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Undifferentiated round cell sarcomas with novel *SS18-POU5F1* fusions

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Abstract

Despite significant recent advances in characterizing the molecular pathogenesis of undifferentiated round cell neoplasms, rare cases remain unclassified. Here, we report two distinctive undifferentiated round cell tumors occurring in young adults. One tumor presented intrabdominally and the other arose within the abdominal wall. One patient died of disease following local and distance recurrence, despite aggressive chemotherapy and radiotherapy. Morphologically, both tumors were similarly composed of primitive round to epithelioid cells arranged in nests, sheets, and trabecular patterns. The cytoplasm was scant and amphophilic, while the nuclei were round and uniform with brisk mitotic activity. Focal necrosis was present. Immunohistochemically, both tumors were variably positive for S100 and EMA, and one case focally expressed cytokeratin and TLE1. Targeted RNA sequencing revealed in both an identical *SS18-POU5F1* fusion gene. Fluorescence in situ hybridization was performed which confirmed *SS18* and *POU5F1* gene rearrangements. Expression data, relative to over 200 other mesenchymal neoplasms that had undergone targeted RNA sequencing on the same platform, suggested the *SS18-POU5F1* tumors cluster with *EWSR1/FUS-POU5F1*-positive myoepithelial tumors. In view of our limited sample size, additional studies are needed to characterize the breadth of clinical and pathologic findings in these neoplasms. In addition, further investigation is necessary to determine whether this entity represents a clinically aggressive and phenotypically undifferentiated variant of myoepithelial tumors, or perhaps an altogether novel category of undifferentiated round cell sarcoma.

Keywords

fusion; myoepithelial tumor; POU5F1; sarcoma; SS18; synovial sarcoma

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

1 | INTRODUCTION

Historically, gene fusions in mesenchymal tumors were considered “disease-defining” and restricted to a specific histotype, such as *EWSR1-FLI1* in Ewing family tumors (EFTs); as a result, these products could be exploited as reliable molecular diagnostic markers. However, with the advent of next generation sequencing, several themes are emerging, including: (a) molecular pleiotropy, whereby the same genetic event may result in multiple phenotypes, driving the pathogenesis of histologically unrelated neoplasms—such as the *EWSR1-CREB* family of tumors, spanning clear cell sarcoma/clear cell sarcoma-like tumor of the gastrointestinal tract, angiomatoid fibrous histiocytoma, and hyalinizing clear cell carcinoma of salivary gland¹; (b) significant infidelity among genes participating in fusions, resulting in numerous possible fusion product permutations, particularly involving *EWSR1* and *FUS* genes, which can associate with tens of different partners resulting in a wide spectrum of tumor types; and, (c) variability in the fusion transcript structure may result in various domain compositions of the fusion oncoproteins. For the latter, one example is a 5′ *SS18*-, which may fuse with 3′ -*SSX1*, -*SSX2*, -*SSX4*, or -*NEDD4*, resulting in synovial sarcoma; ^{2,3} alternatively, a 3′ -*SS18* may fuse with 5′ *CRTC1*- resulting in an undifferentiated small round cell sarcoma.⁴

Moreover, the relationship between fusion gene and tumor histotype is presumed to be influenced by countless other factors, such as (stem)cell of origin, epigenetic changes, and various stochastic events. While some have questioned the specificity of certain gene fusions as diagnostic markers in mesenchymal neoplasms, it is impossible to overstate the value of these fusion products in morphologically and immunohistochemically undifferentiated neoplasms, such as undifferentiated small round cell sarcomas.

The appropriate classification of undifferentiated small round cell sarcomas has recently come to the forefront with important debates concerning the relationship of EFTs and so-called Ewing-like sarcomas.⁵ Despite a shared round cell morphology, and occasional overlap in clinical presentation, additional transcriptional, and epigenetic investigations have clearly established, for example, that tumors with *CIC-DUX4* and *BCOR* genetic alterations warrant classification as discrete pathologic entities, separate from classic EFTs.^{6–8} However, even with the tremendous advances in the molecular classification of soft tissue tumors, challenges still remain on what constitutes a distinct pathologic entity in the context of primitive phenotypes and overlapping gene products. This study underscores this point, and the diagnostic value of RNA sequencing, with the report of two undifferentiated round cell sarcomas arising in young adults, which harbor novel *SS18-POU5F1* fusion genes.

2 | MATERIALS AND METHODS

In the clinical work-up of two cases with an undifferentiated round cell phenotype, lacking known genetic alterations, targeted RNA sequencing was performed for more definitive subclassification. In both cases this revealed a novel *SS18-POU5F1* gene fusion, prompting an in-depth review of the morphology and immunophenotype of these tumors. Follow-up

information was available in both cases, although admittedly limited for one patient. This study was approved by the Institutional Review Boards at our institutions.

