Recent Advances In Select Round Cell Sarcomas

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Translocation-Associated Sarcomas

• Monomorphic histomorphology
• Simple karyotype
• Distinctive clinical features
SRCS in Younger Patients

- Significant subset are small round cell sarcomas
  - ARMS
  - Myxoid/round cell liposarcoma
  - DSRCT
  - PDSS
  - Ewing family tumors (EFT)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>SCCA</th>
<th>MM</th>
<th>ML</th>
<th>Ewing/PNET</th>
<th>RMS</th>
<th>PDSS</th>
<th>DSRCT</th>
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<tr>
<td>PANK</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>Rare</td>
<td>+</td>
<td>+</td>
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<tr>
<td>S-100</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>Rare</td>
<td>+/-</td>
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<td>CD45</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>TdT</td>
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<td>+/-</td>
<td>Rare</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD99</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
<td>Rare</td>
</tr>
</tbody>
</table>

Ab, antibody; SCCA, small cell carcinoma; MM, melanoma; ML, lymphoma; PNET, primitive neuroectodermal tumor; RMS, rhabdomyosarcoma; PDSS, poorly differentiated synovial sarcoma; DSRCT, desmoplastic small round cell tumor
Ewing Sarcoma/PNET
Ewing Family Tumors (EFT)
• Rare highly malignant SRBCT of bone or ST
• Large rapidly growing mass
• Children & adolescents, but occurs at any age
• Extraskeletal ES/PNET most common in deep ST of extremities, but any site including superficial
• 10-year SR 60% with multimodality Tx
EWS (22q12) Break-apart probe

- t(11;22), t(21;22) – EWS/PNET (FLI1/EWS, ERG/EWS)
- t(11;22), t(21;22) – DSRCT (WT1/EWS, E66/EWS)
- t(12;22) – Clear Cell Sarcoma (ATF1/EWS)
- t(9;22) – ES Myxoid Chondrosarcoma (CHN/EWS)
- t(16;22) – Myxoid/Round Cell Liposarcoma (CHOP/EWS)
Primary Cutaneous Ewing Sarcoma

Pan-CK

pCK

EMA
Promiscuous partnerships in Ewing's sarcoma

Sadia Sarkar, Stephen L. Lessnick

Cancer Genetics

Review

Ewing's sarcoma is a highly aggressive bone and soft tissue sarcoma of children and young adults. At the molecular level, Ewing's sarcoma is characterized by balanced reciprocal translocations, t(11;22)(q24;q12), which encode an oncogenic fusion protein and transcription factor EWS-ETS. The fusion-specific genomic lesion results in the aberrant expression of the EWS-ETS fusion, which is a member of the TFIID family of RNA-binding proteins and the catalytic subunit of P-TEFb, a member of the ETS family of transcription factors. In addition to P-TEFb, several transcription factors and cofactors have been implicated in the pathogenesis of Ewing's sarcoma and some of these have been shown to be associated with the disease. EWS-ETS fusion expression is driven by the EWS-ETS fusion protein and translocation. This review aims to summarize the growing list of fusion proteins that characterize Ewing's sarcoma and to highlight important questions that need to be addressed to better understand the biology underlying this disease.

Keywords: Ewing's sarcoma, transcription factors, fusion proteins
Difficult To Classify SRCSTS

- Group of poorly characterized small round cell soft tissue sarcoma (SRCSTS)
- Subset with t(4;19)(q35;q13.1) translocation (CIC-DUX4)
### Ewing & Ewing-like family tumors translocations

