically arising intramuscularly in the extremities of middle-aged adults. This tumor is characteristically hypocellular and consists of multiple lobules separated by bands of fibrosis. The background matrix is distinctly myxoid and somewhat reminiscent of cartilage. The tumor cells are small, round to oval with a rim of eosinophilic (pink) cytoplasm, and are arranged in single-file cords or small clusters. Focal areas of hemorrhage and necrosis are not uncommon. Occasionally, ESMC can be hypercellular and show epithelioid or rhabdoid differentiation.[61] In the majority of cases, ESMC is associated with a reciprocal translocation event involving *EWSR1* on chromosome 22 and the transcriptional activator gene *NR4A3*, although other rare variant fusions have been reported.[61] The chromatin remodeling protein SMARCB1/INI1, which is also on chromosome 22, is lost in a subset of ESMC cases, especially those with rhabdoid features.[62]

Alveolar soft part sarcoma

A rare malignant sarcoma of unknown histogenesis, *alveolar soft part sarcoma (ASPS)* tends to arise in the lower extremities and head and neck of young children, especially females.[63] In ASPS, tumor cells are large and epithelioid with round nuclei, prominent nucleoli, and characteristic granular, eosinophilic cytoplasm. The cells are arranged in back-to-back nests separated by thin vascular channels. As the name implies, it classically exhibits an alveolar pattern, where centrally-located tumor cells degenerate to form empty spaces resembling lung alveoli. Alveolar soft part sarcoma is associated with a translocation involving *ASPSCR1* on the X chromosome and *TFE3* on chromosome 17. The diagnosis is supported by immunohistochemistry for TFE3 or studies to detect the translocation itself.

Desmoplastic small round cell tumor

Desmoplastic small round cell tumor is a highly aggressive, treatment-resistant sarcoma typically arising in the abdomen of young men.[64] Histologically, the tumor is composed of nest or sheets of undifferentiated small cells set within a prominent desmoplastic or fibrous stroma. The cells are quite mitotically active, and areas of central tumor necrosis are common. *Desmoplastic small round cell tumor* is associated with a recurrent rearrangement of *EWSR1* on chromosome 22 and *WT1* on chromosome 11. One unusual feature of this tumor is that it expresses markers of multiple cell lineages, including epithelial (keratins), mesenchymal (vimentin), myogenic (desmin), and neural (neuron-specific enolase) markers.[65] Immunohistochemistry for the C-terminus of WT1 is positive in most cases.[66]

Epithelioid sarcoma

Epithelioid sarcoma is a soft tissue tumor with a predilection for the hands and wrists of young adults, particularly males. This tumor is classified based on its location: conventional-type for those on the extremities, and proximal-type for axial sites. Microscopically, epithelioid sarcoma is comprised of large, somewhat pleomorphic epithelioid cells with cleared out chromatin, large nucleoli, and abundant eosinophilic cytoplasm. Areas of degeneration and necrosis are frequently encountered, and chronic inflammatory cells surround the tumor. By immunohistochemistry, epithelioid sarcoma expresses cytokeratin, in keeping with epithelial differentiation, along with CD34 in a subset of cases. Loss of protein expression of the chromatin remodeling protein SMARCB1/INI1 is characteristic of this tumor and helps establish the diagnosis when found to be absent immunohistochemically.[67] Epithelioid sarcoma is one of a growing list of SMARCB1/INI-deficiency tumors that are linked to alterations in chromosome 22q.[68]

Malignant rhabdoid tumor

Once thought to be a variant of Wilms tumor, *malignant rhabdoid tumor (MRT)* was initially reported as a highly aggressive kidney tumor occurring in infancy and childhood.[69] It is now known to occur in a wider age range and to arise at virtually any site ("extrarenal rhabdoid tumor"); a form of this tumor even occurs in the central nervous system, where it has been labeled "atypical teratoid/rhabdoid tumor." Histologically, these tumors are characterized by nests of rhabdoid cells – large epithelioid cells with eccentrically-placed nuclei, prominent nucleoli, and abundant eosinophilic cytoplasm. Classically, the tumor cells have perinuclear intracytoplasmic inclusions, corresponding to whorls of intermediate filaments.[70] Since many tumors can have rhabdoid features, ancillary tests are recommended to confirm the diagnosis. MRT is notorious for its multilineage immunophenotype – it can express epithelial, mesenchymal, neural, and neuroectodermal markers.[71] Akin to epithelioid sarcoma, loss of SMARCB1/INI1 is a consistent finding in MRT, secondary to mutations in the *SMCARB1* gene.[72] In fact, MRT was the first tumor in which pathogenic *SMARCB1* alterations were identified.[73]

