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Is undifferentiated sarcoma with *EWSR1-POU5F1* fusion a molecularly defined entity?



CAP TODAY and the Association for Molecular Pathology have teamed up to bring molecular case reports to CAP TODAY readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. The following report comes from Washington University School of Medicine in St. Louis and Detroit Medical Center, Wayne State University School of Medicine. If you would like to submit a case report, please send an email to the AMP at amp@amp.org. For more information about the AMP and all previously published case reports, visit www.amp.org.

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About 30 percent of soft tissue sarcomas are associated with chromosomal translocations leading to the formation of chimeric fusion genes that modify cellular function, leading to dysregulation of proliferation and resulting in clinically and genetically distinct neoplasms. *EWSR1* is a commonly rearranged gene in sarcomas that can fuse as the 5' partner with various genes. *EWSR1*-rearranged sarcomas include angiomatoid fibrous histiocytoma, clear cell sarcoma of soft parts, Ewing sarcoma, desmoplastic small round cell tumor (DSRCT), extraskeletal myxoid chondrosarcoma, myxoid liposarcoma, clear cell sarcoma-like tumors of the gastrointestinal tract, as well as round cell sarcoma with *EWSR1*-non-*ETS* fusions.^{1,2}

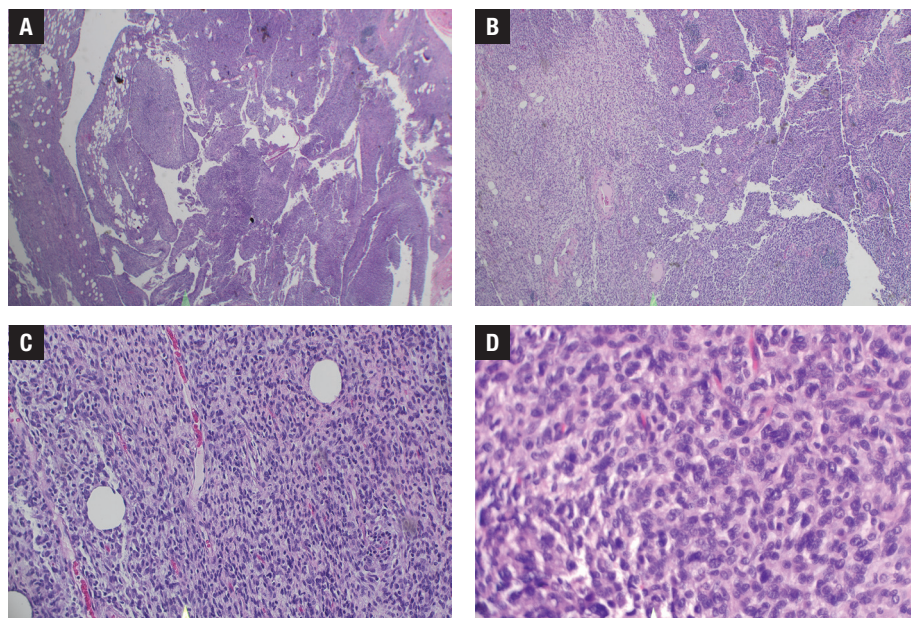


Fig. 1. Undifferentiated sarcoma with *EWSR1-POU5F1* fusion. H&E 20×(A), 40×(B), 100×(C), and 200×(D) magnification.

Herein, we report an unusual case of *EWSR1-POU5F1* fusion in an undifferentiated sarcoma. It has previously been described in myoepithelial tumors,³ but it has been described in undifferentiated sarcomas only a couple of times in the literature, to the best of our knowledge.^{4,5}

Case. A 12-year-old girl presented with an 11.5- × 7-cm mass on the right buttock, which was excised. On gross morphology, it consisted of a variegated edematous, hemorrhagic, necrotic tan-grey, multicystic cut surface. It was reported to progressively enlarge over the prior eight months. Hematoxylin and eosin sections showed a well-demarcated neoplasm comprising cysts of varying sizes with septae showing neoplastic cells with

spindled and round morphology. A few of the tumor cells were mildly pleomorphic (Fig. 1A–D). On immunohistochemistry the tumor cells showed vimentin, S100, and cyclin D1 positivity, and negative staining for CD99, desmin, cytokeratin AE1/AE3, TLE1, BCL-2, CD68, NSE, GFAP, MyoD1, myogenin, and CD34. INI-1 expression was not lost. Despite the relatively low mitotic count and small foci of coagulative necrosis, the histology was in favor of an aggressive sarcoma, infiltrating the surrounding adipose tissue, fascia, and tendon. Importantly, the tumor was subjected to molecular analysis.

