What's new in Ewing(-like) sarcoma

Cristina Antonescu, MD
Department of Pathology
Memorial Sloan Kettering Cancer Center, New York, NY, USA

Genetic Classification of Small Blue Round Cell Sarcomas

- Ewing Sarcoma (EWSR1-ETS)
- Ewing Sarcoma-like Tumors (CIC-DUX4, BCOR)
- Desmoplastic Small Round Cell Tumor (EWSR1-WT1)
- Alveolar Rhabdomyosarcoma (PAX3/7-FKHR1)
- Small Cell Osteosarcoma
- Mesenchymal Chondrosarcoma (HEY1-NCOA2)
- Myxoid/Round cell sarcoma (FUS-DDIT3)
- Poorly Differentiated Synovial Sarcoma (SSX1/2-SS18)

Ewing Sarcoma

- Genetic Definition: positive for EWSR1-ETS (FUS-ETS) fusions
- Family members:
  - Ewing sarcoma
  - PNET
  - Adamantinoma-like Ewing sarcoma
- Morphology
  - Classic/atypical/ with complex epithelial differentiation
- IHC: CD99 strong and diffuse membranous staining

Ewing Sarcoma - Pathology
Ewing Sarcoma - Immunoprofile

Note: 20% positive for CK, rare cases positive S100 protein, desmin

Peripheral Neuroectodermal Tumor (PNET)

CD99/O13

Genetic Hallmark – Classic Ewing Sarcoma

EWSR1-ETS Fusions

Cytogenetic Variability:
- 90% t(11;22)(q24;q12): EWSR1-FLI1
- 5-8% t(21;22)(q22;q12): EWSR1-ERG
- 1-2% other ETS genes: ETV1, FEV, E1AF

Molecular Variability EWSR1-FLI1 (different exon composition):

Molecular Diagnosis RT-PCR vs FISH

- RT-PCR: primer design, contamination
- FISH: No info on the partner gene

Fusion Structure Variability of EWSR1-FLI1
From Ladanyi M, Cancer Biol Ther 2002
**EWSR1 gene family**

- **EWSR1-ETS** (FLI1; ERG; ETV1)
- **EWSR1-WT1**
- **EWSR1-ATF1**
- **EWSR1-WT3A4**
- **EWSR1-CHOP**
- **EWSR1-CREB1**
- **EWSR1-POUSF1/PUF1/ZNF444**

**PROMISCUITY** of one gene partner / **SPECIFICITY** of the other = novel transcription factor

- **Ewing sarcoma/PNET**
- **DSRCT**
- **Clear cell sarcoma**
- **Extraskeletal Myxoid CS**
- **Myoid/Round cell Liposarcoma**
- **AFH/GI Clear cell sarcoma**
- **Myoepithelial tumors**

**When is the Molecular Diagnosis required?**

**Diagnostic Pitfalls:**
- Atypical morphology
- Unusual Immunoprofile
- Insufficient / Inadequate Material
- Unusual clinical presentation

**Differential Diagnosis:**
- Lymphoma
- Neuroendocrine Ca
- other primitive sarcomas (DRCT, PD Synov Sarcoma)

**EWSR1-ERG positive Ewing sarcoma**

- Incidence: 5-8%
- Similar clinical and pathologic findings as EWS-FLI1 fusion
- IHC: CD99 & ERG strong reactivity

**EWSR1-ERG positive Ewing sarcoma - Molecular Diagnostic Pitfalls**

- Unbalanced Fusions – False Negative EWSR1 FISH break-apart

- Chen S, Genes Chromosomes Cancer 2016

**EWSR1-ERG Positive**

- Break/inversion – EWSR1 and ERG = opposite directions of transcriptions
**FUS rearranged Ewing sarcomas**

- EWSR1 & FUS: RNA binding proteins with structural and functional overlap:
  - Myxoid liposarcoma
  - Angiomatoid fibrous histiocytoma
  - Ewing sarcoma
- Incidence: 15 cases reported
  - Our study: 7/85 (8%) of SBRCT cases lacking fusions
  - 8 reported cases in the literature
- FUS-positive Ewing’s gene partners: **ERG, FEV, NFATC2**
- Similar to EWSR1-positive Ewing sarcoma