Other malignant soft tissue tumors

Gastrointestinal stromal tumor

Gastrointestinal stromal tumor (GIST) is a mesenchymal neoplasm of the tubular GI tract, particularly the stomach, small bowel, and colorectum, existing along a broad spectrum of clinical behavior from benign to malignant.[74,75] This is a histologically diverse tumor that can have predominantly spindle- or epithelioid-shaped cells, or a mixture of both (Fig 11). The more common spindle cell variant is characterized by sheets or fascicles of uniform-appearing cells with cigar-shaped nuclei and pale pink cytoplasm. The cells can arrange in side-by-side fashion pointing in the same direction, called palisading, and vacuoles are often seen around nuclei, especially in gastric GISTs. Haphazard packets of coarse collagen fibers, called skeinoid fibers, are seen with some bias for GIST arising in small bowel. By contrast, the less common epithelioid variant has larger round cells, still uniform, with clear to eosinophilic cytoplasm and occasional perinuclear vacuoles. GISTs are believed to originate from the interstitial cell of Cajal, the pacemaker of the autonomic motor system of the GI tract.[76] There are established criteria to predict malignant potential of GISTs, which can help guide therapeutic decision-making. This risk stratification system takes into account mitotic activity, tumor size, and anatomical origin.[77]

The vast majority of GISTs are associated with activating mutations in the tyrosine kinase genes *KIT* or less often *PDGFRA*.[74] The remaining cases have been linked to neurofibromatosis type 1 or succinate dehydrogenase enzyme complex deficiency. The diagnosis of GIST can be supported by demonstrating expression of KIT (CD117) or DOG-1 (*discovered on GIST 1*) by

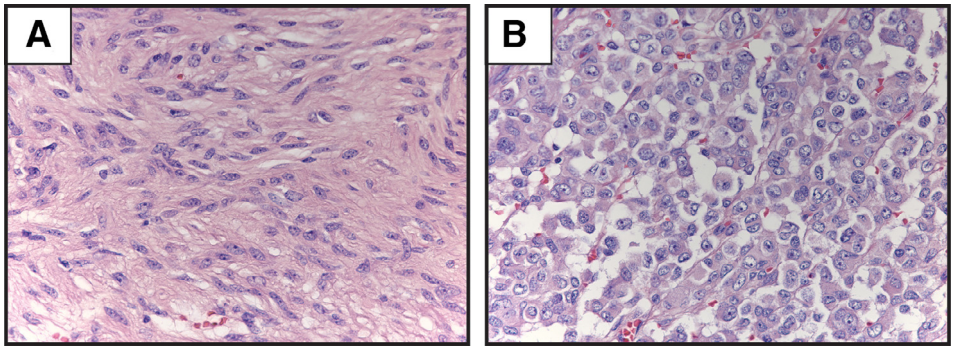

Fig. 11. Gastrointestinal stromal tumor. (A) Gastrointestinal stromal tumors with spindle cell morphology often have lightly eosinophilic, fibrillar cytoplasm, and indistinct cell borders. The cells show some degree of parallel arrangement, giving them a bundled or fascicular appearance (400x, H&E). (B) In epithelioid gastrointestinal stromal tumors, the cell borders are more distinct, the nuclei are more round, and the cells aggregate in sheets, cords, and small clusters (400x, H&E).

immunohistochemistry,[78] reflecting a gene expression signature characteristic of the interstitial cells of Cajal from which GIST is thought to derive, or by direct sequencing of *KIT*/*PDGFRA*.

