Histologic Examination and Ancillary Studies in Soft Tissue Pathology

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2.1 Pathologist Approach to Diagnosis

Soft tissue biopsies are often challenging specimens to diagnose. There is an almost overwhelming amount of entities to consider, many of which are very rare. Moreover, numerous ancillary studies, such as immunohistochemical or molecular tests, are often necessary for appropriate investigation [1].

Visualization of a tumor on an hematoxylin and eosin (H&E) slide remains central to the diagnosis of soft tissue neoplasms. This H&E staining technique, which is over 100 years old, allows the pathologist to examine tumor cells by highlighting nuclei in blue (by hematoxylin) and cytoplasm in red (by eosin).

When viewing an H&E slide, a pathologist assesses the overall architecture at a low power magnification and then analyzes the cytological features of the tumor cells at higher power. Groups of soft tissue tumors manifest characteristic patterns that can be utilized by pathologists in considering diagnostic possibilities. Familiarity with these patterns and associated terminology can lend insight into the diagnostic process [2]. Frequent histologic patterns include spindle cell, epithelioid, round cell, pleomorphic, myxoid, cartilaginous, osseous, and vascular.

Spindle Cell Tumors

One of the most frequent morphologies encountered in soft tissue tumors is a spindled cell pattern, in which the tumor cells exhibit slender and elongated nuclei and cytoplasmic borders. These spindle cells can be arranged in haphazard manner (Figs. 2.1 and 2.2) as seen in nodular fasciitis or placed in organized bundles (often termed a "fascicular" or "herringbone pattern") as seen in malignant peripheral nerve sheath tumor (Fig. 2.3). Finally, the spindle cells can be arranged in a whirling or storiform architecture, as seen in dermatofibrosarcoma protuberans (Fig. 2.4).

Epithelioid Tumors

Although mesenchymal in nature, soft tissue tumor cells can have an epithelioid appearance. Morphologically, these cells have cytoplasmic and nuclear borders that

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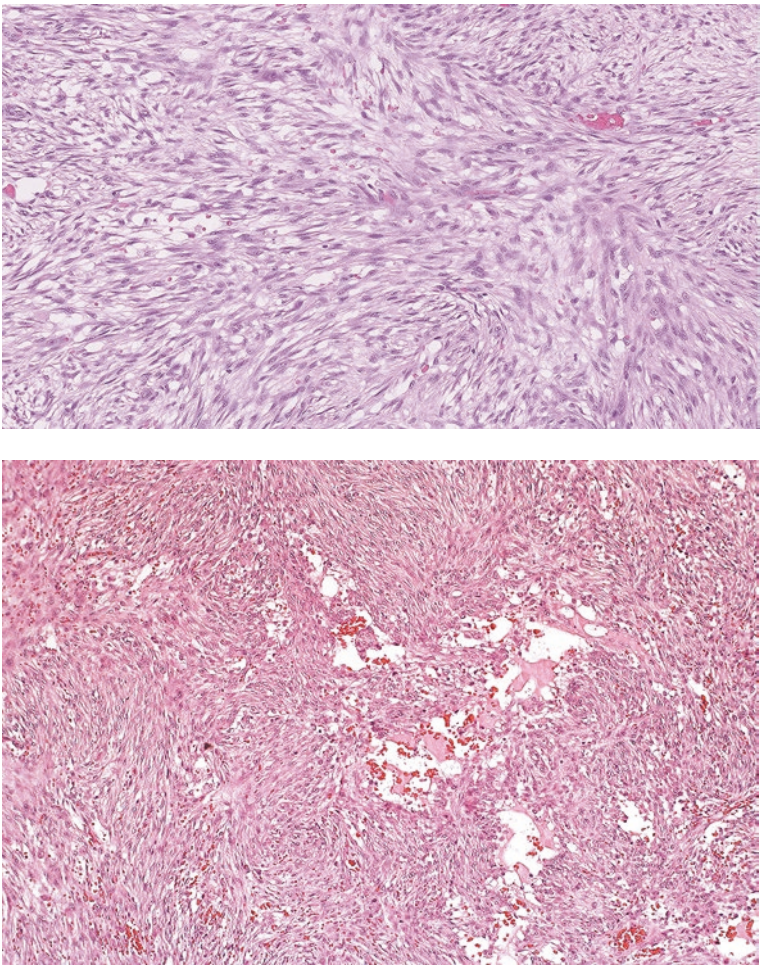

Figs. 2.1 and 2.2 Nodular fasciitis with haphazard arrangement of spindle cells

are round or oval (Fig. 2.5). Examples of epithelioid type soft tissue neoplasms include epithelioid sarcoma, epithelioid hemangioendothelioma, and epithelioid gastrointestinal stromal tumor. Epithelioid-type sarcomas can be mistaken for poorly differentiated carcinomas, particularly if the pathologist is unaware of the anatomic location or clinical history of the lesion.