The next-generation sequencing solid tumor comprehensive panel showed *EWSR1-POU5F1* t(6;22)

(p21;q12) fusion in the undifferentiated sarcoma. The fusion transcripts were between 5' *EWSR1* [EWS RNA binding protein 1] exon 6 and 3' *POU5F1* [POU class 5 homeobox 1] exon 1, resulting in an in-frame *EWSR1::POU5F1* fusion. The fusion breakpoints are both within the coding regions of their respective genes (Table 1). It was consistent with break apart fluorescence in situ hybridization (FISH), which showed *EWSR1* rearrangement in 92 percent of cells. Based on the constellation of findings, the case was diagnosed as an "undifferentiated sarcoma with *EWSR1* gene rearrangement, low grade."

5' Partner			3' Partner		
Gene	Transcript: exon	Fusion junction	Gene	Transcript: exon	Fusion junction
<i>EWSR1</i>	NM_013986.3: exon 6	Chr22: 29674177	<i>POU5F1</i>	NM_002701.5: exon 1	Chr6: 31138264

Table 1. *EWSR1::POU5F1* fusion breakpoints.

Discussion. The *EWSR1* gene is located on chromosome 22q12. It encodes a nuclear protein with 656 amino acids involved in meiotic division, mitotic spindle stability, microtubule dynamics, DNA repair, and cellular aging. Its C-terminal region, encoded by exons 11 to 13, contains an 87 amino acid RNA binding domain crucial for protein-RNA interactions, transcription, and RNA metabolism. *EWSR1* is a part of the TET family of RNA-binding proteins, grouped with FUS (TLS) and TAF15. These can behave as interchangeable fusion partners in various sarcomas. Commonly, *EWSR1* exon 7 serves as the fusion breakpoint for Ewing sarcoma (*EWSR1-ETS* fusions), myxoid liposarcoma (*EWSR1-DDIT3*), DSRCT (*EWSR1-WT1*), and primary pulmonary myxoid sarcoma (*EWSR1-CREB1*). Other notable fusion sites include exon 8 in clear cell sarcoma (*EWSR1-ATF1*) and exon 12 in ex-

traskeletal myxoid chondrosarcoma (*EWSR1-NR4A3*).

Molecular studies are invaluable as many tumors have overlapping histological features and IHC staining patterns. Morphology itself does not differentiate DSRCT from intra-abdominal Ewing sarcoma, and identifying fusion partner genes helps in making this distinction. *EWSR1-CREB1* is the most prevalent fusion in angiomatoid fibrous histiocytoma (AFH); however, *EWSR1-ATF1* is more common in AFH located in visceral sites.²

EWSR1-POU5F1 fusions have been well established as being associ-

ated with soft tissue myoepithelial tumors, many with histology showing undifferentiated round cells. These cases showed evidence of epithelial differentiation, however, having been positive for EMA and/or pan-cytokeratin. As this case was uniformly negative for EMA and pan-cytokeratin, it was diagnosed as an undifferentiated sarcoma instead of a myoepithelial carcinoma.

A study by Antonescu CR, et al., analyzed 66 myoepithelial tumors arising in soft tissue, bone, and visceral sites to investigate their genetic alterations. Using FISH and molecular assays, the authors found that about 45 percent of these tumors harbored *EWSR1* gene rearrangements, identifying several fusion partners, including *POU5F1*, *PBX1*, and *ZNF444*. The *EWSR1-POU5F1* fusion was recurrent, especially in pediatric soft tissue tumors with clear cell morphology. However, tumors without *EWSR1*

rearrangements were mostly benign and located superficially and had differentiation in ductal lineage. The findings demonstrated that *EWSR1* rearrangements are common and characteristic in extra-salivary myoepithelial tumors, distinguishing them genetically from their salivary gland counterparts and providing a useful molecular marker for diagnosis.³

