An update on ancillary techniques in the diagnosis of soft tissue tumors

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INTRODUCTION

Mesenchymal neoplasms represent unique diagnostic challenges to the pathologist because of the rarity, large number of discrete entities and need for highly specific diagnoses on ever decreasing sample sizes. Bone and soft tissue tumors represent less than 1% of neoplasia with over 100 unique diagnoses in soft tissue alone.[1] Although routine H&E diagnosis, combined with gross, clinical and radiographic correlation, remains the mainstay of bone and soft tissue pathology, in an effort to improve sensitivity on small specimens and specificity within the myriad of entities with overlapping histologic features, ancillary techniques are often necessary.

In the middle of the last century, tissue culture and electron microscopy became available to supplement routine histologic sections for diagnosis. However, the application of immunohistochemistry (IHC) toward the end of the 20th century quickly became the standard ancillary technique for the evaluation of sarcomas. Especially with the introduction of monoclonal antibodies and automated staining platforms, IHC became routinely used in most histology laboratories. Useful antibodies for mesenchymal tumors target (1) lineage specific proteins and (2) proteins that result from tumor-specific genetic or molecular abnormalities.

IHC- LINEAGE SPECIFIC PROTEINS

Two approaches exist to identify putative lineage specific proteins for IHC.

1) tumors that recapitulate normal mesenchymal cells or their precursors and express proteins, detectible by IHC, specific to that lineage.

2) profiling a tumor for highly expressed gene(s) that are not expressed in morphologic mimics. IHC to detect the protein products of these genes, even if the functions are unknown, can be diagnostically useful.

The earliest antibodies used to help in the diagnosis of bone and soft tissue tumors include the cytoplasmic lineage-specific antibodies: intermediate filaments (keratin, vimentin, desmin), calcium binding proteins (S-100 protein, calretinin) and cell-adhesion molecules (CD34, CD31). Generally, however, these markers lack high specificity. For example, IHC staining for desmin can be identified in tumors of smooth muscle, skeletal muscle, glomus cells, and also in unrelated entities such as tenosynovial giant cell tumor and a variety of tumors of the specialized stroma of the
vulvovaginal region. Furthermore, many sarcomas do not recapitulate any normal mesenchymal lineage so they are negative for lineage-specific cytoplasmic proteins.

The more recent discovery of nuclear proteins, predominantly transcription factors, that control the differentiation of mesenchyme offer advantages over cytoplasmic proteins. Often, nuclear proteins are expressed early in differentiation of a cell lineage when a differentiated phenotype (e.g. cross striations in skeletal muscle precursors) is not evident on H&E slides. Also, the microscopic interpretation of a nuclear stain is sometimes less ambiguous than a cytoplasmic stain.

The prototypical example of a lineage-specific transcription factor is myogenin (myf4 is the most commonly used monoclonal antibody). Myogenin is expressed in all subtypes of rhabdomyosarcoma[2] and in tumors with heterologous rhabdomyoblastic differentiation.[3-5] Not only is it one of the most specific IHC markers in soft tissue pathology, but the pattern of staining can also distinguish between alveolar and embryonal rhabdomyosarcoma, which show diffuse and patchy positivity, respectively.[6]

Other lineage-specific transcription factors, while not achieving the specificity of myogenin, nonetheless provide useful diagnostic clues to diverse tumor types. The most common category are the homeobox genes, which control specific stages of embryonic development. Of these, two SOX (SRY-related HMG-box) proteins, SOX9 and SOX10, are useful in cartilage and neural crest tumors, respectively.[7, 8] SOX10 stains benign and malignant melanocytic and nerve sheath tumors. Although SOX10 is positive in only ~50% of malignant peripheral nerve sheath tumors, it is virtually always negative in the most common morphologic mimic, synovial sarcoma.[9] Transcription factors that highlight endothelial cells (ERG) and osteoblasts (SATB2) are recently described and hold promise diagnostically.[10, 11]

