The spectrum of myoepithelial (ME) tumors represents a family of lesions with variable terminology, based on anatomic location: such as pleomorphic adenoma in the salivary gland, benign mixed tumor in the skin, and myoepithelial tumor/parachordoma in the soft tissue. Although genetic studies in pleomorphic adenoma have shown frequent rearrangements of \textit{PLAG1} and \textit{HMGA2} \cite{1-3}, similar abnormalities have not been identified in myoepithelial lesions from other tissues \cite{4}. Thus it remains unclear if the myoepithelioma subsets described above share a common pathogenesis.

Until recently, the genetic hallmark of soft tissue ME tumors was still under investigation, with only two cases analyzed cytogenetically, reporting disparate chromosomal translocations. A t(1;22)(q23;q12) resulting in an \textit{EWSR1-PBX1} fusion was first described as a sole cytogenetic event in a soft tissue ME tumor, arising in the foot of a 59-year old female \cite{5}, while a 2\textsuperscript{nd} case, of an occipital soft tissue ME carcinoma, arising in a 40-year old female, showed a t(19;22)(q13;q12), resulting in an \textit{EWSR1-ZNF444} fusion \cite{6}. In addition, \textit{EWSR1} gene rearrangement by FISH has been reported in two ME tumors in the pediatric age group \cite{7}.

In a recent study we undertook a systematic molecular analysis of a large spectrum of ME tumors, including lesions from a variety of anatomic locations, age groups and lesions with differing biologic potential \cite{8}. Minimum criteria for confirming the morphologic diagnosis included the co-reactivity for EMA +/- cytokeratin and S100+/- GFAP. During this talk I will be summarizing our findings.

**\textit{EWSR1} rearrangement is a common event in deep-seated soft tissue and bone ME tumors.** Thirty (45\%) of the 66 cases tested showed the presence of an \textit{EWSR1} gene rearrangement by FISH. More than half of the cases (n=16) occurred in children and young adults. The most common presentation was in the deep-seated soft tissues of the extremities, followed by the head and neck location. Five of the six osseous ME tumors showed an \textit{EWSR1} rearrangement. Four of the six lung ME tumors were positive, while only two of the six cutaneous tumors showed an \textit{EWSR1} break-apart signal by FISH.

Morphologically, in the \textit{EWSR1} rearranged tumors there were several different patterns identified: (1) tumors composed mainly of small blue cells with scant cytoplasm, monotonous cytomonophoria and ill-defined cell borders, arranged in solid sheets; (2) tumors with a predominantly epithelioid or rhabdoid appearance, with moderate to abundant eosinophilic or clear cytoplasm and eccentric nuclei; or (3) tumors composed of spindle or ovoid cells embedded in a prominent sclerotic stroma. None of the \textit{EWSR1} positive tumors showed the presence of ductal or glandular differentiation or cartilage/bone matrix formation. Immunohistochemically, all tumors showed S100 protein staining, typically strong and diffuse, as well EMA and/or cytokeratin AE1/AE3.

**\textit{EWSR1-POUSF1} fusion is identified in a subset of deep soft tissue ME tumors with distinctive clear cell morphology.** The five positive tumors had striking similarities, all of them presenting in the deep soft tissues of extremities, in children or young adults, and microscopically composed predominantly of epithelioid cells with abundant clear
cytoplasm, arranged in a nested growth pattern. These tumors were strongly and diffusely positive for both EMA and S100 protein, but lacked OCT4 expression, which seems surprising since this is the transcription factor encoded by POU5F1. Interestingly, a similar fusion between EWSR1 and POU5F1 has been reported previously in a case of undifferentiated bone tumor of the pelvis, carrying a t(6;22)(p21;q12). The tumor was composed of both primitive round cells and short spindle cells and reportedly was immunopositive for S100 protein and focally for cytokeratin. These morphologic features raise the possibility of an intra-osseous ME tumor.