<table>
<thead>
<tr>
<th>Gene fusions, chromosomal translocations</th>
<th>Karyotype</th>
<th>Anatomic location</th>
<th>Morphology</th>
<th>Expression markers</th>
<th>references</th>
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</thead>
<tbody>
<tr>
<td>EWSR1-FLI1</td>
<td>t(11;22)(q24;q12)</td>
<td>Bone or soft tissues</td>
<td>+++</td>
<td>FLI1</td>
<td>Delattre et al. (1994)</td>
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<tr>
<td>EWSR1-ERG</td>
<td>t(21;22)(q22;q12)</td>
<td>+++</td>
<td>ERG</td>
<td>Sorensen et al. (1994)</td>
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<tr>
<td>EWSR1-ETV1</td>
<td>t(7;22)(p22;q12)</td>
<td>Bone or soft tissues</td>
<td>+</td>
<td>NSE, S100, DES, EMA</td>
<td>Jeon al. (1995)</td>
</tr>
<tr>
<td>EWSR1-ETV4</td>
<td>t(7;22)(p21;q12)</td>
<td>Extraosseous</td>
<td>+++</td>
<td>Kaneko et al. (1996)</td>
<td></td>
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<tr>
<td>EWSR1-FEV</td>
<td>t(2;22)(q35;q12)</td>
<td>Extraosseous</td>
<td>+++</td>
<td>Peter et al. (1997)</td>
<td></td>
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<tr>
<td>EWSR1-NFATC2</td>
<td>t(20;22)(q13;q12)</td>
<td>Bone</td>
<td>So-called 'atypical Ewing sarcoma'</td>
<td>+++</td>
<td>None</td>
</tr>
<tr>
<td>EWSR1-SP3</td>
<td>t(2;22)(q31;q12)</td>
<td>Bone or soft tissues</td>
<td>So-called 'atypical Ewing sarcoma'</td>
<td>+++</td>
<td>NSE</td>
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<tr>
<td>EWSR1-PATZ1</td>
<td>inv(22) in t(1;22)</td>
<td>Chest wall</td>
<td>PNET</td>
<td>+++</td>
<td>DES, keratins</td>
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<tr>
<td>EWSR1-SMARCA5</td>
<td>t(4;22)(q31;q12)</td>
<td>Lumbar spine</td>
<td>So-called 'atypical Ewing sarcoma'</td>
<td>+++</td>
<td>NSE, Synaptophysis</td>
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<tr>
<td>Round cell sarcoma with EWSR1-non ETS rearrangement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EWSR1-ETV4</td>
<td>t(2;16)(q35;p11)</td>
<td>Bone (clavicle)</td>
<td>Ewing sarcoma</td>
<td>+++</td>
<td>None</td>
</tr>
<tr>
<td>CIC-DUX4</td>
<td>t(4;19)(q35;q13)</td>
<td>Soft tissues</td>
<td>URCS or so-called 'atypical Ewing sarcoma'</td>
<td>Weak focal WT1</td>
<td>Kawamura-Saito et al. (2006)</td>
</tr>
<tr>
<td>t(10;19)(q26;q13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BCOR-CCNB3</td>
<td>inv(X)(p11)</td>
<td>Bone</td>
<td>URCS</td>
<td>50%+++</td>
<td>CCNB3</td>
</tr>
<tr>
<td>Undifferentiated round cell sarcoma</td>
<td>None (yet!)</td>
<td>Non recurrent</td>
<td>Variable</td>
<td>URCS</td>
<td>- Variable</td>
</tr>
</tbody>
</table>

**Abbreviations:**
- PNET, peripheral neuroectodermal tumor
- URCS, undifferentiated round cell sarcoma
- +++, strong membranous expression
- -, no expression.

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### Undifferentiated / unclassified sarcomas

#### 2013

- Undifferentiated spindle cell sarcoma
- Undifferentiated pleomorphic sarcoma
- Undifferentiated round cell sarcoma
- Undifferentiated epithelioid sarcoma
- Undifferentiated sarcoma NOS

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### Undifferentiated / unclassified sarcomas

- Previously included in the fibrohistiocytic tumours, namely “malignant fibrous histiocytomas”
- Lack identifiable line of differentiation when characterized by presently available technology
- Dedifferentiated types of specific sarcomas are not included in this category
- Lack distinct clinical or morphological characteristics
- Genetic subgroups are emerging within this family……stay tuned
**Undifferentiated round cell and spindle cell sarcoma**

- **EWSR1** involved in non-ETS fusions with: 
  - *PATZ1*, *POUSF1*, *SMARCA5*, *NFATC2* or *SP3*
- Another recurrent rearrangement involves **CIC-DUX4** fusion gene/protein upregulates genes of the **PEA3** subclass of **ETS** family
- **BCOR-CCNB1** fusions
- One or more separate entities, or best classified as variants of Ewing sarcoma?