The alternative approach, that is identifying candidate tumor specific proteins by gene expression profiling, assumes nothing about histogenesis. Proteins such as MUC4, DOG1 and TLE1 were identified this way and can be targeted by IHC. MUC4 is a transmembrane glycoprotein present on normal glandular epithelium. Though the mechanism is unknown, it is highly expressed in tumors harboring a FUS-CREB3L2 fusion including low-grade fibromyxoid sarcoma (LGFMS), hyalinizing spindle cell tumor with giant rosettes (likely just a variant of LGFMS) and a subset of sclerosing epithelioid fibrosarcoma but is negative in perineureoma, MPNST, desmoid fibromatosis and myxofibrosarcoma.[12, 13] DOG1 is a nuclear protein that is very sensitive and specific for gastrointestinal stromal tumors (GIST), including GISTs that have PDGFR rather than KIT mutations.[14] TLE1 is sensitive for synovial sarcoma but its specificity is controversial.[15, 16]
IHC - INDICATORS OF GENETIC CHANGES

Lineage-specific markers, though useful, are limited in several respects. As mentioned above, many sarcomas are poorly differentiated and therefore express no such markers. Conversely, the diagnostic challenge in some tumors may not be lineage but the distinction between benign or malignant (e.g. lipoma versus liposarcoma). Many mesenchymal tumors harbor reproducible large scale (chromosome or gene-level) or smaller (nucleotide level) genetic changes that can be detected by direct genetic methods (described later below) or indirectly by IHC methods.

Amplification of a region in the long arm of chromosome 12 (12q13-15) is a reproducible change seen in most well-differentiated and dedifferentiated liposarcomas, but not in other liposarcoma types nor in benign lipomas. The 12q13-15 region occupies some 10 million bases with dozens of genes. However, overexpression of two gene products, MDM2 and CDK4, is readily detected by IHC. The immunophenotype of combined strong, diffuse MDM2 and CDK4 nuclear expression is useful to discriminate well-differentiated liposarcoma from lipoma and dedifferentiated liposarcoma from other sarcomas, respectively. Interestingly, low-grade osteosarcomas both of the intramedullary and parosteal variety also show amplification of 12q13-15 and nuclear expression of MDM2 and CDK4. However, staining is not completely specific, especially when only one of the two markers is present, and can be seen in other sarcoma types.[17, 18]

Reciprocal translocations of genes that regulate cell-cycle and signal transduction characterize a large subset of bone and soft tissue tumors. If the resulting fusion proteins are abnormally expressed, they can, at least in theory, be detected by IHC. For example, the most common gene fusion in Ewing sarcoma, EWSR1-FLI1 causes expression of FLI1 protein in the primitive round cells of this tumor.[19] Similarly, the ASPSCR1-TFE3 fusion results in expression of TFE3 protein in alveolar soft parts sarcoma.[20] Since both FLI1 and TFE3 are expressed in the nucleus of a variety of non-neoplastic cells, the specificity is not very high and a positive result must be interpreted with caution. By contrast, IHC detection of STAT6 expression in solitary fibrous tumor with NAB2-STAT6 gene fusion is more specific. This is because STAT6, although also expressed in various cell types, remains in the cytoplasm unless activated. The NAB2-STAT6 fusion protein, however, accumulates in the nucleus. Therefore, strong nuclear expression of STAT6 appears to be highly sensitive and specific.[21] Since the STAT6 gene maps in close proximity to CDK4, STAT6 is sometimes amplified along with MDM2 and CDK4 in dedifferentiated liposarcoma.
resulting in positive IHC staining for STAT6, including nuclear staining in some cases.[22]

**INI1**, also known as SNF5/SMARCB1 is a component of a chromatin remodeling complex that plays a critical role in transcription.[23] Deletion of both copies of INI1 is found in epithelioid sarcoma, but not carcinomas or other sarcomas with epithelioid cytomorphology.[24] Interestingly, a variety of tumors with "rhabdoid" cytomorphology, in addition to proximal type epithelioid sarcoma, such as atypical teratoid/rhabdoid tumor of the CNS, renal and extrarenal rhabdoid tumor in infants also lack INI1 expression. The list of tumors with INI1 loss continues to grow and now includes poorly differentiated chordoma.[25]