Round Cell Tumors

Round cell tumors encompass a broad range of soft tissue neoplasms that are made up of cells that have a high nuclear to cytoplasmic ratio, similar to the appearance of a lymphocyte. As hematoxylin will stain the nucleus of a cell violet or blue, round cell neoplasms generally appear blue at low magnification. Examples of round cell

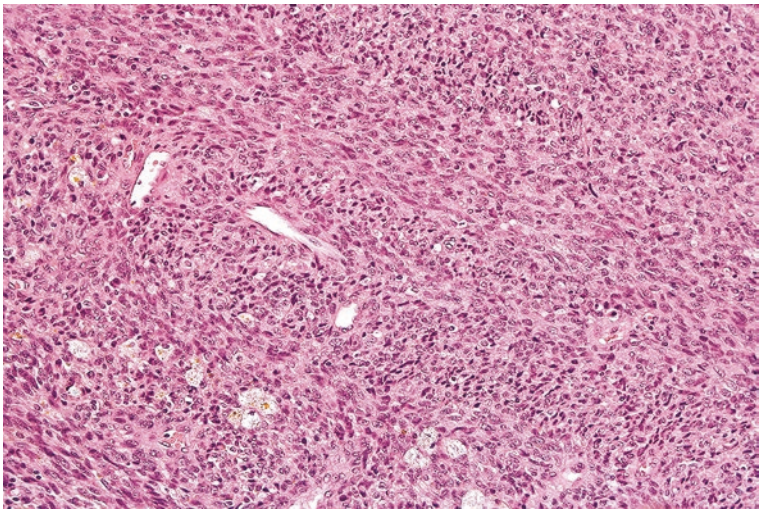

Fig. 2.3 Malignant peripheral nerve sheath tumor with spindle cells that are organized in bundles, often called a fascicular pattern

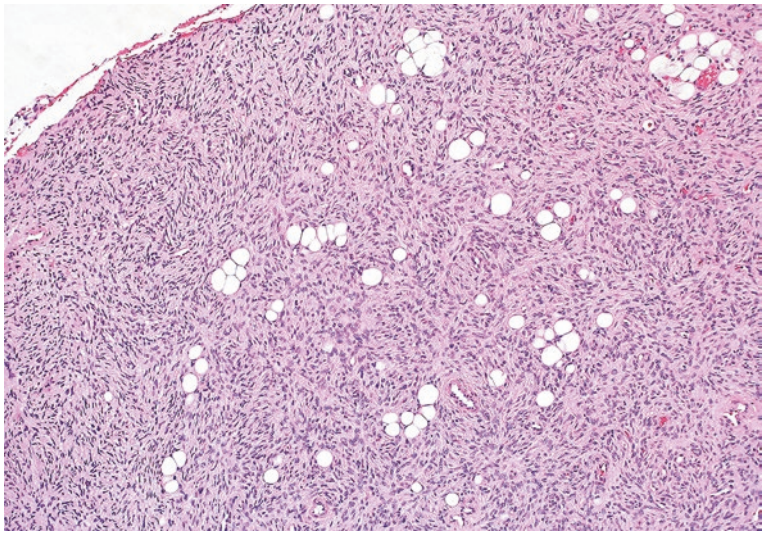

Fig. 2.4 Dermatofibrosarcoma protuberans with spindle cells arranged in a vague whirling or storiform pattern

soft tissue neoplasms include embryonal rhabdomyosarcoma or Ewing sarcoma (Fig. 2.6). Although these neoplasms have substantially overlapping morphologic appearances, many exhibit unique genetic features that facilitate diagnosis. Round cell tumors can also be confused for neuroendocrine carcinomas or lymphomas.

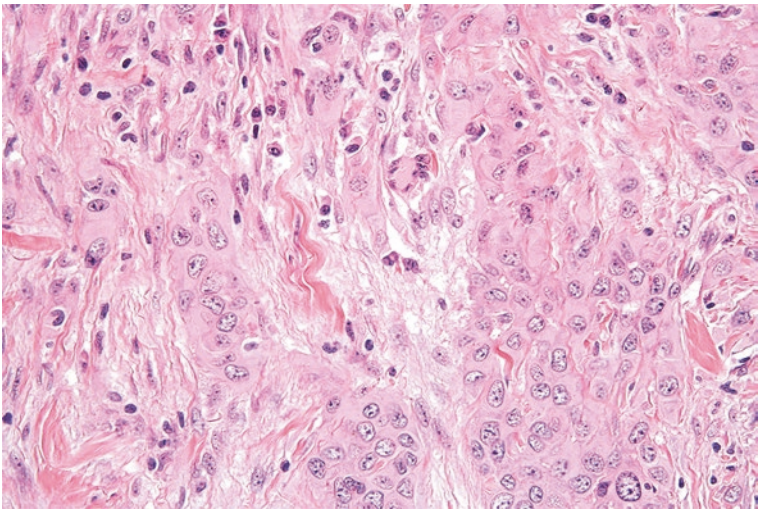

Fig. 2.5 This epithelioid sarcoma contains epithelioid-appearing tumor cells with round to ovoid nuclei

