Small Round Cell Tumors

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Small round cell tumors: definition - patternless sheets of small cells with dense cellularity and high N:C ratio
Small round cell tumors
Defining features

• Highly malignant
• Largely occur in children, 0-20 years-old
• Composed of primitive cells with minimal or no differentiation
• “Size” is relative term
Time to start playing the blues!
Types of round cell tumors
The Big Four

- Hematopoietic: lymphoma and leukemia
- Neuroblastoma
- Rhabdomyosarcoma
- Ewing sarcoma
Types of small cell tumors
Tumors occurring primarily in bone

- Poorly differentiated chordoma
- Melanotic neuroectodermal tumor
- Mesenchymal chondrosarcoma
- Small cell osteosarcoma
Types of small cell tumors
Those occurring mostly in specific sites

• Desmoplastic small round cell tumor
• Germ cell tumors
• \textit{NUT} translocation carcinoma
Types of small cell tumors
Organ-specific blastomas

- Wilms’ tumor (nephroblastoma)
- Hepatoblastoma
- Sialoblastoma
- Pancreatoblastoma
- Pleuropulmonary blastoma
Types of small cell tumors
Those occurring in diverse sites

- Synovial sarcoma
- Rhabdoid tumor
- Undifferentiated sarcoma
Small Round Cell Tumors
Genetic Factors

- Constitutional mutations
  - Deletions
  - Single nucleotide substitutions
    - Truncated protein
  - Epigenetic factors
    - Loss of imprinting
    - Loss of heterozygosity
    - Methylation
    - Histone acetylation
    - sRNAs, siRNAs
Small round cell tumors

- Acquired mutations
  - “Second hits”
  - Clonal progression
    - Chromothripsis
    - “Hot spots”
    - Drug resistance changes
  - Translocations
    - Chimeric proteins
    - Altered promoter region
Ewing Sarcoma Family of Tumors
Defining features

• All poorly differentiated
• Arise in bone and soft tissue (mostly bone)
  – rarely in organs, e.g. kidney
• Characteristic genetic fusions of \textit{EWS} gene with \textit{ETS} group of genes
  – Rare substitution of \textit{FUS} for \textit{EWS} (<1%)
**Ewing Sarcoma Family of Tumors**

**Racial predilection**

<table>
<thead>
<tr>
<th>Location</th>
<th>Incidence Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA, Caucasian, SEER data</td>
<td>2.7 per million</td>
</tr>
<tr>
<td>USA, African American, SEER data</td>
<td>0.3 per million</td>
</tr>
<tr>
<td>USA, Caucasian, New York</td>
<td>2.8 per million</td>
</tr>
<tr>
<td>USA, African American, New York</td>
<td>0.3 per million</td>
</tr>
</tbody>
</table>
Ewing Sarcoma Family of Tumors
Clinical Features

- Mostly adolescents and young adults
  - Less common in young children and older adults
- Slightly more common in boys
- Bone tumors: any bone of body affected
- Organs: kidney most common
- Second most common primary pediatric cancer in
  - Bone
  - Soft tissue
Ewing sarcoma: Site distribution

- **Bone tumors:**
  - Mostly diaphyseal
  - Large soft tissue component

- **Locations:**
  - Paravertebral
  - Retroperitoneum
  - Chest wall
  - Extremities
Ewing Sarcoma
Radiological Features
Ewing Sarcoma: Microscopic Features

• Three major varieties
  – Classical Ewing sarcoma
  – Atypical Ewing sarcoma
  – Peripheral neuroectodermal tumor (PNET)
Ewing sarcoma: classical
Ewing sarcoma: large cell
Ewing sarcoma: PNET with rosettes
Ewing sarcoma: PNET with spindle cells
## Ewing Sarcoma: Microscopic Features

<table>
<thead>
<tr>
<th>Feature</th>
<th>Typical</th>
<th>Atypical/PNET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear size</td>
<td>Small</td>
<td>Large</td>
</tr>
<tr>
<td>Mitotic index</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Nuclear contours</td>
<td>Regular</td>
<td>Irregular</td>
</tr>
<tr>
<td>Rosettes</td>
<td>No</td>
<td>Yes in PNETs</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>Inconspicuous</td>
<td>Prominent</td>
</tr>
</tbody>
</table>
Ewing Sarcoma: Electron Microscopy

- Not often used in standard practice
- Pools of glycogen
- Primitive neural differentiation
# Ewing sarcoma: Immunohistochemistry