The **Rb** protein, a product of the retinoblastoma gene is a tumor suppressor that has long been studied for its role in cell-cycle regulation and senescence. Whereas loss of Rb function definitely plays a role in retinoblastoma, osteosarcoma and some carcinomas, its loss is less common in sarcomas as a whole. However, recent evidence indicates that complete loss of Rb expression, for example from gene deletion, is detected in spindle cell lipoma and pleomorphic lipoma.[26] IHC for Rb is useful to confirm these diagnosis as nuclear expression is retained in other benign and malignant lipomatous tumors and solitary fibrous tumor which are important morphologic mimics. Intriguingly, mammary type myofibroblastoma and cellular angiofibroma of the perineal region, two lesions with striking morphologic similarity to spindle cell lipoma, also lack nuclear expression of Rb suggesting that all of these lesion may belong to the same family of neoplasms.[27]

Point mutations and short nucleotide insertions or deletions are also tumorigenic in mesenchymal neoplasms. **β-catenin** is the protein product of the *CTNNB1* gene and is involved in the Wnt signaling pathway. Point mutations in the *CTNNB1* or *APC* genes are present in sporadic and familial (i.e. Gardner syndrome) forms of desmoid fibromatosis, respectively. These mutations produce an abnormally activated β-catenin protein resulting in translocation of β-catenin from the plasma membrane to the nucleus. IHC detection of *nuclear* β-catenin is, thus, a sensitive and, compared to other intra-abdominal spindle cell lesions, specific confirmatory test for desmoid tumor.[28-30]

Isocitrate dehydrogenases (**IDH1** and **IDH2**) are enzymes of the Krebs cycle. Mutations of these genes are thought to produce oncogenic metabolites. More specifically, a substitution of Arginine to Histidine in IDH1 is found in glioblastomas and leukemias.[31, 32] Monoclonal antibodies for Histidine containing IDH1 area available and detect the mutant protein by IHC.[33] Benign and malignant cartilage tumors also demonstrate a substitution at Arginine. However, the resulting amino acid substitution is more often Arginine → Cysteine than Arginine→Histidine so the most widely used monoclonal antibody is still of limited use. However, a new antibody that
recognizes a variety of mutations was recently reported to work in ELISA and may be useful in the diagnosis of cartilage tumors.[34]

Activating mutations of the \textit{BRAF} gene, specifically a missense substitution of valine by glutamic acid (V600E) can be detected by a monoclonal antibody. In addition to prognostic and therapeutic implications in colorectal carcinoma, papillary thyroid carcinoma and melanoma, [35, 36] IHC may be diagnostically useful in bone biopsies for Langerhans histiocytosis especially if tissue decalcification precludes molecular studies. [37]

\textbf{GENETIC AND MOLECULAR TESTING}

Genetic and molecular techniques may not be available at all pathology laboratories, but all practicing pathologists should be familiar with the genetic abnormalities in bone and soft tissue tumors and the benefits and limitations of specific techniques of cytogenetics and molecular pathology. At the coarsest level, mesenchymal tumors can be divided into those with complex genomic abnormalities and those with relatively few abnormalities. The former category includes most of the high-grade adult sarcomas such as pleomorphic undifferentiated sarcoma (PUS), pleomorphic rhabdomyosarcoma, leiomyosarcoma etc. The confirmation of a complex genotype, therefore, even if not revealing a specific, reproducible genetic event, can at least categorize a sarcoma into this broad category. Tumors with more simple genetic events are often reproducible between tumors within a category and, as such, are diagnostically useful. Table 1 lists the current list of such genetic events. It should be noted that the table is current as of the writing of this handout but the catalog is ever-expanding. The current list of commercially available tests at reference labs can be found at: \url{http://www.amptestdirectory.org/directory/st_test_list.php}

