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Molecular characterization of an *EWSR1–POU5F1* fusion associated with a t(6;22) in an undifferentiated soft tissue sarcoma

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We report a soft tissue sarcoma from the thigh with morphologic features resembling Ewing sarcoma, clear cell sarcoma, and myoepithelial tumor of soft tissue. In addition, the genetic and immunohistochemical findings do not correspond to any established pattern, so the tumor does not clearly fit into any one classification. The karyotype analysis revealed a rare chromosomal rearrangement, t(6;22)(p22;q12), that previously has been reported in bone and epithelial tumors. Molecular studies confirmed the presence of an *EWSR1–POU5F1* fusion creating a chimeric gene with the N-terminal transcriptional activation domain of *EWSR1* and the C-terminal POU DNA binding domain of *POU5F1*. This report is novel in that to our knowledge, it is the first complete molecular characterization of an *EWSR1–POU5F1* fusion in a soft tissue sarcoma. Evaluation of existing data on the known *EWSR1–POU5F1* tumors suggests that the fusion gene functions in a wide variety of cell types and may modify the differentiation state of cells, resulting in susceptibility to tumorigenesis.

Keywords Chromosome 22, chromosome 6, *EWSR1*, *POU5F1*, soft tissue sarcoma

We describe a soft tissue tumor associated with an *EWSR1* rearrangement involving a rare fusion partner, *POU5F1*. Chromosomal rearrangements of the *EWSR1* gene are well known and usually involve one of the several members of the ETS family of transcription factors, including FLI1, ERG, ETV1, ETV4, EIAF, FEV, and ZSG (1), resulting in small round cell tumors of the bone and soft tissue (Ewing family tumors). Depending on the specific rearrangement, the resulting chimeric protein is likely a causative factor in the malignant transformation (1–3). At first glance, the *EWSR1–POU5F1* translocation, t(6;22)(q22;q12), seems to be a variant *EWSR1* rearrangement with a new translocation partner on the short arm of chromosome 6, but more detailed analyses have revealed that the resulting tumors do not fit the classic pattern of *EWSR1* gene rearrangements.

Morphologically and phenotypically, the tumor presented here seems to be an undifferentiated round cell sarcoma, although it has some features suggestive of clear cell sarcoma, Ewing sarcoma, or a myoepithelial tumor of soft tissue, which is a generalized classification that combines into one group a variety of tumors that had previously been categorized as separate entities (4). The unusual molecular genetic findings further complicate the picture and suggest that the tumor may belong to a new class of malignancy. The clinical and pathologic features of this pediatric tumor are discussed.

Materials and methods

Patient data

A 7-year-old previously healthy girl presented with a lump in the left anterior proximal thigh. The mass was mildly tender, and the overlying skin was slightly purple. A magnetic resonance image of the region showed an enhancing 4.0 × 2.0 cm lesion over the tensor fascia latae and gluteus medius muscle

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(Figure 1); a left inguinal lymph node (~0.7 cm) was noted. A positron emission tomographic scan revealed only the primary tumor. Metastatic evaluation, including chest, abdominal, and pelvic computed tomographic scans, bone scan, and bone marrow biopsy, were negative for the disease. The patient had no significant medical or family history. Surgical resection of the mass was performed, and because the resection margins were focally involved by tumor, a repeat excision was performed. Pathologic examination of this second specimen revealed a small metastatic tumor focus in one of two lymph nodes, but no residual tumor was found in the surgical bed.

The patient received seven cycles of therapy at three week intervals after surgery, following the Children's Oncology Group trial 9553 for non-rhabdomyosarcoma tumors (regimen C). The agents were ifosfamide (3 g/m^2/day intravenously over 4 hours for 3 days) and doxorubicin (37.5 mg/m^2/day continuous infusion over 48 hours). Two cycles (ifosfamide and doxorubicin) were administered before radiotherapy (179 cGy in 37 daily fractions over 51 days; cumulative dose of 6,623 cGy). The radiation field included the involved lymph node. Ifosfamide alone was given for cycles 3–4 (during radiotherapy). Ifosfamide and doxorubicin were administered for cycles 5–6. Doxorubicin alone was administered for cycle 7 (final cumulative dose of 375 mg/m^2). The patient was free of disease 24 months after completing therapy.

Immunohistochemistry

Specimens were fixed in formalin and routinely processed for hematoxylin and eosin staining. Immunohistochemistry was performed with an UltraView universal DAB detection kit (Ventana, Tucson, AZ) with hematoxylin counterstain. The following panel of antibodies was used: S-100 (NeoMarker, Rocklin, CA), vimentin (Ventana, Tucson, AZ), desmin (Ventana, Tucson, AZ), myogenin (NeoMarker, Rocklin, CA), myo-D1 (NeoMarker, Rocklin, CA), synaptophysin (Ventana, Tucson, AZ), chromogranin (Ventana, Tucson, AZ), NB84a (NeoMarker, Rocklin, CA), GFAP (Ventana, Tucson, AZ), HMB45 (Ventana, Tucson, AZ), MART-1 (Ventana, Tucson, AZ), CD45 (LCA) (Ventana, Tucson, AZ), CD99 (Vector, Burlingame, CA), CD34 (Ventana, Tucson, AZ), epithelial membrane antigen (EMA) (Ventana, Tucson, AZ), and cytokeratin AE1/AE3 (NeoMarker, Rocklin, CA).