Malignant peripheral nerve sheath tumor

Malignant peripheral nerve sheath tumor (MPNST) is a high-grade neoplasm that arises from a preexisting nerve or a benign nerve sheath neoplasm such as neurofibroma or less commonly schwannoma. They tend to occur in the proximal extremities of young to middle-aged adults and about 50% of cases are associated with neurofibromatosis type 1. Histologically, the tumor is composed of malignant spindle cells arranged in alternating hypercellular and hypocellular fascicles. Larger, plump tumor cells tend to aggregate near vessels. Nuclear pleomorphism, mitotic activity, and necrosis are common.

These lesions can be a challenge to diagnose if there is no associated benign nerve sheath tumor. Immunohistochemical studies are not always helpful: in one-third of cases, S100/SOX10 exhibit a focal pattern of expression, representing a vestige of nerve sheath-type differentiation.[79] A proportion of cases harbor mutations in *SUZ12*, which encodes a chromatin modifying protein.[80] This mutation leads to downstream aberrations in the enzymatic methylation of histone H3 at lysine 27 (H3K27) and resultant loss of trimethylated H3K27 is seen in about 50% of MPNSTs.

Epithelioid MPNST is a rare, aggressive variant with high metastatic potential.[81] This type is composed of polygonal cells with prominent nucleoli and even rhabdoid features. Unlike classic MPNST, epithelioid MPNST is often strongly and diffusely positive for S100 expression. Similar to other epithelioid mesenchymal neoplasms, a subset of epithelioid MPNSTs are deficient in SMARCB1/INI1 secondary to *SMARCB1* alterations.[82,83]

Undifferentiated pleomorphic sarcoma

Undifferentiated pleomorphic sarcoma is a high-grade sarcoma with no known line of differentiation, and is considered a diagnosis of exclusion. This tumor tends to arise in the deep soft tissue of the extremities in older adults, but can occur anywhere. UPS is believed to represent the final pathway of tumor progression or dedifferentiation from other high-grade sarcomas.[84] The histologic features of UPS can be impressive: markedly pleomorphic spindled to epithelioid cells with bizarre nuclei, abundant mitotic figures including atypical forms and necrosis. Immunohistochemistry is only helpful in ruling out other high-grade malignancies, as UPS characteristically

lacks more than focal expression of lineage-specific markers. Prior studies have pointed to a potential histiocytic etiology,[85] which inspired the historical name "malignant fibrous histiocytoma"; later work debunked this theory and the WHO recommended the current terminology in 2002.[86] As might be expected given the pleomorphic morphology, the molecular genetics of UPS are complex and no recurrent mutations have been identified, although fusions involving *PRDM10* have been reported in up to 5% of cases.[87]

Summary and future directions

Molecular classification of sarcoma

The increasing availability, efficiency, and affordability of high-throughput nucleic acid sequencing techniques has led to a proliferation of information regarding the molecular characteristics of soft tissue sarcomas. With the advent of such information, there has been substantial progress initially toward integrating molecular descriptions of sarcomas with the existing histology-based classification and now toward the application of these molecular classifications in daily diagnostic practice. In the case of some entities, such as SFT, a morphologically well-defined tumor type has been found to associate closely with a pathognomonic translocation involving *NAB2* and *STAT6*. Diagnostic assessment of the genetic translocation (or a surrogate marker of the translocation such as STAT6 overexpression) thus helps to instill confidence in the diagnosis in morphologically ordinary cases, helps to identify histologically aberrant cases that otherwise may have been unclassified or misclassified, and, lastly, helps to exclude morphologic mimics that would have been wrongly diagnosed without knowledge of the underlying molecular lesion. Other entities, such as dedifferentiated liposarcoma, are associated with recurrent genetic abnormalities (ie, *MDM2* amplification) that are not entirely specific for an individual neoplasm. In the case of dedifferentiated liposarcoma, for instance, *MDM2* amplification is also seen in intimal sarcoma, low-grade central type osteosarcoma, and various carcinomas, among other entities. For such tumors with recurrent, but nonspecific, genetic lesions, clearly the molecular features in isolation are insufficient for definitive classification, but remain as valuable diagnostic clues in the context of a more comprehensive histologic, radiographic, and clinical assessment.