Genetic and molecular characterization of a tumor provides prognostic and predictive information. For example, alveolar rhabdomyosarcoma has a significantly worse prognosis than embryonal rhabdomyosarcoma so confirming the presence of \textit{PAX3} or \textit{PAX7} gene rearrangement is vital to correct management.[38]

The approaches commonly used to study the chromosomal and gene-level changes in the laboratory can be divided into cytogenetic and molecular methods.[39, 40] Cytogenetics includes conventional \textit{karyotyping} and \textit{fluorescence in-situ hybridization} (FISH). Both of these techniques offer the advantage that a direct correlation can be made between histomorphology and genetic changes. Karyotyping is time consuming (1-2 weeks to obtain a result), requires specially trained technical experts to interpret and the sample must contain viable, dividing cells. This means the pathologist has to make the decision to obtain a karyotype at the time of surgery,
usually based on a combination of clinical suspicion and intraoperative frozen section. The advantage of karyotyping is that any chromosomal abnormality, provided it is sufficiently large, will be detected without any specific target in mind. Thus karyotyping is most useful in cases where the differential diagnosis is very broad.

FISH, on the other hand, is performed on paraffin embedded tissue. Cytologic smears with a monolayer of cells are especially good samples for FISH. However, unlike karyotyping, the test is directed at a specific abnormality. Therefore, the pathologist must have already narrowed the differential so that a positive result yields diagnostically useful information. FISH is also limited in that most commercially available probe sets detect only the presence of a rearrangement or copy-number at a single locus. In most cases, the presence of a rearrangement in the suspected gene (e.g. SS18) is sufficient to confirm the suspected diagnosis (e.g. synovial sarcoma), but this is not always true. The most obvious exception is the EWSR1 gene, which, while fused to FLI1 in the majority of Ewing sarcomas, can be fused to other genes (Table 1) in a variety of other bone and soft tissue tumors.[41] FISH probes can be designed to detect both partners in a translocation to offer more specificity,[42] but even this approach does not avoid examples where two distinct clinicopathologic entities (e.g. clear cell sarcoma and angiomatoid fibrous histiocytoma) have identical gene fusions (e.g. EWSR1-ATF1).

Molecular approaches rely on purification of nucleic acids from tissue followed by PCR amplification and detection using sequencing or other sequence-specific methods. As such, there is no direct correlation between the molecular result and histomorphology. Therefore, the pathologist must exercise great care in selecting appropriate material for testing to ensure it is representative of the tumor. The tissue does not need to be viable, but in general, flash frozen tissue is preferred to paraffin embedded tissue. Acid decalcification is especially problematic for molecular testing because it quickly fragments nucleic acids, especially RNA.[43] If possible, some tissue should be set aside without decalcification if there is a possibility of downstream molecular testing. The advantage to most molecular methods is the high resolution and, therefore, high specificity. Molecular methods can reveal not only that the suspected genes are involved in a translocation but even which exons are involved. Other methods can detect single nucleotide substitutions (e.g. BRAF V600E).[44] Most tests are also rapid and inexpensive.

The latest approaches employ array or “high-throughput” molecular methods, sometimes referred to as “-omics”. Instead of targeting a specific gene or exon, new technology can examine the entire genome of a tumor in parallel.[45] The data obtained from these arrays is highly complex and requires specialized bioinformatics software to interpret. Array based methods are not yet in routine clinical use for bone and soft tissue tumors, but these methods are leading to the discovery of diagnostically useful genetic abnormalities at a geometric rate.
Table 1. Chromosomal and genetic events in bone and soft tissue tumors.

