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

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Pathology
Disclosures

I have nothing to disclose.
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
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
Myogenin

Alveolar RMS

Embryonal RMS

Myogenin

Myogenin
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%)
SOX10

MPNST

Synovial sarcoma

SOX10

SOX10
IHC: Lineage specific proteins, gene expression profiling

- **MUC4**
  - Glycoprotein on glandular epithelium
  - Highly expressed in
    - Low-grade fibromyxoid sarcoma
    - Hyalanizing spindle cell tumor with giant rosettes
    - Sclerosing epithelioid fibrosarcoma t(7;16) positive

- **Negative**
  - Perineurioma, MPNST, desmoid, myxofibrosarcoma
Low-grade fibromyxoid sarcoma
IHC: Indicators of genetic changes

- Amplification
  - MDM2, CDK4

- Chromosomal translocations
  - STAT6
  - Others: FLI1, TFE3

- Deletion
  - INI1
  - Rb

- Point mutation
  - β-Catenin
  - Others: IDH1, BRAF
- **Liposarcoma**
  - Well-differentiated
  - Dedifferentiated

- **Osteosarcoma**
  - Parosteal
  - Central low-grade

**IHC: Amplification**

Diagram showing the amplified region on 12q 13.3 - 12q 15.

- 56Mb
- 67Mb
- DDIT3
- CDK4
- HMGA2
- MDM2

Graphical representation indicating the amplified regions associated with various subtypes of sarcoma.
MDM2 / CDK4

Well-differentiated liposarcoma

De-differentiated liposarcoma
Parosteal osteosarcoma

MDM2, CDK4

MDM2 / CDK4
IHC: Chromosomal translocation

- STAT6
- Transcription factor, moves to nucleus when activated (phosphorylated)
- Fusion $NAB2-STAT6$ in solitary fibrous tumor $\rightarrow$ abnormal nuclear localization of STAT6
  - Sensitivity 98%
  - Specificity $>90$
    - Dedifferentiated liposarcoma
STAT6

Solitary fibrous tumor
- Dedifferentiated liposarcoma
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 (?)
INI1 (loss)

Extrarenal rhabdoid tumor
IHC: Gene deletion or mutation

- 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
Rb (complete loss)

Spindle cell lipoma
IHC: Point mutation

- β-catenin
- Encoded by *CTNNB1* gene, Wnt signaling pathway
- Desmoid tumor: 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></th>
<th>Karyotype</th>
<th>FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue source</td>
<td>Fresh, dividing cells</td>
<td>FFPE, cyto smears, frozen</td>
</tr>
<tr>
<td>Turnaround</td>
<td>&gt;1 wk</td>
<td>~ 1 wk</td>
</tr>
<tr>
<td>Specificity</td>
<td>Shotgun approach</td>
<td>Directed approach</td>
</tr>
<tr>
<td>Advantages</td>
<td>Direct correlation between morphology and genetics</td>
<td></td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Low resolution</td>
<td></td>
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</tbody>
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Cytogenetics:
33 year old woman, knee mass
Karyotyping: Synovial sarcoma

\( t(X;18) \)
Cytogenetics: FISH

Fluorescence In Situ Hybridization

Labeling with fluorescent dye

Denature & Hybridize

probe DNA

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</th>
<th>Diagnosis</th>
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<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></td>
<td>Angiomatoid fibrous histiocytoma</td>
</tr>
<tr>
<td>NR4A3</td>
<td>Extraskeletal myxoid chondrosarcoma</td>
</tr>
<tr>
<td>WT1</td>
<td>Desmoplastic small round cell tumor</td>
</tr>
<tr>
<td>DDIT3</td>
<td>Myxoid liposarcoma</td>
</tr>
<tr>
<td>POU5F1</td>
<td>Myoepithelial tumors</td>
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### Molecular genetics

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

SYT-SSX fusion

Extraskeletal myxoid chondrosarcoma

EWSR1-NR4A3 fusion
PCR: translocation

“High throughput” assays

- Array based sequencing
New markers identified by high throughput methods

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<tr>
<th>Genetics</th>
<th>Diagnosis</th>
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<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></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>
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Molecular genetics of sarcoma

![Graph showing the increase in the number of tumors and genetic/molecular aberrations over time.]

- Green line: # of tumors characterized
- Yellow line: # of genetic/molecular aberrations

Time:
- 1960
- 1970
- 1980
- 1990
- 2000
- 2010
- 2020
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
Solid Tumors Test Directory

http://www.amptestdirectory.org/directory/st_test_list.php