Given that molecular alterations are now so closely linked to specific diagnostic entities, it is not surprising that on occasion there is disagreement between classification based on molecular findings and classification according to histologic features. Although these situations often beckon the question of whether to lean more on the histology or on the molecular biology, the answer of course is that an individualized approach is required to weigh the two in conjunction with clinical and radiologic factors *en route* to a final classification. Still, we know that there are certain situations in which either the histologic characteristics or the molecular characteristics ought to carry more weight than the other. An obvious diagnostic dilemma in which the histologic assessment matters most is one where the molecular findings are expected to be similar among the entities under consideration. The distinction between well-differentiated and dedifferentiated liposarcoma, for example, is for now entirely dependent on histology. Conversely, classification of ARMS is one instance in which we know that the identification of a pathogenic translocation (ie, *PAX3/7-FOXO1*) trumps the finding of alveolar morphology. In particular, morphologically alveolar-appearing RMS that lacks the translocation is more similar, both biologically and clinically, to its ERMS counterpart that also has no translocation than it is to the classical translocation-positive alveolar cases.[88,89] Therefore, the classification of pediatric RMS now primarily takes into account the presence or absence of a *PAX3/7-FOXO1* translocation when considering the appropriate diagnosis for treatment planning.

Therapy-centered diagnostics

As diagnostic entities become increasingly aligned with specific therapies designed to target characteristic molecular abnormalities, it stands to reason that there may be additional diag-

nostic deference to the druggable molecular lesion. For instance, immune checkpoint inhibitor therapies have been shown to be effective in many distinct diagnostic entities, reflecting a general susceptibility of tumors to enhanced surveillance by the endogenous immune system. As these therapies tend to work better in tumors with microsatellite instability or with elevated expression of the immunomodulatory protein PD-L1, the characterization of these features that predict therapy response can in some cases become more clinically important than the diagnostic classification itself. In that sense, assays to evaluate predictive markers of therapy response could hold greater value than assays to determine the line of cytodifferentiation, for instance. Eventually, there may even be reason to redistribute diagnostic groupings according to shared therapeutic susceptibilities rather than shared morphologies or presumed tissues of origin. We now know, for example, that fairly rare, but diverse, tumors ranging from secretory carcinoma of the breast to infantile fibrosarcoma are driven by activating mutations of NTRK receptors and that, despite their histologic diversity, these tumors generally can be treated with targeted inhibitors of NTRK. Ultimately, the evolution of a diagnostic framework centered on treatment decisions will depend on the continued growth in our understanding of oncogenic mechanisms and the development of new methods to target these mechanisms.

Epigenetic diagnosis

Although the large majority of advances in the molecular classification of sarcomas to date have derived from traditional cytogenetics and the more recent advent of high-throughput sequencing technologies, molecular characterizations that go beyond the tumor's DNA sequence (hence "epigenetic") have evolved in such a way that they are now being applied to clinical diagnosis. These epigenetic characteristics include patterns of gene expression, modifications of the DNA (ie, methylation), the structural organization of DNA, and modifications of the chromatin scaffold of DNA (ie, histone acetylation). The use of these features for clinical diagnosis has been ushered in by the development of feature-specific techniques to interrogate tumor cells. As an example, chromatin immunoprecipitation coupled with DNA sequencing (ChIP-seq) employs antibodies specific to certain histone modifications to capture fragments of DNA that can then be sequenced. It turns out that the pattern of some types of histone modification, when analyzed genome-wide, can serve as a sort of fingerprint for tumors. The same is true of DNA modifications such as methylation. In fact, DNA methylation signatures can accurately classify the morphologically ambiguous group of "small round blue cell" sarcomas according to their pathognomonic cytogenetic abnormalities.[90] The advent of epigenetic-based diagnostic techniques raises the question of why one might bother to pursue more complicated (and, for now, costly) methods to detect downstream effects of a molecular lesion that could be probed directly. Interestingly, the answer may lie in burgeoning evidence that the signature epigenetic effects of oncogenic DNA mutations or rearrangements could offer a more robust readout than assessing for the mutation or rearrangement itself.[91] The robustness of the epigenetic signature may relate to the fact that it is the shared manifestation of a potentially diverse array of underlying genetic drivers, or to the fact that a multivariable outcome is less susceptible to technical error than assays of single gene alterations. Whatever the reason, clinical assays of epigenetic features are likely to become more common as the cost of those assays decreases over time.

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