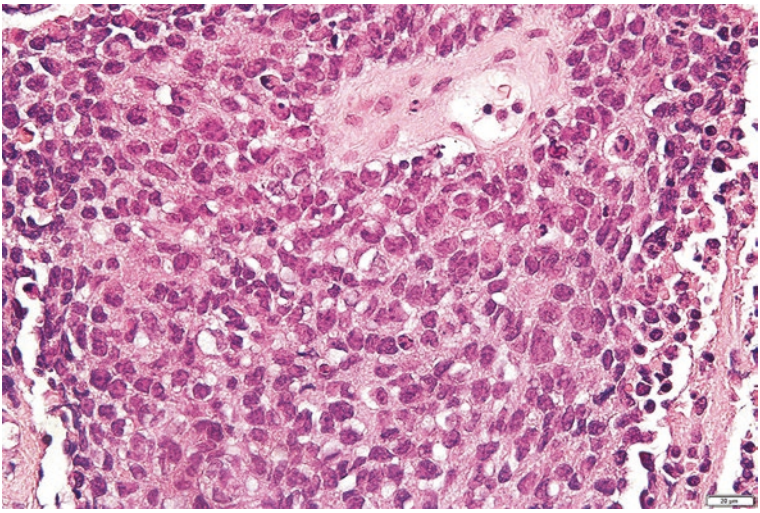

Fig. 2.6 Ewing sarcoma containing round tumor cells with a high nuclear to cytoplasmic ratio

Pleomorphic Tumors

Many high-grade sarcomas can exhibit pleomorphic or bizarre-appearing cells. The tumor cells of this pattern demonstrate substantial variation in the size and shape of the nuclei (Fig. 2.7). Highly atypical mitoses can often be identified. One of the most frequently occurring pleomorphic soft tissue neoplasms is an undifferentiated high-grade pleomorphic sarcoma, previously designated as "high-grade malignant fibrous histiocytoma (MFH)." Pleomorphic sarcomas must be distinguished from pleomorphic carcinoma, hematolymphoid neoplasms, or melanomas that can have a similar appearance.

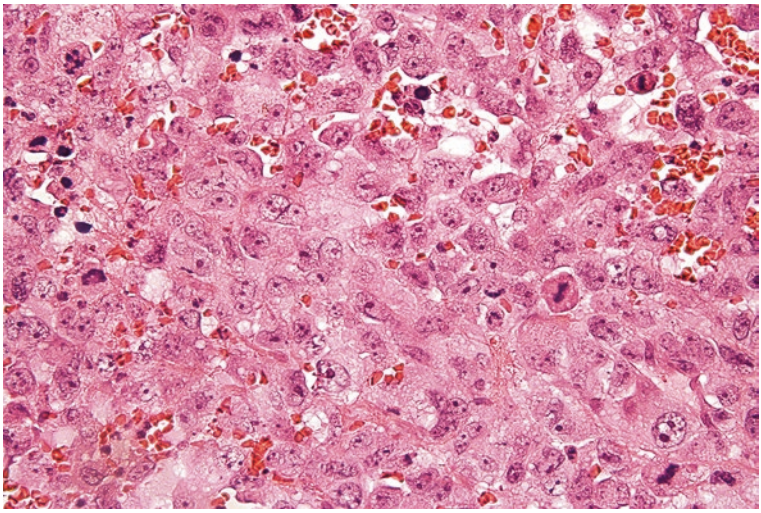

Fig. 2.7 Undifferentiated pleomorphic sarcoma with tumor cells that contain large and irregular nuclei with increased mitoses

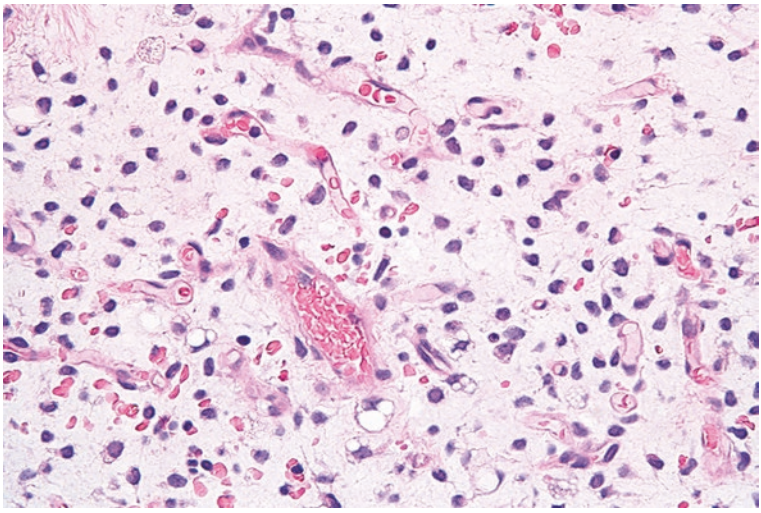

Fig. 2.8 Myxoid liposarcoma with substantial amount of background myxoid material and delicate capillaries

Myxoid Tumors

Myxoid soft tissue tumors exhibit varying amounts of a background bluish mucoid-like substance (Fig. 2.8). The neoplasms in this pattern can be difficult to differentiate based on architecture, as the tumor cells often freely float in this myxoid material. Examples of these tumors include myxoid liposarcoma, myxofibrosarcoma, and aggressive angiomyxoma.