Chen S, Genes Chromosomes Cancer 2016

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**Ewing sarcoma with unusual pathologic features, but typical genetics**

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**Ewing Sarcomas with FUS-ERG & FUS-FEV fusion**

- **Ewing Sarcoma with Complex Epithelial Differentiation**
  - Adamantinoma-like Ewing sarcoma (Bridge JA, AJSP 1999)

- 3/F knee soft tissue
FISH Analysis: EWSR1 and FLI1 gene rearrangements

**Ewing Sarcoma with Complex Epithelial Differentiation**

**Adamantinoma-like Ewing Sarcoma**

**Head and Neck Adamantinoma-like Ewing Sarcoma**

Differential Diagnosis:
- NUT midline carcinoma
- Anaplastic carcinoma
- Basal cell adenocarcinoma
- Adenoid cystic carcinoma
- SNUC
- Olfactory neuroblastoma
- Medullary carcinoma
- CASTLE

Head and Neck Adamantinoma-like Ewing Sarcoma

- Squamous pearls
- Nested or micro-cystic growth
- Intra-epithelial growth

# Site  Age  Sex
1  Ethmoid 37  F
2  Ethmoid 21  M
3  Orbit 7  F
4  Parotid 56  F
5  Parotid 40  F
6  Thyroid 19  M

Lezcano C, Head Neck Pathol 2015; Bishop JA, AJSP 2015

Lezcano C, Head Neck Pathol 2015; Bishop JA, AJSP 2015
Head and Neck Adamantinoma-like ES

Molecular confirmation: EWSR1-FLI1 fusion

Ewing sarcoma-like tumors
- lacking the classic EWSR1-ETS fusion -

2013 WHO – Undifferentiated Round Cell and Spindle Cell Sarcomas

1. EWSR1 fusion to an non-ETS transcription factor
2. Alternative (non-EWSR1) fusions
3. Fusion-negative

Adamantinoma-like Ewing sarcoma with FUS-FEV fusion

Ewing Sarcoma-like Tumors

1. EWSR1 fusion with a non-ETS transcription factor
   - EWSR1-NFATc2 (TF, T cell & neuronal development)
     Szuhai K et al. Clin Cancer Res 2009
   - EWSR1-SMARCA5 (chromatin remodeling gene)
     Sumegi J et al. Modern Pathol 2011
   - EWSR1-SP3 (ZNF-transcription factor)

23/M, anterior mediastinum (NUT neg carcinoma)
Ewing Sarcoma-like Tumors

2. Alternative Fusions (non-EWSR1/non-ETS)

- CIC-DUX4/CIC-FOXO4/CIC-NUTM1
- BCOR abnormalities
  - ABCOR-CCNB3 inversion
  - ABCOR-MAML3 fusions
  - ABCOR Internal Tandem Duplications (ITDs)

Sarcomas With CIC-rearrangements are a Distinct Pathologic Entity With Aggressive Outcome
A Clinicopathologic and Molecular Study of 115 Cases

- Age range: 6-81 years, mean 32 (22% pediatric)
- Gender: 55% males, 45% females
- Tumor location:
  - Soft tissue (86%): equal distribution extremity/trunk
  - Visceral (12%)
  - Bone (3%)
- Tumor size: 64% >5 cm

Antonescu CR, AJSP 2017

CIC-DUX4 positive SBRCT

59/M, Scapular/axillary mass >10 cm

s/p neoadjuvant chemo; poor pathologic response (20-30% necrosis)
CIC-DUX4 positive SBRCTs

21/M, neck ST

24/F, buttock

Immunoprofile in CIC-DUX4 fusion positive SBRCTs

CD99 Immunostaining Variability

23% diffuse
61% patchy
16% negative
Immunoprofile in **CIC-DUX4** fusion positive SBRCs

WT1 and ETV4 highly sensitive immunomarkers

Specht K, Genes Chromosome Cancer 2014
Hung YP, Modern Pathology 2016
Kao YC, Genes Chromosome Cancer 2017