Subsequently, a similar EWSR1-POU5F1 fusion was identified in 3 hidradenomas of the skin and 1 case of mucoepidermoid carcinoma of salivary gland, which, in contrast to our findings, showed OCT4 expression immunohistochemically. Although not identical, the transcript composition of the EWSR1-POU5F1 reported in these cases was quite similar with the one identified in our ME tumor. These two epithelial tumor types have been previously shown to be molecularly related, both entities showing a CRTCI-MAML2 fusion in approximately half of the cases. A FISH investigation of 10 additional hidradenomas lacking the CRTCI-MAML2 fusion transcript identified two cases with EWSR1 gene rearrangement. However, none of the 5 eccrine hidradenomas and 6 salivary gland mucoepidermoid carcinoma tested in our study showed an EWSR1 rearrangement. The relationship between these EWSR1-fusion positive skin adnexal tumors and tumors reported in this series with features of superficial myoepithelioma and cutaneous benign mixed tumor requires additional investigation. However, most of the cutaneous ME tumors and the lesions displaying ductal/glandular differentiation in this study were negative for EWSR1 gene rearrangement, suggesting an alternative pathogenesis. Furthermore, the presence of EWSR1 gene rearrangement in only one of the three mucoepidermoid carcinoma studied by Moller and coworkers and none of the five in the present series suggests that other mechanisms may be involved in the pathogenesis of that tumor type. In addition, none of the 5 myoepithelial carcinomas of salivary gland studied showed involvement of the EWSR1 gene, suggesting that at least a subset of ME tumors arising in salivary gland may not be related to their soft tissue counterparts.

POU5F1 is a transcription factor and its expression is restricted to germ cells in mature adult tissue and in human tumors is typically present in germ cell tumors. The multiple cell types seen in the composition of tumors positive for the EWSR1-POU5F1 fusion in our study may be indicative of a multi-phenotypic differentiation. ME tumor is a prototypical example of dual differentiation to both epithelial and mesenchymal lineages. POU5F1 expression as evidenced by OCT4 immunoreactivity was documented in the EWSR1-POU5F1 positive hidradenomas – however, it was negative in all 5 ME tumors in the present study, using the same antibody.

**EWSR1-PBX1 fusion is present in a subset of ME tumors associated with a bland sclerotic appearance or clear cell morphology.** Morphologically, 3 of the tumors, located in the foot, hip and pelvic bone, showed a deceptively bland appearance, being composed mainly of spindle cells embedded in a fibrotic stroma, resembling in areas desmoid-type fibromatosis. The other two cases, located in the forearm and lung, were composed of epithelioid or ovoid uniform cells with abundant clear cytoplasm.

**EWSR1-ZNF444 fusion is a rare occurrence in ME tumors.** Only one tumor had this rearrangement, which occurred in the lung of a 64-year old female. The
morphologic appearance was quite typical, with predominantly epithelioid cells with scant, clear cytoplasm, arranged in Indian-files or pseudo-rosettes separated by prominent sclerotic stroma. A focal spindle cell component was also noted. Tumor cells were positive for cytokeratin AE1:AE3 and S100 protein, but negative for EMA.

**Salivary gland myoepithelial carcinomas nor the other related tumors showed an EWSR1 gene rearrangement.** None of the tumors included in the control groups, including: 5 salivary gland myoepithelial carcinomas ex-pleomorphic adenoma; 6 salivary gland mucoepidermoid carcinoma; 5 cutaneous eccrine hidradenomas; 3 ossifying fibromyxoid tumors and 2 chordoma periphericum showed an EWSR1 abnormality.

**Differential Diagnosis.** The differential diagnosis of ME tumors is typically quite broad and may vary depending on patient age and anatomic location. Ossifying fibromyxoid tumor (OFMT), an S100 protein-positive soft tissue tumor with an uncertain line of differentiation, shares significant morphologic overlap with ME tumors. Although only 3 OFMTs were included for genetic analysis, the lack of EWSR1 abnormalities suggests that this entity may not be related to ME tumors.