Cartilaginous, Osseous, Adipocytic, and Vascular Tumors

The endothelial nature of vascular tumors is frequently apparent by the formation of infiltrative vascular channels (Fig. 2.9). Adipocytic tumors can often be identified by obvious fat cells or lipoblasts that contain large clear vacuoles in the cytoplasm (Fig. 2.10). Cartilaginous tumors will exhibit deposition of a blue or pink background chondroid-like matrix (Fig. 2.11). Osseous tumors show at least focal dense and eosinophilic extracellular osteoid material (Fig. 2.12).

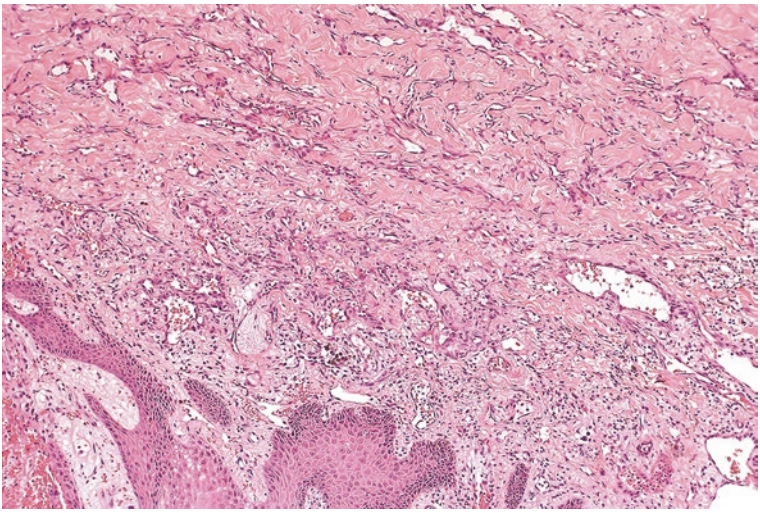

Fig. 2.9 Well-differentiated angiosarcoma with vascular channels that dissect through tissue

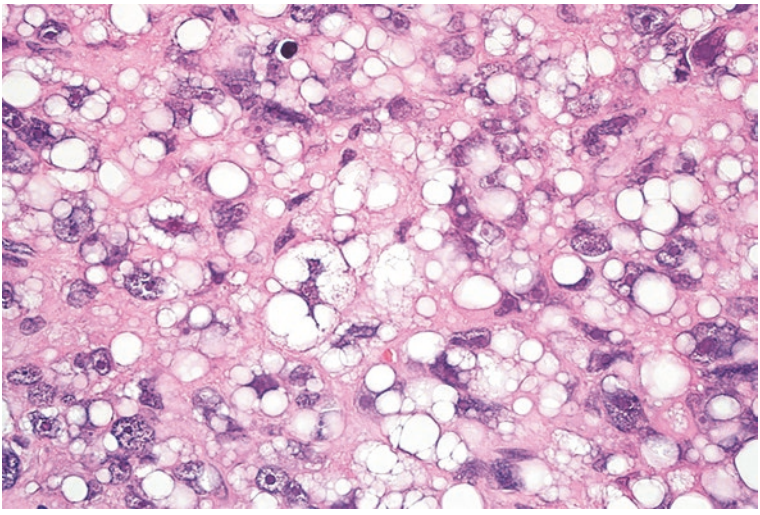

Fig. 2.10 Lipoblasts seen in a pleomorphic liposarcoma

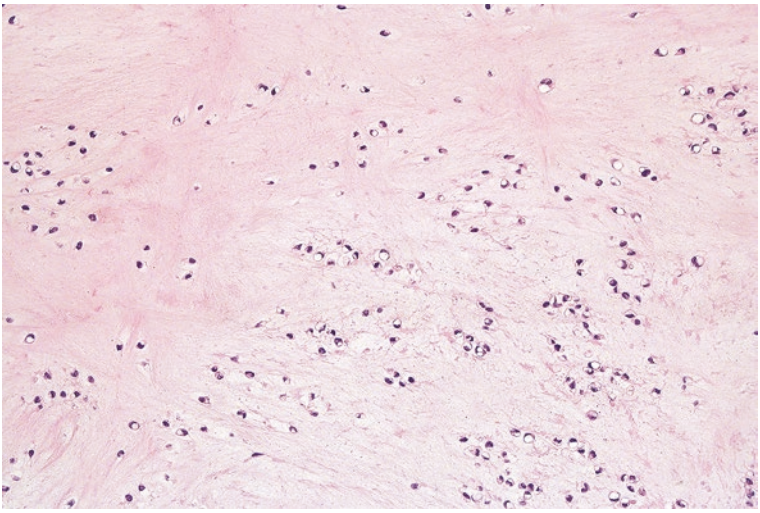

Fig. 2.11 Variably blue and pink background chondroid matrix in a soft tissue chondroma