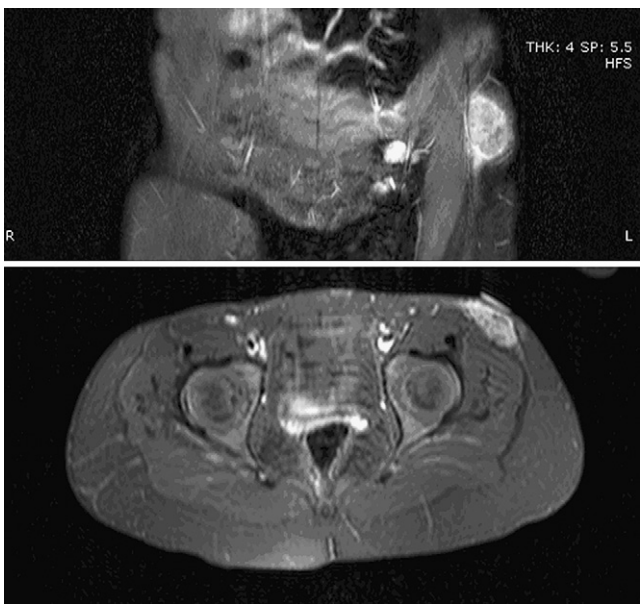

Figure 1 Magnetic resonance image of the upper thighs shows a mass (measuring 4.0 × 2.0 cm) overlying the left tensor fascia latae and gluteus medius musculature. The tumor demonstrates post-gadolinium enhancement. Small inguinal lymph nodes are present; the largest measures ~0.7 cm in the left inguinal chain.

Cytogenetic and molecular analysis

For cytogenetic studies, fresh specimens were disaggregated with collagenase, and the cells were cultured in Ham F-10 medium supplemented with 20% fetal bovine serum, glutamine, and antibiotics. After the cells were harvested, G-band karyotype analysis was performed on Wright stained preparations. Unstained slides for fluorescence in situ hybridization (FISH) were hybridized with the LSI EWSR1 dual-color break-apart probe (Abbott, Des Plaines, IL). FISH was also performed on formalin-fixed, paraffin-embedded sections.

Total RNA was isolated from a fresh frozen tumor specimen with the Pinpoint Slide RNA Isolation System I (Zymo Research, Orange, CA). Reverse transcription (RT)–polymerase chain reaction (PCR) was performed with the Qiagen One-Step RT-PCR kit (Qiagen, Valencia, CA). The PCR primers for the chimeric *EWSR1–POU5F1* fusion were 5′-AATGGCGTCCA CGGATTACAG-3′ (forward; EWS55F) and 5′-TCAGTTTGAA TGCATGGGAGAG-3′ (reverse; POU1125R) (5).

Results

The tumor seemed to originate from the fascia and revealed a well-circumscribed (4.0 × 3.0 × 2.5 cm), lobulated, tan-gray solid mass. Hematoxylin and eosin–stained histologic sections revealed a cellular neoplasm with prominent nesting pattern, infiltrating the adjacent adipose tissues. The tumor nests were separated by bands of collagen and were composed of a uniform population of round to polygonal epithelioid cells with vesicular nuclei, prominent nucleoli, and abundant, clear, vacuolated cytoplasm (Figure 2A–C). Areas of necrosis were present. Mitotic figures were abundant (~7 mitoses per 10 high-power fields). The morphologic appearance of this tumor had some aspects that are consistent with clear cell sarcoma, while other areas were more reminiscent of a Ewing sarcoma/primitive neuroectodermal tumor or a myoepithelial tumor of soft tissue (4,6).

Immunohistochemical studies revealed diffuse strong reactivity with S-100 protein (Figure 2D) and vimentin. No immunoreactivity was observed with other tested markers, including EMA, cytokeratin AE1/AE3, desmin, myogenin, myo-D1, synaptophysin, chromogranin, NB84a, HMB45, MART-1, OCT3/4, CD45 (LCA), CD99, or CD34.

Karyotype analysis of tumor tissue revealed a translocation between the short arm of chromosome 6 and the long arm of chromosome 22, t(6;22)(p22.1q12), in 16 of 20 metaphase cells (Figure 3; see also Supplemental Figure 1). The remaining 4 cells showed a 46,XX chromosome complement. The breakpoint on chromosome 22q was confirmed by FISH analysis to be at the *EWSR1* locus,