<table>
<thead>
<tr>
<th>Marker</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD56 (NCAM)</td>
<td>Typically negative</td>
</tr>
<tr>
<td>CD99</td>
<td>Positive (non-specific)</td>
</tr>
<tr>
<td>Chromogranin</td>
<td>Typically negative</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>Occasionally positive</td>
</tr>
<tr>
<td>Desmin</td>
<td>Rarely positive</td>
</tr>
<tr>
<td>FLI1</td>
<td>Positive (non-specific)</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>Often focal</td>
</tr>
</tbody>
</table>
Ewing sarcoma: NKX2.2, a new marker

• Transcription factor for neuronal development
• Target gene product of EWS-FLI1 fusion protein
• 80% sensitivity, 84% specificity for fusion-positive Ewing sarcomas
• Positive in olfactory neuroblastoma, some mesenchymal chondrosarcoma, and some adult round cell tumors (carcinomas).
• Specificity is improved with concurrent use of CD99 (98%).
The new microscope

Gene chip with classic monocular microscope
Courtesy of Tim Triche, MD, PhD
Ewing Sarcoma: Genetics (TET-ETS fusions)

• Karyotype
  – \( t(11;22) \sim 85\%: \) EWS-FLI1
  – \( t(21;22) \sim 10-15\%: \) EWS-ERG
  – Other (rare)
    • \( t(2;22) \) - EWS-FEV
    • \( t(7;22) \) - EWS-ETV1
    • \( t(17;22) \) - EWS-E1AF
Chimeric proteins: the original chimera

• “A creature fearful, great, swift-footed and strong, who had three heads, one of a grim-eyed lion; in her hinderpart, a dragon; and in her middle, a goat, breathing forth a fearful blast of blazing fire.” Homer, The Iliad

Apulian plate, c. 350–340 BC (Louvre)
Genetic promiscuity

- Zeus: a promiscuous god
- EWS: a promiscuous gene, i.e., one that fuses with multiple partners
Genetic diagnosis

- **Karyotyping**
  - Fusion discovery (new fusion variants, non-Ewing translocations)
  - Imprecise (Does not identify fusion at molecular level)
  - Untimely (results after diagnosis)
  - Insensitive (substantial no-growth, normal cell overgrowths)

- **RT-PCR**
  - Requires recognition of entire fusion
    - Requires consensus primers, multiple reactions
  - Allows subtyping by fusion length
    - No longer indicated
Genetic diagnosis

• FISH
  – Recognizes rearrangement of EWS, most sensitive
  – May require addition of FUS
  – Does not identify partner gene
  – Does not exclude other sarcomas with EWS translocations
    • clear cell sarcoma
    • extramedullary myxoid chondrosarcoma
    • myoepithelial carcinoma of soft tissue
FISH vs RT-PCR

• FISH (+) and RT-PCR (+)
  – Majority of cases
• FISH (+), RT-PCR (-)
  – Recognition of all potential fusions
  – Involvement of variant partner
  – Low RNA expression of fusion gene (requires “nested technique”)
• FISH (-), RT-PCR (+)
  – Problems with FFPE processing
  – Sampling issues
Ewing sarcoma
FUS fusions

• FUS partners with Ewing sarcoma genes
  – FUS-ERG
  – FUS-FEV

• Analogous to several other sarcomas with FUS or EWS fusions
  – Myxoid liposarcoma
  – Angiomatoid fibrous histiocytoma
  – Low grade fibromyxoid sarcoma
Ewing Sarcoma: Prognosis and Outcome

- Addition of ifosfamide and etoposide has improved outcome (2003 data)
- 70% survival non-metastatic tumors
- 20% survival metastatic tumors
Other prognostic factors

- Tumor size (<8 cm, ≥ 8 cm)
- Tumor site (distal v. proximal extremity v. pelvis)
- Age (>10 y v. 10-18y v ≥ 18 y)
- Histological effect (necrosis vs. viable tumor)
  - Data less compelling

- Controversial factors (no consistent data)
  - Histology (PNET vs Ewing histology)
  - Bone vs. soft tissue
Ewing Sarcoma Family of Tumors
Differential Diagnosis

• In bone consider
  – Lymphoma: CD3, CD20, CD43, TdT
  – Osteosarcoma: osteoid SALLB4
  – Mesenchymal chondrosarcoma: cartilage, HPC-like foci
  – Invasive soft tissue sarcoma like synovial sarcoma
  – Undifferentiated sarcoma: “Ewing-like tumors”
Undifferentiated “Ewing-like” sarcoma

- Those with EWS fusion with non-ETS family member
- Those without EWS fusion but with a similar genetic profile
- The clinical setting and therapeutic responses are heterogeneous and unpredictable.
- At present time, the rarity of these lesions has precluded extensive understanding, so a pragmatic approach is often necessary.
Rare birds

California condor

http://nwpassages.files.wordpress.com/2010/12/condor.jpg
EWS-non ETS fusions

- EWS-transcription factors
- EWS-non-transcription factors
- Some show typical Ewing features
- Most show atypical Ewing features
- Osseous and extraosseous tumors
- Variable CD99 positivity
EWS-non ETS fusions