**Molecular Diagnosis of CIC-DUX4 positive SBRCs**

FISH break-apart assay of CIC gene

FISH CIC-DUX4 fusion assay

**Ewing Sarcoma-like Tumors – with BCOR gene abnormalities**

1. BCOR-CCNB3 inversion
2. BCOR-MAML3 fusions
3. BCOR Internal Tandem Duplications
1. Ewing Sarcoma-like Tumors – with BCOR-CCNB3 Fusions

- BCOR-CCNB3 (testis-specific cyclin B3) overexpression
- Pierron G et al. Nature Genetics 2012

- EWS-Fli1
- BCOR-CCNB3
- Intra-X- chromosome paracentric inversion
- 24 cases
- 80% bone
- 67% males
- 75% children

Kao YC et al, AJSP 2018

- Age: mean 15 (range 2-44)
- Gender striking male predominance M:F= 31:5
- Location: bone (55%), soft tissue (39%)
- IHC: BCOR overexpression
- Outcome: 72% 5-year overall survival

BCOR-CCNB3 positive Ewing Sarcoma-like Tumor

Femoral tumor with extraosseous extension
**IHC of BCOR-CCNB3 sarcomas**

- BCOR
- CCNB3
- SATB2
- TLE1
- Cyclin D1
- CD99

**FISH Fusion Assay for Detection of Break/Inversion**

BCOR-CCNB3 positive Ewing Sarcoma-like Tumor

Normal

Red T', CCNB3
Green T' BCOR
(9 Mb away, opposite directions)

**BCOR-CCNB3 sarcomas have favorable outcome**

Kao YC, AJSP 2018
2. SBRCs with BCOR Gene Rearrangements

- BCOR break-apart abnormalities were detected in 11% (8/75) of EWSR1/FUS/CIC-negative SBRCs:
  - BCOR-MAML3 (n=2)
  - BCOR-ZC3H7B (n=2)
  - BCOR (n=4)

- Morphology: round & spindled, no difference between genetic subgroups

- CD99 immunohistochemistry variable

3. BCOR Internal Tandem Duplications (ITD) in a Subset of Infantile Soft Tissue SBRCs

- 41% (9/22) infantile soft tissue SBRCs (< 1 year-old)
- Location: Trunk / Retroperitoneum / Pelvic cavity / Head and neck
High Sensitivity of BCOR IHC Overexpression in BCOR-ITD SBRCT

Chest wall, 2wk/F

Anti-BCOR monoclonal antibody C-10 (sc-514576)

Larynx, S mo/M


Clear Cell Sarcoma of Kidney (CCSK) Genetics

Consistent in-frame internal tandem duplications (ITD) of BCOR characterize clear cell sarcoma of the kidney

100% (20/20) CCSK had ITD in exon 16 of BCOR

PrIMITIVE MYXOID MESENCHYMAL TUMOR OF INFANCY (PMMTI)

N=6/7 (2 previously reported) positive for BCOR ITD

Overlapping Morphologic Features

CCSK

BCOR-ITD SBRCT

Courtesy Pedram Argani

Uniform round cells, arborizing capillaries
Overlapping Morphologic Features

Cellular septa

Myxoid stroma, micro-cystic change

BCOR overexpression unifies different sarcomas with BCOR genetic abnormalities into one sarcoma family.
Summary

- Classic Ewing sarcomas with *EWSR1-ETS* fusions show a monotonous cytomorphology and diffuse CD99 reactivity.

- Ewing sarcoma-like tumors with *CIC-DUX4* are common in adults, soft tissue location, a less uniform cytomorphology, variable CD99 staining and often WT1 & ETV4 nuclear expression. They typically follow an aggressive clinical course and less sensitive to cytotoxic chemotherapy.

Summary

- The histologic and genetic overlap of a subset of infantile SBRCT and PMMTI with CCSK suggests a single pathologic entity.

- *BCOR-ITD* infantile SBRCT/CCSK also share morphologic features and expression profiles with *BCOR-CCNB3* and *BCOR-MAML3* sarcomas, suggesting that *BCOR overexpression* is a critical pathogenetic driver in this group of tumors.