The distinction from extraskeletal myxoid chondrosarcoma (EMC) can often be quite difficult, especially in large, deep-seated soft tissue tumors associated with myxoid changes. The presence of EWSR1 gene rearrangement, used previously to support the diagnosis of EMC, is now demonstrated in both tumor entities, in a significant number of cases. In spite of the morphologic overlap and EWSR1 gene abnormality, we do not believe the two tumors are related. The consistently strong EMA and S100 protein co-reactivity, the common clear cell changes and nested growth pattern seen in ME tumors, but not in most EMC, should help in the distinction. Furthermore a predominant myxoid stroma throughout the tumor is rarely seen in ME lesions.

In EWSR1-positive tumors having a small cell/undifferentiated appearance, an alternative Ewing sarcoma/PNET diagnosis was excluded either by negativity for CD99 immunostaining or by RT-PCR for EWSR1-FLI1 and EWSR1-ERG. The presence of EWSR1 gene rearrangement in about half of ME tumors which can be readily detected by FISH analysis can serve as a powerful diagnostic tool in challenging cases. However, this finding adds ME tumors to an already growing family of EWSR1 gene-rearranged tumors, which are often considered in the differential diagnosis, especially in the pediatric age group. ME tumors with EWSR1 gene rearrangement often have uniform rounded cell morphology and clear cytoplasm, presenting in the deep soft tissues of the extremities. These findings pose significant overlap both microscopically and clinically with other pediatric tumors, especially with Ewing sarcoma, also characterized by recurrent translocations involving the EWSR1 gene. In difficult cases which are positive for EWSR1 rearrangement, efforts to identify the translocation partner should be undertaken by RT-PCR methods for a definitive diagnosis and to avoid unnecessary systemic treatment.

These findings reinforce the fact that recurrent chromosomal translocations involving EWSR1 do not occur only in aggressive high grade sarcomas, but also in tumors with low or undetermined malignant potential, like ME tumors and so-called angiomatoid fibrous histiocyotma. Furthermore, these results provide solid evidence for a unifying concept of soft tissue ME with similar tumors arising in bone and at visceral
locations. However, the data presented here do not support a pathogenetic relationship between soft tissue ME tumors and their salivary gland counterparts. Additional cases will need to be analyzed in order to further investigate the relationship of soft tissue ME lesions and cutaneous benign mixed tumors.

References


Myoepithelial Tumors of Soft Tissue and Bone

Cristina Antonescu, MD

Department of Pathology
Memorial Sloan-Kettering Cancer Center, New York

2/11/2011
Background – Spectrum of ME Tumors

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

• Definition: immunohistochemical evidence of myoepithelial differentiation (EMA/CK, S100)

• Uncertain if any/all have a common pathogenesis
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 $ZNF444$
Molecular Study of Large Spectrum of ME Tumors/Patients

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

- Age distribution: 15 children, 11 young adults (<30 years), 40 adults

- Gender distribution: 36 females/30 males

Antonescu CR Genes Chromosomes Cancer 2010
Control Group

Other Related or Morphologically Similar Entities

- Salivary gland myoepithelial carcinoma (example: pleomorphic adenoma) (n=5)
- Salivary gland mucoepidermoid carcinoma (n=6)
- Cutaneous eccrine hidradenomas (n=5)
- Ossifying Fibromyxoid Tumor (n=3)
- Chordoma Periphericum (n=2)
Results: \textit{EWSR1} Rearrangement in 45\% of ME tumors

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

2/11/2011
ME tumor with *EWSR1* rearrangement

1. Small blue cell phenotype

53 yr F, R index finger, superficial

IHC: s100 & EMA positive

2/11/2011
ME tumor with *EWSR1* rearrangement

2. Epithelioid morphology with clear or eosinophilic cytoplasm

20y M, 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**

3’RACE & RT-PCR for *EWSR1-POU5F1*

1. ME1
2. Negative Contr M. marker

**ME1-POU5F1 Telomeric/Centromeric**

**FISH**

2/11/2011
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 is Fused to POU5F1 in a Bone Tumor with Translocation t(6;22)(p21;q12)