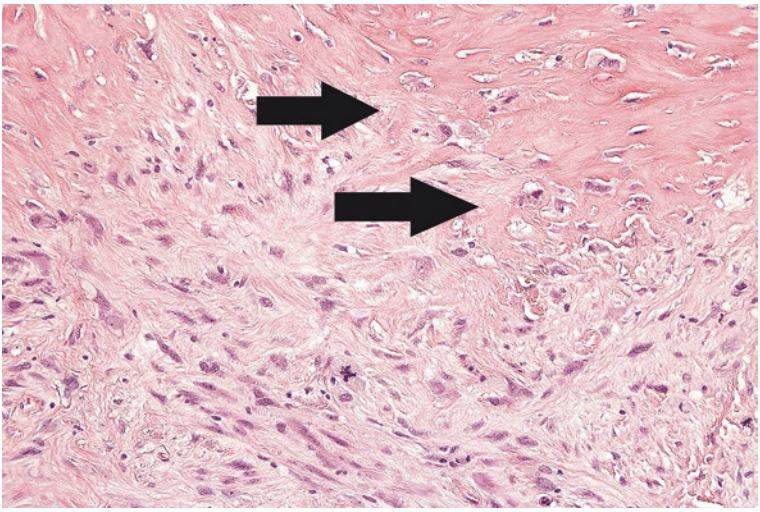

Fig. 2.12 Extraskeletal osteosarcoma with deposition of pink osteoid material (*arrows*)

It is important to understand that these patterns serve as a starting point in investigating the ultimate differentiation and diagnosis of a particular soft tissue tumor. At the microscope, an individual tumor may manifest multiple patterns, such as a synovial sarcoma, which can contain spindle cells, epithelioid cells, and round cells in the same tumor (Fig. 2.13a–c.) After assessing for these patterns and features, the pathologist can then progress to a more detailed examination and incorporate various ancillary tests to evaluate specific diagnostic considerations.

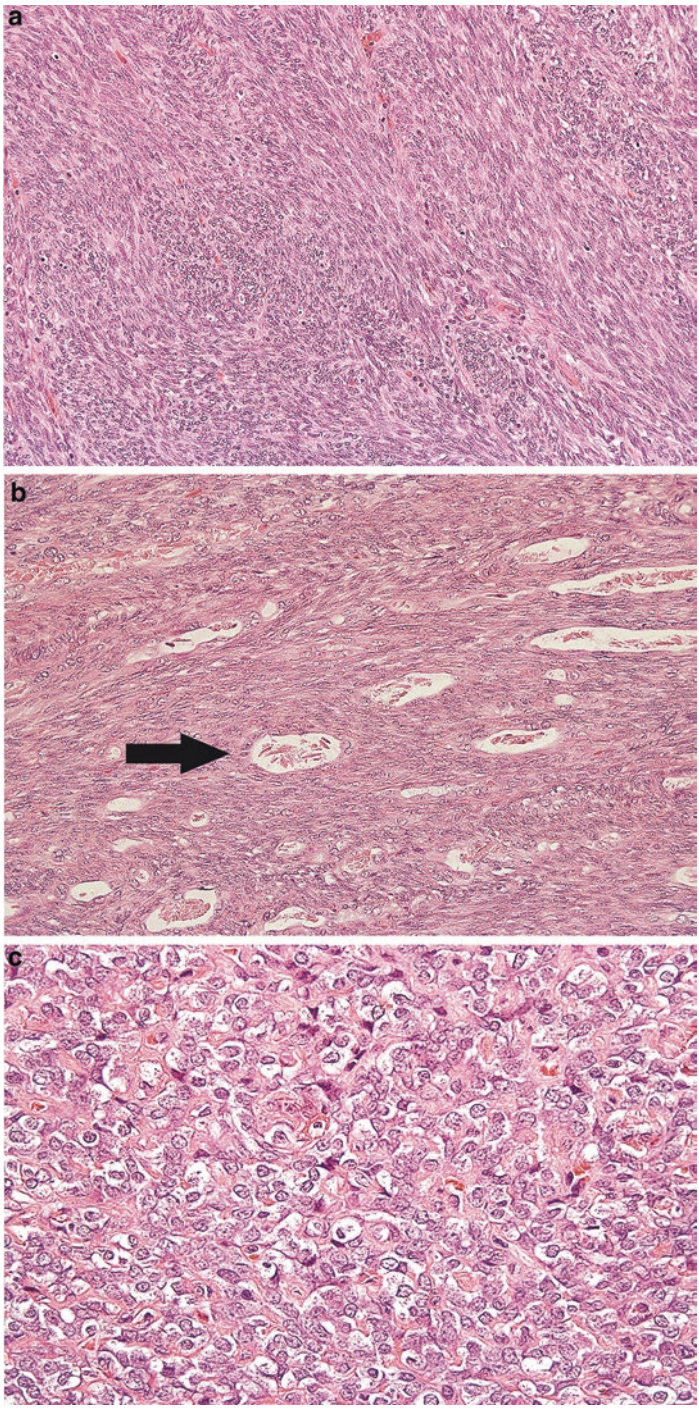

Fig. 2.13 (**a**) Synovial sarcoma showing a spindle cell pattern, (**b**) Epithelioid-like cells in a synovial sarcoma forming glandular appearing spaces (*arrow*), (**c**) round cells in a poorly differentiated synovial sarcoma

2.2 Diagnostic Ancillary Studies

While many tumors can be diagnosed from their appearance on an H&E slide, numerous soft tissue neoplasms have overlapping microscopic features that can complicate interpretation. Fortunately, there is now a large arsenal of ancillary studies available to facilitate classification. Familiarity with these different techniques can be helpful in understanding the particular approach to a diagnosis or potential issues when submitting a specimen to the pathology lab. Frequently used ancillary studies include immunohistochemistry, chromosomal karyotyping, fluorescence in situ hybridization (FISH), and reverse transcription PCR.