<table>
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<tr>
<th>Tumor</th>
<th>Chromosomal changes</th>
<th>Genes involved</th>
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<td><strong>SMALL ROUND BLUE CELL TUMORS</strong></td>
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<tr>
<td>Ewing sarcoma / PNET</td>
<td>t(11;22)</td>
<td>EWS-FLI1</td>
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<td></td>
<td>t(21;22)</td>
<td>EWS-ERG</td>
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<td></td>
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<td></td>
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<td>EWS-FEV</td>
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<td></td>
<td>t(17;22)</td>
<td>EWS-E1AF</td>
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<td></td>
<td>inv(22)</td>
<td>EWS-ZSG</td>
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<tr>
<td>Ewing-like sarcoma</td>
<td>Xp11 t(4;19)</td>
<td>BCOR-CCNB3</td>
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<td></td>
<td></td>
<td>CIC-DUX4</td>
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<tr>
<td>Desmoplastic small round cell tumor</td>
<td>t(11;22)</td>
<td>EWS-WT1</td>
</tr>
<tr>
<td>Mesenchymal chondrosarcoma</td>
<td>inv(8q)</td>
<td>HE1:NCOA2</td>
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<td><strong>SKELETAL MUSCLE</strong></td>
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<tr>
<td>Alveolar rhabdomyosarcoma</td>
<td>t(2;13) t(1;13)</td>
<td>PAX3-FKHR</td>
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<td>PAX7-FKHR</td>
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<td><strong>VASCULAR</strong></td>
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<td>Pseudomyogenic hemangioendothelioma</td>
<td>t(7;19)</td>
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<tr>
<td>Epithelioid hemangioendothelioma</td>
<td>t(1;3)</td>
<td>WWTR1-CAMTA1</td>
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<td><strong>ADIPOCYTIC</strong></td>
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<tr>
<td>Lipoma</td>
<td>6p21 12q15</td>
<td>HMGA1</td>
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<td></td>
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<td>HMGA2</td>
</tr>
<tr>
<td>Spindle cell/pleomorphic lipoma</td>
<td>del 13q</td>
<td>Rb loss</td>
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<tr>
<td>Hibernoma</td>
<td>11q13-21</td>
<td>MEN1, AIP</td>
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<tr>
<td>Lipoblastoma</td>
<td>8q11-13</td>
<td>PLAG1</td>
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<tr>
<td>Well-differentiated liposarcoma</td>
<td>Ring 12, 12q+</td>
<td>MDM2, CDK4</td>
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<tr>
<td>Dedifferentiated liposarcoma</td>
<td>Ring 12, 12q+</td>
<td>MDM2, CDK4</td>
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<tr>
<td>Myxoid liposarcoma</td>
<td>t(12;16) t(12;16)</td>
<td>FUS-DDIT3</td>
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<td>EWS-DDIT3</td>
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<tr>
<td>Spindle cell/pleomorphic lipoma</td>
<td>del 13q</td>
<td>Rb loss</td>
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<td>Hibernoma</td>
<td>11q13-21</td>
<td>MEN1, AIP</td>
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<tr>
<td><strong>FIBROBLASTIC and MYOFIBROBLASTIC</strong></td>
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<tr>
<td>Nodular fasciitis</td>
<td>t(22;17)</td>
<td>MYH9-USP6</td>
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<tr>
<td>Inflammatory myofibroblastic tumor</td>
<td>t(var;2)</td>
<td>various-ALK1</td>
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<tr>
<td>Myxoinflammatory fibroblastic</td>
<td>t(1p22;10q24)</td>
<td>?</td>
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<tr>
<td>sarcoma</td>
<td>karyotype</td>