Shuichi Yamaguchi,1 Yukari Yamazaki,1 Yuichi Ishikawa,2 Noriyoshi Kawaguchi,3 Hiroyuki Mukai,4 and Takuro Nakamura1

- 39y F, undifferentiated sarcoma of the pubic bone (negative for O13)
- highly aggressive, DOD in 6 mo
POU5F1, encoding a key regulator of stem cell pluripotency, is fused to EWSR1 in hidradenoma of the skin and mucoepidermoid carcinoma of the salivary glands

E Möller,1,* G Stenman,2 N Mandahl,1 H Hamberg,2 L Molne,2 JJ van den Oord,4 O Brosjo,5 F Mertens1 and I Paragopoulo1

- close to half of hidradenomas and MECs display the same CRTC1–MAML2 fusion gene
- HA composed of 3 cell types: clear, cuticular, poroid
- MEC composed of 3 cell types: mucus-forming, intermediate, squamous

- EWSR1-POU5F1 (+) in 3 HA and 1 MEC

EWSR1 (exon 6) → POU5F1 (exon 2)

Hidradenoma with t(6;12)(p21;q12) and EWSR1–POU5F1 gene fusion
ME Tumor with *EWS-POU5F1* (3’RACE, RT-PCR, FISH)

Nested epithelioid appearance with clear cytoplasm

9 yr Male, deep soft tissue, arm

2/11/2011
EWS-POU5F1 fusion positive ME tumor

S100 protein

EMA

2/11/2011
**EWSR1-POU5F1 fusion positive**

Nested, diffuse clear cell changes

34 yr F, wrist, deep, malignant

IHC: s100 & EMA positive; OCT4 negative

2/11/2011
**EWSR1-POU5F1 Positive ME Tumors**

- N = 5 cases
- Age: 7-34 yrs (mean 21 yrs), 2 children
- Location: deep soft tissue extremities (5/5)
- Morphology: extensive clear cell change (4/5)
- Malignant potential: 4/5
- IHC: positive for S100 & EMA, OCT4 negative (5/5)
EWSR1-PBX1 positive ME Tumor
**EWSR1-PBX1** fusion positive ME tumors

- N=5 (3F, 2 M)
- Age: mean 45 (range 11-75 years)
- Location: 3 soft tissue, 1 bone, 1 lung
- 3 cases with bland spindle cell proliferation in a sclerotic background, resembling fibromatosis
- 2 cases with abundant clear cell morphology (one spindle, one epithelioid)
- 3 benign/2 malignant
- IHC: 5/5 positive S100 & EMA/CK
ME tumor with *EWSR1-PBX1* fusion

Bland, ‘Fibromatosis’-like

37 yr Male, L hip mass

S100 & EMA positive

2/11/2011
ME Tumor with EWSR1-PBX1 fusion

Bland, sclerotic areas

59 yr Female, foot, subcutaneous, benign

S100 & EMA positive
Summary

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

• ME tumors should be considered in the differential diagnosis of *EWSR1* rearranged tumors.

• *EWSR1-POU5F1* fusion was identified in 5 (8%) ME tumors, involving the extremity deep soft tissues, occurring in children or young adults and displaying a prominent clear cell morphology.
Summary

- *EWSR1-PBX1* and *EWSR1-ZNF444* fusions, previously reported in ME tumors, were detected in additional 6 cases of both soft tissue and visceral sites.

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

- Additional studies are required to identify other *EWSR1* fusion partners involved in ME tumors.
Summary

• No *EWSR1* gene rearrangements were detected in any of the salivary gland tumors, or morphologically similar tumors tested.

• These findings question a pathogenetic link between soft tissue ME tumors and the more common salivary gland or cutaneous counterparts.