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**1(q41): A Recurrent Change in Primitive Mesenchymal Tumors?**

K. E. Richkind, S. G. Romainsky, and J. Z. Finklestein

**Abstract**

We report a recurring balanced (1;19)(q41;q31) on the long arm of chromosome 1 in a patient with a midline, non-specific, small round cell neoplasm. The lesion was identified in a multiparametric study of ultrastructural features in a patient with a midline non-specific neoplasm of the anterior mediastinum. The recurrent change in the patient's tumor shows a structural similarity to the recurrent (1;19)(q41;q31) found in the human testicular seminoma. The (1;19)(q41;q31) translocation in the patient's tumor is consistent with a developmental abnormality in the medullary retinoblastoma cell line that is a cell of primitive mesenchymal origin.


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**Fusion between CIC and DUX4 up-regulates PEA3 family genes in Ewing-like sarcomas with t(4;19)(q35;q13) translocation**

Mitsunori Iwai, Kiyotaka Nakamura, Misaki Matsuda, Yuki Nishimori, Hiroshi Nishimura, Tetsuya Nakamura, Hiroshi Nakamura, Tetsuya Nakamura, Masahide Takasu, Takashi Takasu, Takahiro Takasu, Takahiro Nakamura

**Abstract**

Fusion between CIC and DUX4 up-regulates PEA3 family genes in Ewing-like sarcomas with t(4;19)(q35;q13) translocation.

Human Molecular Genetics 2006; Vol. 15, No. 11 2299-2307
Advance Access published on May 22, 2006

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CIC-DUX4 oncogene

N-terminus  CIC  DUX4  C-terminus

CIC (loc: 19q13.2)
- Sox-related HMG subfamily
- Intact HMG box DNA-binding domain

DUX4 (loc: 4q35.2)
- DUX family, 2 homeobox domains
- Loss of DNA-binding homeodomains

Transcription factor: Upregulate expression PEA3 family genes
- ETV1, ETV4, ETV5 (ERM) (may be translocation partners for EWSR1)


<table>
<thead>
<tr>
<th>Article</th>
<th>Pediatric</th>
<th>Adult</th>
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</thead>
</table>

Total # cases: 8 7

Undifferentiated Small Round Cell Sarcoma With t(4;19)(p16;q13.2) Chromosomal Abnormality: A Novel Highly Aggressive Soft Tissue Tumor With Distinctive Histopathology

Purpose

- Further characterize CIC-DUX4 SRCSTS
  - Clinicopathologic features
  - Cytogenetic
  - Molecular
  - Immunohistochemical

Database Search

- U of M & William Beaumont Hospital
- Small round cell soft tissue sarcomas
  - Difficult to precisely classify
  - Lacked molecular or cytogenetic evidence of known sarcoma-associated translocations
- 20 candidates with FFPET and/or FFT
- CIC-DUX4 fusion +
  - Histologic, IHC & clinical features evaluated
Study Design

RT-PCR

RNA isolated from FFPE & FFT
Reverse transcriptase
Nested PCR for CIC-DUX4 transcript
  • Amplicons identified by AGE
  • + rxns Sanger sequenced

Study Design

RT-PCR

Assays to exclude other SRCSTS
  • EWS-FLI1 (exons 7/exon 6; exon 7/exon 9)
  • EWS-ERG (exon 7/ exon 6)
  • EWS-WT1
  • SYT-SSX1 and SYT-SSX2
  • GAPDH (house-keeping gene)

Interphase FISH
Translocation Probes

Fusion Probes
Single or dual fusion (increased specificity)