POU5F1 (OCT3/4) is a key transcription factor that maintains pluripotency in germ and embryonic stem cells. Yamaguchi and colleagues were the first to describe this novel fusion of *EWSR1* and *POU5F1* with a t(6;22)(p21;q12) translocation in an undifferentiated sarcoma. A 39-year-old woman presented with undifferentiated sarcoma of the right pubic bone. The *EWSR1-POU5F1* fusion juxtaposed the transcriptional activation domain of *EWSR1* comprising exons 1 to 6 and the DNA-binding domains of *POU5F1* comprising exons 2 to 5 and part of exon 1. Histology demonstrated undifferentiated spindle-shaped tumor cells with oval nuclei, scant cytoplasm, and diffuse growth pattern. Some areas showed hypercellularity but no bone formation. The IHC findings were similar to those in the current case. It was positive for vimentin, S100, and neuron-specific enolase. The tumor was predominantly cytokeratin negative. It was negative for MIC2 and muscle actin. The resulting chimeric protein likely functioned as an oncogenic transcription factor, driving the undifferentiated, immature characteristics of this bone and soft tissue tumor, which did not fit into any known tumor category.⁵

Deng FM, et al., was the first to completely characterize this fusion in a tumor from the thigh of a seven-year-old girl that showed features of an undifferentiated round cell sarcoma. H&E showed a cellular neoplasm infiltrating the adjacent adipose

tissues. The predominant histological pattern was nesting, separated by bands of collagen. It was composed of a population of uniform round to polygonal clear epithelioid cells with vesicular nuclei, prominent nucleoli, and abundant, vacuolated cytoplasm. There were areas of tumor necrosis and several mitotic figures. The morphological features were overlapping with several soft tissue sarcoma types (resembling Ewing sarcoma, clear cell sarcoma, and myoepithelial tumors) but did not fit an established classification. The IHC studies were positive for S100 and vimentin but negative for cytokeratin AE1/AE3, similar to the findings in our case. Cytogenetic analysis revealed a rare translocation, t(6;22) (p22;q12). Molecular testing demonstrated a fusion between the *EWSR1* gene and *POU5F1* (*EWSR1-POU5F1*), specifically juxtaposing the *EWSR1* N-terminal transactivation domain and the *POU5F1* DNA-binding domain. Interestingly, this fusion may act in diverse cell types and likely affects differentiation states, thereby promoting susceptibility to malignant transformation.⁴

Conclusions. New *EWSR1* fusion associations continue to emerge, and their number and diversity are expected to grow as molecular diagnostic techniques become more advanced and sensitive. Herein, we

describe a rare case of undifferentiated sarcoma with *EWSR1-POU5F1* fusion, which has been described only a few times in the literature. With the widespread availability of NGS, undifferentiated sarcomas with this fusion may emerge as a distinct subclassification of sarcomas. □

1. Romeo S, Dei Tos AP. Soft tissue tumors associated with *EWSR1* translocation. *Virchows Arch.* 2010;456 (2):219–234.
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3. Antonescu CR, Zhang L, Chang NE, et al. *EWSR1-POU5F1* fusion in soft tissue myoepithelial tumors. A molecular analysis of sixty-six cases, including soft tissue, bone, and visceral lesions, showing common involvement of the *EWSR1* gene. *Genes Chromosomes Cancer.* 2010;49(12):1114–1124.
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Test yourself

Here are three questions taken from the case report. Answers are online now at www.amp.org/casereports and will be published next month in CAP TODAY.

1. *EWSR1* rearrangements are characteristically seen in all of the following entities except which one of the following?
 - a. Desmoplastic small round cell tumor.
 - b. Primary pulmonary myxoid sarcoma.
 - c. High-grade endometrial stromal sarcoma.
 - d. Extraskelatal myxoid chondrosarcoma.
2. Which of the following statements is most accurate regarding myoepithelial tumors?
 - a. Less than 20 percent of these tumors harbored *EWSR1* gene rearrangements.
 - b. *EWSR1-POU5F1* fusion is recurrent in pediatric soft tissue tumors with clear cell morphology.
 - c. Myoepithelial tumors demonstrated a lack of epithelial differentiation, having been negative for EMA and/or pan-cytokeratin.
 - d. *EWSR1* rearrangements are not found in extra-salivary myoepithelial tumors.
3. Among the fusion product and soft tissue tumor pairs listed, which of the following are incorrectly paired?
 - a. *EWSR1-DDIT3* and myxoid liposarcoma.
 - b. *EWSR1-CREB1* and angiomatoid fibrous histiocytoma.
 - c. *EWSR1-POU5F1* and undifferentiated round cell sarcoma.
 - d. *EWSR1-NR4A3* and desmoplastic small round cell tumor (DSRCT).