2.2.1 Immunohistochemistry

Immunohistochemical staining and interpretation have profoundly impacted the diagnosis of soft tissue neoplasms. The core concept of immunohistochemistry is to take an antibody that is specific for a particular cell protein and attach a chromogenic enzyme such as peroxidase [3]. If the enzyme-attached antibody binds to a cell of interest, a coloring reaction allows direct visualization under the microscope (Fig. 2.14a, b).

Immunohistochemical staining has its pitfalls. Insufficient or delayed fixation can result in a false positive or negative staining pattern. Failure to interpret the appropriate cells or misinterpret a nonspecific staining pattern can result in an erroneous diagnosis. Most immunohistochemical stains will highlight multiple types of tumor cells. For example, an S100 immunohistochemical stain will highlight neoplastic cells from both a benign nerve sheath tumor or a malignant melanoma. Consequently, appropriate use and interpretation of this test in the context of the morphologic findings is critical.

2.2.2 Karyotyping

Numerous soft tissue tumors have been found to manifest recurring chromosomal and genetic aberrations (Table 2.1).

Over the past few decades, pathologists have been able to identify genetic mutations by submitting fresh tumor tissue for cell culture and subsequent chromosomal analysis. With this technique, a representative fragment (about a cubic centimeter) of viable tumor is submitted to a cytogenetic laboratory in the appropriate culture medium as soon as possible. Genetic mutations can be deduced from the chromosomal aberrations identified [4].

Unlike many genetic tests, chromosomal karyotyping allows for a broad analysis of suspected or unsuspected cytogenetic abnormalities. This can be helpful when the histologic impression offers no clues to tumor differentiation or numerous diagnostic possibilities are being simultaneously considered. However, as this process requires culturing tissue, a fresh, representative, and viable specimen is required.

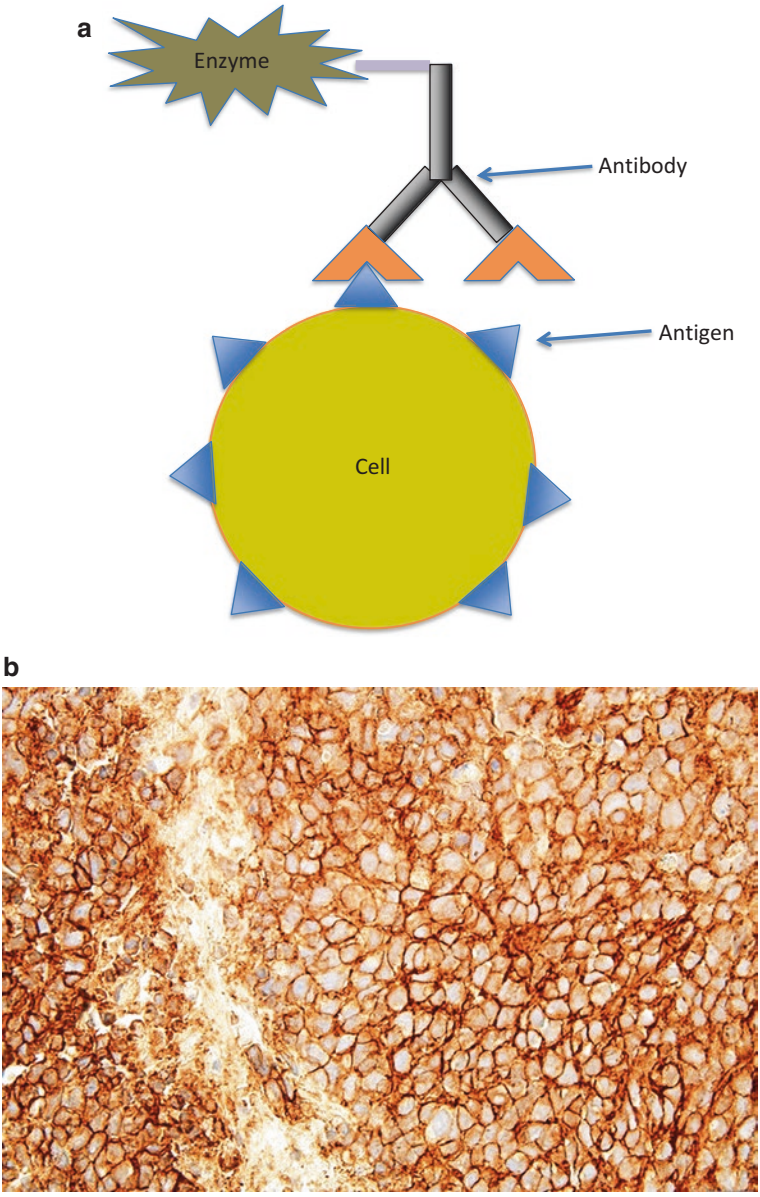

Fig. 2.14 (**a**) Illustration of the underlying principle of immunohistochemistry. An enzyme attached to an antibody signals the presence of a specific nuclear, cytoplasmic, or membranous antigen associated with a cell, (**b**) Positive immunohistochemical staining of tumor cells