<td>rearrangement</td>
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<td>----------------------------------------------</td>
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<tr>
<td>Low-grade fibromyxoid sarcoma</td>
<td>t(7;16)</td>
<td>FUS-CREB3L1/2</td>
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<tr>
<td>Dermatofibrosarcoma protuberans</td>
<td>t(17;22)</td>
<td>COLA1-PDGFB</td>
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<td>Giant cell fibroblastoma</td>
<td>t(17;22)</td>
<td>COLA1-PDGFB</td>
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<tr>
<td>Infantile fibrosarcoma</td>
<td>t(12;15)</td>
<td>ETV6-NTRK3</td>
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<tr>
<td>Mammary type myofibroblastoma</td>
<td>del 13q</td>
<td>Rb</td>
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<tr>
<td>Cellular angiofibroma</td>
<td>del 13q</td>
<td>Rb</td>
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<tr>
<td>Angiofibroma of soft tissue</td>
<td>t(5;8)</td>
<td>AHRR-NCOA2</td>
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<tr>
<td><strong>UNCERTAIN DIFFERENTIATION</strong></td>
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<tr>
<td>Extrarenal rhabdoid tumor</td>
<td>del 22q</td>
<td>INI1</td>
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<tr>
<td>Angiomatoid fibrous histiocytoma</td>
<td>t(12;16)</td>
<td>FUS-ATF1</td>
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<tr>
<td></td>
<td>t(12;22)</td>
<td>EWS-ATF1</td>
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<tr>
<td>Mixed tumor, myoepithelial tumor</td>
<td>t(12;8) etc</td>
<td>EWS-POU5F1</td>
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<tr>
<td>Hemosiderotic fibrolipomatous tumor</td>
<td>t(1p22;10q24)</td>
<td>?</td>
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<tr>
<td>Tenosynovial giant cell tumor</td>
<td>t(1;2)</td>
<td>CSF-COL6A3</td>
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<tr>
<td>Aneurysmal bone cyst</td>
<td>t(var;17p13)</td>
<td>various-USP6</td>
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<td>Ossifying fibromyxoid tumor</td>
<td>6p21</td>
<td>PHF1</td>
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<tr>
<td>Synovial sarcoma</td>
<td>t(X;18)</td>
<td>SYT-SSX</td>
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<td>Clear cell sarcoma</td>
<td>t(12;22)</td>
<td>EWS-ATF1</td>
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<tr>
<td>Extraskeletal myxoid chondrosarcoma</td>
<td>t(9;22)</td>
<td>EWS-NR4A3</td>
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<td></td>
<td>t(9;17)</td>
<td>RBP56-NR4A3</td>
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<tr>
<td>Alveolar soft part sarcoma</td>
<td>t(X;17)</td>
<td>ASPL-TFE3</td>
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<td>PEComa</td>
<td>del 16p</td>
<td>TSC2</td>
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<tr>
<td>Solitary fibrous tumor/hamangiopericytoma</td>
<td>12q13</td>
<td>NAB2-STAT6</td>
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<tr>
<td><strong>OTHER</strong></td>
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<tr>
<td>Endometrial stromal sarcoma</td>
<td>t(7;17)</td>
<td>JAZF1-JJA1Z1</td>
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<tr>
<td>Mesoblastic nephroma</td>
<td>t(12;15)</td>
<td>ETV6-NTRK3</td>
</tr>
<tr>
<td>Poorly differentiated chordoma</td>
<td>dell 22q</td>
<td>INI1</td>
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References
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Introduction
- Bone and soft tissue tumors are rare (<1% of neoplasms)
- >100 unique soft tissue diagnoses in WHO 2013
- Goal of diagnosis: reproducible classification of lesions with differing clinical behavior and prognosis
- H&E might not be enough
  - Sensitivity: smaller biopsies
  - Specificity: overlapping histologic features

