The Genetics of Myoepithelial Tumors: salivary glands, soft tissue and bone

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Disclosure

• Nothing to declare
Spectrum of Myoepithelial (ME) Tumors

- **Family of tumors** with variable terminology based on anatomic location:
  - pleomorphic adenoma (salivary gland)
  - benign mixed tumor (skin)
  - soft tissue myoepithelioma

- **Definition**: immunohistochemical evidence of myoepithelial differentiation (EMA/CK, S100/calponin)

- Uncertain if any/all have a common pathogenesis
Altered Myoepithelial Cells – Neoplastic Conditions

High morphologic plasticity:
- Spindle shaped or myoid cells
- Hyalin or plasmacytoid cells
- Clear cells

Pleomorphic Adenoma, Parotid
Salivary Gland Myoepithelial Tumors - Immunoprofile

Hybrid epithelial and mesenchymal properties

AE1:AE3  
S100  
Calponin
<table>
<thead>
<tr>
<th>Antigen</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
<th>Total+</th>
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<tr>
<td>PAN-K</td>
<td>23</td>
<td>10</td>
<td>13</td>
<td>8</td>
<td>17</td>
<td>48/71</td>
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<tr>
<td>AE1/AE3</td>
<td>15</td>
<td>17</td>
<td>10</td>
<td>5</td>
<td>19</td>
<td>51/66</td>
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<tr>
<td>CK8/18</td>
<td>20</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>7</td>
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<tr>
<td>CK14</td>
<td>26</td>
<td>6</td>
<td>4</td>
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<td>2</td>
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<tr>
<td>EMA</td>
<td>31</td>
<td>7</td>
<td>16</td>
<td>6</td>
<td>23</td>
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<td>9</td>
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<td>12</td>
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<td>9</td>
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<tr>
<td>Calponin</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>30</td>
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<td>7</td>
<td>4</td>
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<tr>
<td>Desmin</td>
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<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
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<td>51</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>15/66</td>
</tr>
</tbody>
</table>

0, no staining; 1+, <5% tumor cells reactive; 2+, 5–25% tumor cells reactive; 3+, 26–50% tumor cells reactive; 4+, >50% tumor cells reactive.
Soft Tissue ME Tumors Genetics

• Few reports of *EWSR1* gene rearrangement

• one case report each, the fusion partner was identified as being either *PBX1* or *ZNF444*
Towards a Molecular Classification of ME Tumors

• Systematic molecular analysis of a large spectrum of ME tumors, including a variety of anatomic locations, age groups and risk of malignancy

• To investigate the pathogenetic relationship with the salivary gland counterpart (PLAG1 & HMGA2 rearrangements in pleomorphic adenoma)

• Unifying concept of ME tumors within soft tissues, bone, and visceral locations
Molecular Study of ME Tumors

• 66 ME tumors with confirmed diagnosis and adequate material for molecular analysis
  – 47 Soft Tissue
  – 7 Cutaneous
  – 6 Bone
  – 6 Visceral (lung)

• Age distribution: 1-75 years;
  – 15 children

• Gender distribution: 36 females/30 males
EWSR1 Rearrangement in 45% of ME tumors

Common presentations:
- Deep soft tissue
- Malignant histology
- Pediatric: 8/15
- Bone: 5/6
- Visceral: 4/6

EWSR1 Telomeric-Centromeric
ME Tumors with *EWSR1* gene rearrangement

**Histologic patterns:**

1. Small blue cell phenotype
2. Epithelioid or rhabdoid histology with eosinophilic or clear cytoplasm
3. Spindle or ovoid appearance in a prominent sclerotic stroma
4. Lack ductal, squamous, or matrix differentiation
ME tumor with *EWSR1* rearrangement

1. Small blue cell phenotype

53yF, R index finger, superficial

IHC: s100 & EMA positive
ME tumor with *EWSR1* rearrangement

2. Epithelioid morphology with clear or eosinophilic cytoplasm

20yM, Left foot, subcutaneous, benign

S100 positive, EMA negative, CK positive
ME tumor with *EWSR1* rearrangement

3. Sclerotic background, mixed spindle & epithelioid cells

32yM, subcutaneous R ankle, benign

S100 & EMA positive
EWSR1 -POU5F1 Fusion in ME Tumors

- Children & young adults
- Deep soft tissue extremities
- Clear cell morphology

3’RACE & RT-PCR

EWSR1 EXON6

POU5F1 EXON2

POU5F1 Telomeric/ Centromeric

Antonescu CR Genes Chromosomes Cancer 2010
EWSR1-POU5F1 fusion positive

Nested, diffuse clear cell changes

34 yr F, wrist, deep, malignant

IHC: s100 & EMA positive; OCT4 negative
POU5F1 (a.k.a OCT3/4)

• encodes a **transcription factor** which binds to the octamer motif (ATGCAAAAT) present in the promoter or enhancer regions of target genes.