Break-Apart Probes
Multiple fusion partners with common breakpoint
FISH Enumeration Probes

- Peri-Centromeric
  - Repetitive (alphoid) sequences

- Locus Specific
  - Specific gene loci

Study Design

**INTERPHASE FISH**

- EWS break apart
- SYT break apart

Negative *EWSR1* Break-apart FISH
Study Design

TWO INTERPHASE FISH STRATEGIES

1. CIC-DUX4 fusion
   - RP11-317E13 (FITC centromeric to CIC)
   - RP11-521G19 (TRITC telomeric to DUX4)
**CIC-DUX4 Fusion FISH**

CIC-DUX4 fusion + = yellow fusion signal in majority of nuclei examined

**Study Design**

**TWO INTERPHASE FISH STRATEGIES**

2. CIC rearrangement (#chr 19 homologs)
   - RP11-46I12 (FITC near chr 19 centromere)
   - RP11-569M1 (TRITC spans entire CIC)

**Chr. 19 Break-apart FISH**

CIC rearrangement = 3 TRITC (red) and 2 FITC (green "control") signals
**CIC Rearrangement FISH**

CIC rearrangement = 3 TRITC (red) and 2 FITC (green "control") signals

**Study Design**

**RT-PCR**

*CIC-DUX4*, nested RT-PCR

Exclusion EWS, Synovial Sarcoma, DSRCT

- EWS-FLI1 (exon 7/exon 6, exon 7/exon 9)
- EWS-ERG (exon 7/exon 6)
- EWS-WT1
- SYT-SSX1
- SYT-SSX2

**Conventional Cytogenetics**

**Study Design**

**Immunohistochemistry**

- CD99
- INI1
- S100
- Desmin
- Myogenin
- TLE1
- Pan-cytokeratin
Results: CIC-DUX4 positive cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Cytogenetics</th>
<th>RT-PCR</th>
<th>FISH</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CIC-DUX4</td>
<td>EWS</td>
</tr>
<tr>
<td>16</td>
<td>t(4;19)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>t(4;19)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>N/A</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td>N/A</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

16 excluded of which 9 had EWS-FLI1 and 7 negative by all assays

Case 17 Pre-treatment Karyotype

46+47,X,add(2)(p16),t(3;7)(p24.2;p13),t(4;19)(q35;q13.1),+8, t(11;17)(q13;q25),add(16)(p13.1),+mar2[cp6]

Case 17

4;19(q13.1)(p13.1)
Case 16
46,XY,t(X;1)(q11.2;p34),+del(1)(p22p36),
t(3;20)(p21;q13.3),+del(1)(p22p36),
del(13)(q12.3q14),
-14[cp17]

RT-PCR for CIC-DUX4 Fusion

Sanger Sequencing Fusion Variant 1
Results: *CIC-DUX4* positive cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Primary tumor location</th>
<th>Size (cm)</th>
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<tbody>
<tr>
<td>16</td>
<td>32</td>
<td>F</td>
<td>Pelvis (R. perineum/gluteal)</td>
<td>14.0</td>
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<tr>
<td>17</td>
<td>25</td>
<td>F</td>
<td>Extremity (R. calf)</td>
<td>11.0</td>
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<tr>
<td>21</td>
<td>43</td>
<td>M</td>
<td>Extremity (L. knee)</td>
<td>9.8</td>
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<tr>
<td>23</td>
<td>20</td>
<td>F</td>
<td>Extremity (L. shoulder)</td>
<td>6.0</td>
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</table>

Case 17

![Case 17 images]
Lobular growth pattern
Geographic necrosis
Closely spaced round cells
Slight nuclear pleomorphism
Course chromatin
Large nucleoli
High mitotic rate
Indistinct cytoplasm
Focal cytoplasmic clearing
Focal myxoid matrix