Table 2.1 Common diagnostic genetic translocations in soft tissue tumors

Tumor type	Chromosomal abnormality	Genetic aberration
Well-differentiated liposarcoma	12q14-15 amplification	*MDM2*, *CDK4* amplification
Dedifferentiated liposarcoma	12q14-15 amplification	*MDM2*, *CDK4* amplification
Myxoid liposarcoma	t(12;16) or t(12;22)	*FUS-DDIT3* or *EWSR1-DDIT3*
Fibroblastic/myofibroblastic tumors		
Nodular fasciitis	t(17;22)	*USP6-MYH9*
Solitary fibrous tumor	12q13	*NAB2-STAT6*
Inflammatory myofibroblastic tumor	Alterations of chromosome 2	Multiple translocations involving *ALK*
Dermatofibrosarcoma protuberans	t(17;22)	*COL1A1-PDGFB*
Infantile fibrosarcoma	t(12;15)	*ETV6-NTRK3*
Low-grade fibromyxoid sarcoma	t(7;16), t(11;16)	*FUS-CREB3L2 or FUS-CREB3L1*
Myxoinflammatory fibroblastic sarcoma	t(1;10)	*TGFBR3-MGEA5*
Skeletal muscle tumors		
Alveolar rhabdomyosarcoma	t(X;17)	*ASPSCR1-TFE3*
Spindle cell rhabdomyosarcoma	t(6;8)	*SRF-NCOA2*
Vascular tumors		
Epithelioid hemangioendothelioma	t(1;3) or t(X;11)	*WWTR1-CAMTA1* or *YAPT1-TFE3*
Chondroid and osseous tumors		
Extraskeletal mesenchymal chondrosarcoma	t(8;8)	*HEY1-NCOA2*
Soft tissue tumors of uncertain differentiation		
Myoepithelioma of soft tissue	Chromosome 22 translocations	Multiple translocations involving *EWSR1*
Ossifying fibromyxoid tumor	t(6;12)	*EP400-PHF1*
Alveolar soft part sarcoma	t(X;17)	*ASPSCR1-TFE3*
Clear cell sarcoma	t(12;22)	*EWSR1-ATF1*
Ewing sarcoma	t(11;22), t(21;22)	*EWSR1-FLI1, EWSR1-ERG*
Desmoplastic round cell tumor	t(11;22)	*EWSR1-WT1*
Epithelioid sarcoma	Del 22q	Inactivation of *SMARCB1*
Extrarenal rhabdoid tumor	Del 22q	Mutation of *SMARCB1*
Synovial sarcoma	t(X;18)	*SS18-SSX1, SS18-SSX2*
Extraskeletal myxoid chondrosarcoma	Chromosome 9 translocations	Multiple translocations involving *NR4A3*

In hospitals or clinics where a biopsy or resection specimen is usually placed in formalin prior to transit, this requires planning prior to the procedure. Critical considerations include timely delivery and use of appropriate transport media. Additionally, the pathologist needs to ensure procurement of sufficient material for routine processing and morphologic assessment.

2.2.3 Molecular Fluorescence In Situ Hybridization

In a method known as fluorescence in situ hybridization (FISH), instead of reviewing an entire set of chromosomes, pathologists can assess for specific genes by using complementary DNA probes. These probe sequences are tagged with dye and bind to genes of interest.

Fluorescence in situ hybridization has many advantages. It can be performed on tumors that have been previously fixed in formalin. Also, the morphology of the tumor cells is retained, allowing the pathologist to be confident that a positive or negative genomic finding is occurring in the actual cells of interest.

This technique also has limitations as it assesses for a single genetic aberration, as opposed to chromosomal karyotyping which assesses for all cytogenetic abnormalities. Therefore, successful use is predicated on appropriate suspicion for a particular diagnosis.

2.2.4 Reverse transcription PCR

Reverse transcription polymerase chain reaction (RT-PCR) is another way a pathologist can confirm or exclude the presence of a genetic aberration in a soft tissue tumor.

Reverse transription PCR has its advantages and disadvantages. It can be performed on paraffin-embedded blocks and is highly sensitive. However, with this sensitivity comes an increased risk for contamination and false positive results. Moreover, unlike FISH and immunohistochemical stains, the underlying cellular morphology is lost during analysis. Consequently, a pathologist has to be careful to submit tissue that is representative of viable tumor, as there is potential for a false negative result from sampling error [1].

2.3 Intraoperative Consultation

Long ago, physicians recognized the value of immediate feedback from a pathologist during a surgical operation. Long ago, pathologists developed a method that would allow for intraoperative microscopic assessment, often referred to as a frozen section procedure [6].

After a surgeon submits tissue with a specific question, the pathologist or pathology assistant will embed the specimen in a gel-like medium that solidifies in

freezing temperatures. This frozen block is then mounted in a cryostat that cuts a thin slice of tissue (approximately 5 μm) that can be placed on a glass slide (Figs. 2.15 and 2.16). The slide is subsequently stained with hematoxylin and eosin and interpreted by a pathologist (Fig. 2.17).