- EWS-NFATC2
- EWS fused with transcription factor
- Not part of ETS family of genes, but shares DNA binding properties
- Activates same downstream pathway as FLI1 and ERG fusions
- Shows recurrent gene amplification, similar to the PAX7-FOXO1 of alveolar rhabdomyosarcoma
EWS-non ETS fusions

- EWS-PATZ1 (EWS-ZNF278)
- EWS-SP3
- Binds EWS to a transcription factor unlike ETS family
- Both involve a zinc finger fusion partner, similar to desmoplastic small round cell tumor (EWS-WT1)
- Shows polyphenotypia similar to DSRCT
- More aggressive and drug resistant similar to DSRCT
EWS-non ETS fusions

- EWS-SMARCA5
- Binds EWS epigenetic factor rather than transcription factor
- Chromatin remodeling gene fusion, similar to INI1 (SMARCB1)
- Suggests a relatedness more to rhabdoid tumor, but not proven
EWS-non ETS fusions

- EWS-POU5F1 (OCT4)
- Binds EWS to transcription factor that regulates stem cells
- Regulates an entirely separate group of genes from ETS transcription factors
- Heterogeneous morphology, with areas containing nested polygonal and spindle cells
- S100 positive
- Probably a form of soft tissue myoepithelial tumors
Ewing-like sarcoma with non-EWS fusions

- CIC-DUX4 fusions
  - t(4;19)
  - t(10;19)
- BCOR-CCNB3 fusion
  - paracentric inv(X)
CIC-DUX4 sarcomas

• Usually extraskeletal
• Most often in extremities
• Aggressive course with early metastasis
• More like atypical Ewing sarcoma with nucleoli, more abundant cytoplasm, and extensive necrosis and mitoses
• Variable CD99 positivity
• Frequently WT1 positive
CIC-DUX4 sarcomas

- Fusion gene contains:
  - The binding site for TLE proteins, similar to synovial sarcoma (CIC).
  - DNA binding site (DUX4)
- Upregulates several ETS family genes, similar to Ewing type fusions
- Alternate partners for CIC have been described, similar to EWS
- CIC-DUX4 appears to be particularly common in EWS FISH negative Ewing-like tumors (two thirds of cases in Memorial series).
Round cell undifferentiated neoplasm
Geographic necrosis
Cytologic features
BCOR-CCNB3 sarcomas

- Usually arise in bone
- Resembles Ewing sarcomas clinically and morphologically
- Rare, <5%
- Fuses BCOR, an epigenetic repressor, to cyclin B3
- Amplifies the ability of cyclin B3 to drive cell cycle events
- Causes loss of function of BCOR, and epigenetic instability
Other mutations of BCOR

- **Constitutional mutations**
  - Skeletal dysplasia (oculofaciocardiodental syndrome)
  - Increased osteogenic potential in mesenchymal stem cells

- **Somatic mutations**
  - AML (deletions and translocations)
    - `t(X;17)`, a PML variant
    - Cytogenetically normal AML
  - Myelodysplastic syndromes
  - Medulloblastoma
  - Endometrial stromal tumor `[t(X;22)]`
  - Ossifying fibromyxoid tumor
Desmoplastic Small Round Cell Tumor (DSRCT) Clinicopathological features

- High grade malignancy that predominately occurs in peritoneal-lined cavities, occasionally elsewhere
- Small cells with prominent stromal desmoplasia
- Polyphenotypia: epithelial, neural, and mesenchymal (dot-like desmin expression)
- EWS-WT1 fusion with WT1 expression
- Aggressive behavior with poor chemotherapy response and peritoneal seeding and metastasis
DSRCT
Additional microscopic features

• Epithelial differentiation
• Glands
• Rosettes
• Rhabdoid cells
• CD99 positivity, sometimes like Ewing sarcoma
• Some Ewing sarcomas (defined by genetics!) have identical phenotype as DSRCT
Immunohistochemistry of DSRCT

• **Mesenchymal**
  – Vimentin
  – Desmin
  – Smooth-muscle actin
  – Muscle-specific actin

• **Epithelial**
  – Keratins
  – Epithelial membrane antigen
Immunohistochemistry of DSRCT

• Neural
  – Neuron-specific enolase
  – CD57 (Leu7)
  – Synaptophysin
  – Chromogranin
  – NB84

• Miscellaneous
  – CD15
  – WT1
  – CD99
  – CA125
  – Ber-EP4
Genetics of DSRCT

- t(11;22)(p13;q12)
- EWS-WT1 fusion
- WT1: a zinc finger protein with DNA binding properties
- Normally acts as a tumor suppressor