• is essential for keeping germ cells and embryonic stem cells in an **immature and pluripotent status**

• *POU5F1* reactivation has been found to be implicated in human cancer: germ cell tumors (OCT3/4 IHC marker), bladder tumors
**EWSR1-PBX1** positive ME Tumors

- Age range 11-75 years
- Location: 3 soft tissue, 1 bone, 1 lung
- Morphology: sclerotic, bland (fibromatosis-like) or clear cell changes
- IHC: (+) S100, EMA/CK
EWSR1-ZNF444 Positive ME Tumor

64yF, Lung

S100 positive, EMA negative
Intra-Osseous Myoepithelial Tumors
Intra-Osseous Myoepithelial Tumors

• 5/6 showed EWSR1 rearrangement
• Location:
  – Vertebral body (L1)
  – Long bones of extremity (humerus, fibula, prox femur)
  – Pelvic Bones
• Morphology: epithelioid, clear cell, spindle
• Malignancy: 5 benign/1 malignant
ME tumor with *EWSR1* rearrangement

45 yF, vertebral body/L1

S100 protein (+)
49yM, Iliac bone lesion – long history followed for presumed FD

CT

MRI, Axial T1, fat saturation

Courtesy Petur Nielsen, MGH
Intra-Osseous Myoepithelial Tumor

Positive for EWSR1 & PBX1 gene rearrangements by FISH
26yM R Prox Tibia
Benign Intra-Osseous Myoepithelial Tumor
Visceral ME Tumors

- Lung ME Tumors (n=6)
  - 4/6 positive for EWSR1 rearrangement
    - 1 EWSR1-PBX1
    - 1 EWSR1-ZNF444
  - 1/2 FUS rearranged

- Breast ME tumors (n=5) – all negative
**EWSR1** negative ME tumors

- Common presentation:
  - superficial
  - cutaneous
  - benign
  - with ductal, squamous or cartilage matrix formation

Relationship with Myoepithelial Tumors of Salivary Gland?
PLAG1 Rearrangements in Cutaneous and Soft Tissue ME tumors/Mixed Tumors

• Benign myoepithelial tumors with prominent tubulo-ductal differentiation
• Lack *EWSR1* gene rearrangement
• Neoplasms with genuine salivary gland-like morphology, so-called soft tissue/cutaneous mixed tumors, are genetically related to their salivary gland counterpart

*Bahrami A, Genes Chromosome Cancer 2012
Antonescu CR, Modern Pathology 2012*
PLAG1 Overexpression and Gene Rearrangement in Skin/Soft Tissue Mixed Tumors
Saliv Gland Tumors containing Myoepithelial cells

Benign
• Myoepithelioma
• Pleomorphic Adenoma

Malignant
• Myoepithelial Carcinoma (de novo or ex-pleomorphic adenoma)
• Epi-Myoepithelial Carcinoma
Myoepithelial Carcinoma Ex-Pleomorphic Adenoma
**PLAG1** gene alterations in salivary gland pleomorphic adenoma and carcinoma ex-pleomorphic adenoma

Martins et al. *Mod Path*, 2005
increasing evidence that epithelial and myoepithelial cell populations share phenotypical and genotypical characteristics supporting the 'modified myoepithelial cell model'

Unified histogenesis: a single pluripotent cell, capable of differentiation into a variety of somatic phenotypes
Criteria for Malignancy – Saliv Gland MET

• **Infiltrative destructive growth**, beyond the tumor capsule into adjacent salivary gland or soft tissue is the most widely accepted criterion
  
  – **Multinodular growth pattern** with tongue-like processes (the most prevalent and characteristic pattern
    
    • Hypercellular periphery and central hypocellular myxoid matrix and/or necrosis
  
  – Sheet-like pattern
Criteria for Malignancy – Saliv Gland MET

• cytologic atypia and mitotic rate have been reported to be useful, without firm guidelines for reaching a diagnosis of myoepithelial carcinoma

(No specific mitotic rate cutoff exists for making this distinction, however, one series reported >7MF/10 HPF was diagnostic of malignancy)  

*Nagao T et al. Cancer 1998*
Criteria for Malignancy – ST MET

• Most morphologically *benign* or low-grade behave in a *benign fashion*, with a low, but unpredictable, risk for local recurrence (approximately 20%).

• MET with at least *moderate cytologic atypia* are clinically *malignant*.

*Hornick JL et al. AJSP 2003*
DIFFERENTIAL DIAGNOSIS
Chordoma Periphericum

CK19

EMA

T-Brachyury

EWSR1 negative (N=2)
Ossifying Fibromyxoid Tumor (OFT)

- S100+ only in 70% of cases
- Desmin+ focally in up to 20%
- CK, SMA rarely +
Ossifying Fibromyxoid Tumor (OFT)

No recurrent cytogenetic abnormalities

Ossifying fibromyxoid tumor: modified myoepithelial cell tumor?

Min KW et al. Ultrastruct Pathol 2005
Extraskletal Myxoid Chondrosarcoma

- Clinical: adults, extremity
- More diffusely myxoid
- IHC: non-contributory (weak/negative S100, EMA)

CHN (NOR1) on 9q22 fusions:
- t(9;22)(q22;q12) EWSR1
- t(9;17)(q22;q11) TAF2N
- t(9;15)(q22;q21) TCF12
- t(3;9)(q11;q22) TFG
Ewing Sarcoma and other Primitive Sarcomas

RT-PCR

| 1 | 2 | 3 | 4 |

FISH: No info RE the gene partner

>18 in-frame chimeric transcripts
Summary

- *EWSR1* gene rearrangement is a common genetic event in soft tissue, bone and visceral ME tumors, particularly in the pediatric and young adults.

- *EWSR1-POU5F1* fusion is present 8-10% of ME tumors, involving the extremity deep soft tissues, occurring in children or young adults and displaying a prominent clear cell morphology.

- *EWSR1-PBX1* detected in ME tumors with sclerosing and ‘fibromatosis’-like morphology.
Summary

• *EWSR1* fusion negative ME tumors are more often benign, cutaneous or superficially located and display ductal or cartilage differentiation.

• *PLAG1* gene rearrangements are noted in 1/3 of tumors lacking *EWSR1*, which are typically benign and have prominent tubulo-ductal differentiation (i.e. mixed tumors)
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