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

Andrew Horvai, MD, PhD
Clinical Professor, Pathology

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
    - Hyalinizing spindle cell tumor with giant rosettes
    - Sclerosing epithelioid fibrosarcoma t(7;16) positive
  - Negative
    - Perineurioma, MPNST, desmoid, myxofibrosarcoma

MUC4

- Low-grade fibromyxoid sarcoma
- 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

MDM2 / CDK4

- Well-differentiated liposarcoma
- De-differentiated liposarcoma
- Parosteal osteosarcoma
**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

---

**STAT6**

- Dedifferentiated liposarcoma

---

**STAT6**

- Solitary fibrous tumor
- Dedifferentiated liposarcoma

---

**STAT6**

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)

Rb
- 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)

Spindle cell lipoma

Rb

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

Desmoid fibromatosis

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>
<thead>
<tr>
<th>Karyotype</th>
<th>FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue source</td>
<td>Fresh, dividing cells, FFPE, cytose smears, frozen</td>
</tr>
<tr>
<td>Turnaround</td>
<td>&gt;1 wk</td>
</tr>
<tr>
<td>Specificity</td>
<td>Shotgun approach, Directed approach</td>
</tr>
<tr>
<td>Advantages</td>
<td>Direct correlation between morphology and genetics</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Low resolution</td>
</tr>
</tbody>
</table>

33 year old woman, knee mass

Cytogenetics: Synovial sarcoma

t(X;18)

Cytogenetics: FISH

Fluorescence In Situ Hybridization

Source: National Human Genome Research Institute
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, Diagnoses</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>
<tr>
<td></td>
<td>Myoepithelial tumors</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**

**PCR: translocation**

25 year old with multiple lytic lesions of bone.

**PCR: BRAF V600E mutation**

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

- Array based sequencing

![Genomic DNA](image)

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>NAB2-STAT6</td>
<td>Hemosiderotic fibrolipomatous tumor</td>
</tr>
<tr>
<td>HEY1-NCOA2</td>
<td>Mesenchymal chondrosarcoma</td>
</tr>
<tr>
<td>CIC-DUX4</td>
<td>Ewing-like sarcoma</td>
</tr>
<tr>
<td>BCOR-CCNB3</td>
<td>Ewing-like sarcoma</td>
</tr>
</tbody>
</table>

Molecular genetics of sarcoma

![Graph](image)

Solid Tumors Test Directory

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