Immunohistochemistry

<table>
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<tr>
<th>Case</th>
<th>CD99</th>
<th>Pan CK</th>
<th>TLE1</th>
<th>S100</th>
<th>Desmin</th>
<th>Myogenin</th>
<th>INI1</th>
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<tbody>
<tr>
<td>16</td>
<td>+**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>+**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>+</td>
</tr>
<tr>
<td>21</td>
<td>+**</td>
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<td>23</td>
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<td>-</td>
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* focal  ** very focal
### Clinical Outcome

<table>
<thead>
<tr>
<th>Case</th>
<th>Recurrence</th>
<th>Metastasis</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Survival (mo)</th>
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<tbody>
<tr>
<td>16</td>
<td>Yes</td>
<td>Lung *</td>
<td>Chemotherapy, Surgical resection, Radiotherapy</td>
<td>DOD</td>
<td>14.1</td>
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<tr>
<td>17</td>
<td>No</td>
<td>Lung *</td>
<td>Chemotherapy, Surgical resection</td>
<td>DOD</td>
<td>10.2</td>
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<tr>
<td>21</td>
<td>No</td>
<td>Pelvis **</td>
<td>Surgical resection, Radiotherapy</td>
<td>DOD</td>
<td>14.2</td>
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<tr>
<td>23</td>
<td>No</td>
<td>Lung *</td>
<td>Chemotherapy, Surgical resection, Radiotherapy</td>
<td>DOD</td>
<td>16.8</td>
</tr>
</tbody>
</table>

* * synchronous  ** metachronous

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### CIC-DUX4 Sarcoma

#### Case Information
- **Cases**: 19
  - 8 Pediatric : 11 Adult
- **Age**: 20 years (6 – 62 years)
- **Sex**: 11 Females : 8 Males
- **Size**: 8.4 cm (1.5 – 14 cm)
- **Location**: Soft tissue: 8 Extremity / 4 Trunk / 5 Pelvis / 2 H&N
- **Metastases**: 11 cases: 10 Lung / 2 Brain / 2 Pelvis / 1 Bone
- **Histology**: Small round cell histology
- **CD99**: 12 weak focal / 4 positive / 2 negative / 1 not done
- **Outcome**: 8 DOD avg 12 (9-17) months
  - 1 AWD 24 months
  - 4 NED 6, 7, 7, 30 months
  - 6 Lost or NA

#### Karyotype

- **Case 16**: 46,XX,i(1)(q11.2),del(1)(p12q22),+8,der(1)(p12q22),t(3;20)(q21q36),t(4;19)(q35;q13.1),+6,del(13)(q12.3q14),+mar[15]
- **Case 17**: 45,XX,i(1)(q11.2),+6,der(1)(p12q22),t(3;7)(p11.2q13),t(4;19)(q13.1q13.3),+8,der(15)(q15.1q15.3),del(13)(q12.3q14),+mar[10]
- **Somers 2004**: 47,XX,i(1)(q11.2),+8,der(1)(p12q22),t(3;7)(p11.2q13),+6,der(15)(q15.1q15.3),+mar[10]
- **Kawamura-Saito 2006**: 48,XY,t(4;19)(q35;q13),+6,17,20
- **Kawamura-Saito 2006**: 48,XY,t(4;19)(q35;q13),+6,17,20
- **Rakheja 2008**: 46,XX,i(1)(q11.2),+6,17,20
- **Ricklin 1996**: 46,XY,i(1)(q11.2),+6,17,20

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UNDIFFERENTIATED “ROUND CELL” SARCOMA WITH BCOR-CCNB3
Conclusions

- CIC-DUX4 SRCSTS is a novel recurrent translocation-associated sarcoma with a distinctive histopathology.
- Studies have confirmed their aggressive clinical behavior and rapid development of resistance to conventional therapy.
- BCOR-CCNB3 (BCS) have histologic, IHC and gene expression profiles distinct from other round cell sarcomas such as ES and CIC-DUX4 tumors.
- Most BCS appear to be chemosensitive to ES-based therapy protocols.
- The OS of BCS seems more favorable than CIC-rearranged sarcomas, but similar to ES and SS.

[Cityscape image]