Ancillary techniques
- Immunohistochemistry
  - Lineage "specific"
  - Indicators of genetic and molecular abnormalities
- Molecular and Genetic testing
  - Available techniques
  - Advantages and Limitations
  - Selected examples

Disclosures
I have nothing to disclose.
Immunohistochemistry (IHC)

1. Lineage specific proteins
2. Indicators of **genetic** and **molecular** abnormalities (amplifications, deletions, translocations, point mutations)

IHC: Lineage “specific” proteins

- Classic approach
  - Cytoplasmic: Desmin, keratin, actins, S-100, CD34, CD31
  - Nuclear transcription factors
    - Skeletal muscle: Myogenin
    - Neural crest: SOX10
    - Others: SOX9, ERG, SATB2
- Gene expression profiling
  - MUC4
  - Others: DOG1, TLE1

Myogenin

- Master regulator of skeletal muscle differentiation
- ≈100% specific (myf4 monoclonal) for rhabdomyoblastic differentiation
  - Rhabdomyosarcoma (all types)
  - Heterologous rhabdomyoblastic differentiation
    - Triton tumor, dedifferentiated liposarcoma, myxoid liposarcoma, Wilms tumor
- Can help distinguish subtypes of rhabdomyosarcoma
SOX10

- SRY-related HMG box transcription factor
- Sensitive for neural crest-derived tumors
  - Melanoma (%)
  - Schwannoma, neurofibroma (>99%)
  - Malignant peripheral nerve sheath tumor (50%, focal)
- Specificity
  - Negative in synovial sarcoma, GIST, smooth muscle
  - Positive in astrocytomas, myoepitheliomas, breast carcinoma (10%)

IHC: Lineage specific proteins, gene expression profiling

- MUC4
- Glycoprotein on glandular epithelium
- Highly expressed in
  - Low-grade fibromyxoid sarcoma
  - Hyalaneizing spindle cell tumor with giant rosettes
  - Sclerosing epithelioid fibrosarcoma t(7;16) positive
- Negative
  - Perineurioma, MPNST, desmoid, myxofibrosarcoma

MUC4
**IHC: Indicators of genetic changes**

- Amplification
  - MDM2, CDK4
- Chromosomal translocations
  - STAT6
  - Others: FLI1, TFE3
- Deletion
  - INI1
  - Rb
- Point mutation
  - β-Catenin
  - Others: IDH1, BRAF

**IHC: Amplification**

- Liposarcoma
  - Well-differentiated
  - Dedifferentiated
- Osteosarcoma
  - Parosteal
  - Central low-grade
IHC: Chromosomal translocation

- STAT6
- Transcription factor, moves to nucleus when activated (phosphorylated)
- Fusion NAB2-STAT6 in solitary fibrous tumor → abnormal nuclear localization of STAT6
  - Sensitivity 98%
  - Specificity >90%
    - Dedifferentiated liposarcoma
**INI1 (SNF5/SMARCB1)**
- Chromatin remodeling, tumor suppressor, constitutively expressed
- **Loss of expression**
  - Epithelioid sarcoma (gene deletion)
  - Atypical teratoid rhabdoid tumor (inactivation)
  - Rhabdoid tumor (inactivation)
  - Poorly differentiated chordoma (?)

**IHC: Gene deletion**

**INI1 (loss)**
- Extrarenal rhabdoid tumor
- Retinoblastoma gene 13q14
- Tumor suppressor
- Deleted or mutated
  - Spindle cell lipoma
  - Pleomorphic lipoma
  - Mammary type myofibroblastoma
  - Cellular angiofibroma
- Retained in
  - Other benign and malignant lipomatous tumors
  - Solitary fibrous tumor

**IHC: Gene deletion or mutation**
Rb (complete loss)

IHC: Point mutation

- β-catenin
- Encoded by CTNNB1 gene, Wnt signaling pathway
- Mutations in CTNNB1 (sporadic) or APC (Gardner syndrome) → abnormal localization
  - Normal cells: membrane
  - Scar, GIST, smooth muscle: membrane
  - Desmoid fibromatosis: nuclear (+ cytoplasm) (70-90%)

Nuclear β-catenin

Genetic and molecular testing

- Purpose
  - Classification:
    - Separation of tumors into clinically meaningful categories based on reproducible changes
  - Prognostic:
    - Alveolar versus embryonal rhabdomyosarcoma
    - Myxoid versus well-differentiated liposarcoma
  - Predictive:
    - Therapeutic target from fusion gene product
- Techniques
  - Cytogenetic: Karyotype, FISH
  - Molecular: RT-PCR, Sanger sequencing, MLPA, array
Cytogenetics