The real-time feedback offered by a frozen section interpretation can greatly assist the surgeon in the key decision points of a procedure, but the method has

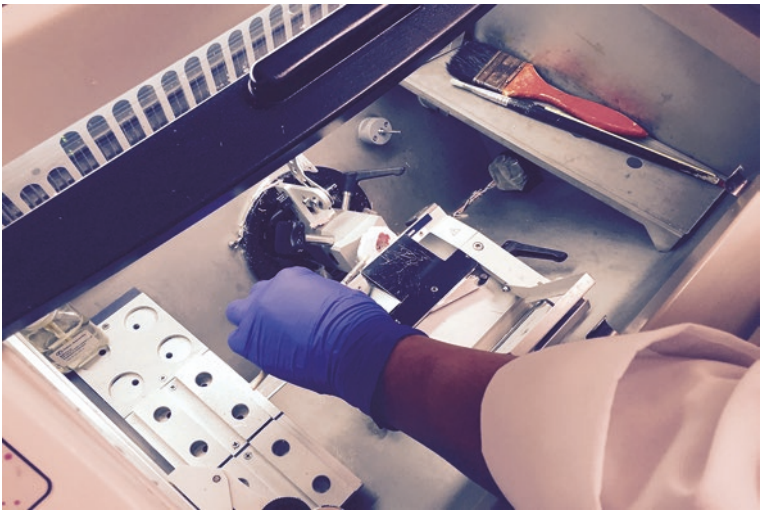

Fig. 2.15 Tissue fragment submitted in gel media that is frozen and being cut by the pathologist assistant

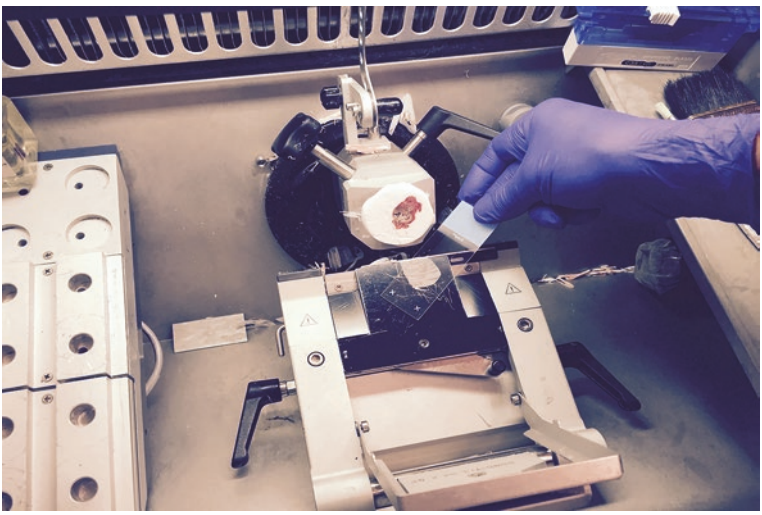

Fig. 2.16 Thin section of frozen tissue is placed on a glass slide

Fig. 2.17 Frozen section slide is being stained with hematoxylin and eosin

limitations. Some tissue, such as adipose tissue, does not freeze well enough for effective cutting in the cryostat, resulting in limited examination on a slide. Moreover, immunohistochemical stains are generally not available during frozen section diagnosis. The ideal turnaround time from receipt to diagnosis for a routine frozen section case with one piece of tissue is approximately 20 min. However, this time can be substantially prolonged if the procedure involves multiple pieces of tissue or the case is especially complex.

In addition to technical issues, there are also interpretive limitations. The nuclei in a frozen section slide are not well preserved and sometimes manifest artifactual atypia. Therefore, it can sometimes be difficult to distinguish involvement by a low-grade tumor such as deep fibromatosis from previous surgical site changes. Similarly, specimens previously subjected to neoadjuvant radiation can exhibit a reactive atypia in the mesenchymal tissue that can complicate an intraoperative diagnosis. Finally, tumor cells which are not present on the frozen section slide will sometimes become apparent when cutting deeper into the tissue after routine processing. Thus, although intraoperative consultations are typically accurate, discrepancies do occur [7].

In light of these considerations, intraoperative consultations have value, but it is prudent to utilize them when the result will impact the course of the procedure. When performing a needle core biopsy of a soft tissue mass, a frozen section will likely not result in a definitive histologic diagnosis. Frozen section interpretation can detect margin involvement by a high-grade sarcoma; however, determining involvement by some soft tissue neoplasms, such as angiosarcoma, is virtually impossible. A presurgical discussion with the pathologist can be helpful in clarifying expectations of a frozen section diagnosis for specific types of tumors.

Facts to Remember

1. When diagnosing a tumor, pathologists will examine a slide to assess for morphologic patterns.
2. Available ancillary tests for diagnosis include karyotyping, immunohistochemistry, FISH, and RT-PCR.
3. These tests are utilized based on the clinical and histologic features of the tumor.
4. A frozen section diagnosis can be of value in soft tissue pathology. However, these preliminary diagnoses have limitations, and consultations should be requested with prudence.

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