<table>
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<tr>
<th>Karyotype</th>
<th>FISH</th>
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<tr>
<td>Tissue source</td>
<td>Fresh, dividing cells, FFPE, cyto smears, frozen</td>
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<tr>
<td>Turnaround</td>
<td>&gt;1 wk</td>
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<tr>
<td>Specificity</td>
<td>Shotgun approach</td>
</tr>
<tr>
<td>Advantages</td>
<td>Directed approach</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Low resolution</td>
</tr>
</tbody>
</table>

Cytogenetics: 33 year old woman, knee mass

Karyotyping: Synovial sarcoma

Cytogenetics: FISH
77 year old man, left femur mass, cough

Small cell carcinoma

Ewing/PNET

Lymphoma (DLBCL)

FISH: Ewing sarcoma

EWSR1 rearrangement
**EWSR1 rearrangements**

<table>
<thead>
<tr>
<th>Partner</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLI1, ERG, ETV1, EIAF, FEV, others</td>
<td>Ewing sarcoma family of tumors</td>
</tr>
<tr>
<td>ATF1, CREB1</td>
<td>Clear cell sarcoma</td>
</tr>
<tr>
<td>NR4A3</td>
<td>Angiomatoid fibrous histiocytoma</td>
</tr>
<tr>
<td>WT1</td>
<td>Extraskeletal myxoid chondrosarcoma</td>
</tr>
<tr>
<td>DDIT3</td>
<td>Desmoplastic small round cell tumor</td>
</tr>
<tr>
<td>POU5F1</td>
<td>Myxoid liposarcoma</td>
</tr>
</tbody>
</table>

**Molecular genetics**

<table>
<thead>
<tr>
<th></th>
<th>RT-PCR</th>
<th>Next gen sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue source</td>
<td>Frozen &gt; FFPE</td>
<td>Frozen, + normal control</td>
</tr>
<tr>
<td>Turnaround</td>
<td>&lt; 1-2 days</td>
<td>&gt; 1 wk</td>
</tr>
<tr>
<td>Specificity</td>
<td>Highly directed</td>
<td>Shotgun approach</td>
</tr>
<tr>
<td>Advantages</td>
<td>High specificity</td>
<td></td>
</tr>
<tr>
<td>Disadvantages</td>
<td>No correlation between morphology and genetics</td>
<td></td>
</tr>
</tbody>
</table>

43 year old woman, thigh mass

**Synovial sarcoma**

**Extraskeletal myxoid chondrosarcoma**
Vergara-Lui M, et al. 25 year old with multiple lytic lesions of bone

Gaucher
Fat atrophy
Xanthoma
Erdheim-Chester

PCR: translocation

PCR: BRAF V600E mutation

- Erdheim-Chester disease
- Langerhans histiocytosis
- Melanoma
- Papillary thyroid carcinoma
- Others
“High throughput” assays

- Array based sequencing

New markers identified by high throughput methods

<table>
<thead>
<tr>
<th>Genetics</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYH9-USP6</td>
<td>Nodular fasciitis</td>
</tr>
<tr>
<td>NAB2-STAT6</td>
<td>Solitary fibrous tumor</td>
</tr>
<tr>
<td>t(1;10)(p22;q24)</td>
<td>Myxoinflammatory fibroblastic sarcoma</td>
</tr>
<tr>
<td>HES1-CCNB3</td>
<td>Ewing-like sarcoma</td>
</tr>
<tr>
<td>BCOR-CCNB3</td>
<td>Ewing-like sarcoma</td>
</tr>
</tbody>
</table>

Molecular genetics of sarcoma

Solid Tumors Test Directory

http://www.amptestdirectory.org/directory/st_test_list.php
Take-home messages

- Lineage-specific is a relative term
- IHC for nuclear transcription factors offer advantages over older cytoplasmic proteins
- IHC can indirectly detect tumor-specific genetic and molecular abnormalities
- Gene and molecular abnormalities can be detected directly by more specialized methods
- High throughput methods can rapidly screen an entire tumor genome and may allow personalized medicine