2.1 | RNA sequencing

RNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue using Amsbio's ExpressArt FFPE Clear RNA Ready kit (Amsbio LLC, Cambridge, MA). One case was tested on the TruSight RNA Fusion Panel (Illumina, San Diego, CA), using 100 ng total RNA for RNA-sequencing libraries preparation.⁹ Fragment length was assessed with an RNA 6000 chip on an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). The sample was subjected to targeted RNA sequencing on an Illumina MiSeq at eight samples per flow cell (~3 million reads per sample). All reads were independently aligned with STAR (version 2.3) and BowTie2 against the human reference genome (hg19) for Manta-Fusion and TopHat-Fusion analysis, respectively. The second case was tested by Anchored Multiplex RNA sequencing assay using the Archer FusionPlex Solid tumor Kit (Archer, Boulder, CO).¹⁰ Anchored Multiplex polymerase chain reaction amplicons were sequenced on Illumina MiSeq, and the data were analyzed using the Archer software.

2.2 | Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) on interphase nuclei from paraffin-embedded 4- μ m sections was performed applying custom probes using bacterial artificial chromosomes (BAC) covering and flanking genes of interest. BAC clones for *SS18* and *POU5F1* were chosen according to UCSC genome browser (<http://genome.ucsc.edu>), as previously described.^{11,12} The BAC clones were obtained from BACPAC sources of Children's Hospital of Oakland Research Institute (CHORI) (Oakland, CA) (<https://bacpacresources.org/>). DNA from individual BACs was isolated according to the manufacturer's instructions, labeled with different fluorochromes in a nick translation reaction, denatured, and hybridized to pretreated slides. Slides were then incubated, washed, and mounted with DAPI in an antifade solution, as previously described.¹² The genomic location of each BAC set was verified by hybridizing them to normal metaphase chromosomes. Two hundred successive nuclei were examined using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany), controlled by Isis 5 software (Metasystems, Newton, MA). A positive score was interpreted when at least 20% of the nuclei showed a split-apart signal in the break-apart assay. Nuclei with an incomplete set of signals were omitted from the score.

3 | RESULTS

3.1 | Case 1

An 18-year-old male presented with diffuse abdominal pain, severe nausea, and pruritis. Magnetic resonance imaging identified a 4.8 $\times$ 3.0 $\times$ 2.7 cm lesion at the neck of the pancreas, extending superiorly to the porta hepatis, with additional peripancreatic masses, and para-aortic and retroperitoneal lymph node involvement. A fine needle aspirate was performed showing a primitive round cell malignancy, for which an EFT was favored, despite negative FISH results for *EWSR1*, *FUS*, *CIC*, and *BCOR* gene rearrangements. The patient received standard VAC-chemotherapy (cyclophosphamide, doxorubicin, and

vincristine) with resolution of the symptoms and a significant decrease in tumor size. However, 1-year later, the patient developed local recurrence and liver metastases for which he received radiation and further chemotherapy (cisplatin and doxorubicin). An excision of one of the liver metastases showed a high-grade undifferentiated round-epithelioid cell malignancy arranged in solid sheets and nests, with focal areas of necrosis (Figure 1). The tumor cells contained scant amphophilic cytoplasm. The nuclei were round with vesicular chromatin and small nucleoli, with relatively minor nuclear pleomorphism.

By immunohistochemistry, the tumor was diffusely positive for EMA, with focal immunoreactivity for keratins (AE1:AE3 and Cam5.2), S100, TLE1 (Figure 1) and CD99, with rare synaptophysin; it was negative for p40, p63, SOX10, HMB45, desmin, WT1, chromogranin, INSM1, trypsin, chymotrypsin, PR, OCT4, AFP, CD30, SALL4, GATA3, B-HCG, and HPL. INI1 expression was retained. The Ki67 Proliferative Index was approximately 90%.

The patient progressed and developed mediastinal lymphadenopathy and additional nodules in the liver, which were treated with irinotecan and temozolomide as third-line chemotherapy. The patient ultimately succumbed to the disease 31 months after diagnosis.

3.2 | Case 2

A 29-year-female was found to have a $2.5 \times 1.5 \times 1.4$ cm subcutaneous left lower quadrant/groin mass, over the pubic symphysis. Clinically it was thought to represent a cyst. The lesion was marginally excised with positive margins. Microscopically, at lower power, the lesion showed a multinodular growth within the subcutis. The tumor was composed of undifferentiated round to epithelioid cells arranged in solid sheets and trabeculae (Figure 2). The cytoplasm was scant and amphophilic. The nuclei were round with mild pleomorphism, clumped chromatin, and prominent small nucleoli; there was brisk mitotic activity (>10 per 10 HPFs).

Immunohistochemically, an extensive panel revealed only patchy expression of S100 and weak/focal immunoreactivity for EMA; it was negative for keratins (panCK, CK7, CK20, CK8/18), SOX10, HMB45, MelanA, WT1, CD117, CD99, TLE1, STAT6, BCOR, desmin, myogenin, synaptophysin, TTF1, PLAP, HCG, ER, CD3, CD20, CD30, CD31, CD34, CD43, MUM1, MPO, TdT, and ERG. Expression of INI1, BRG1, and BRM was retained.

FISH studies were performed, but no abnormalities were detected in *EWSR1*, *FUS*, *CIC*, *BCOR*, *NUTM1*, *YWHEA*, or *NCOA2* genes.

The patient received adjuvant chemotherapy and is currently alive with no evidence of disease 10 months after the initial diagnosis.

3.3 | Molecular findings

Targeted RNA sequencing in both cases revealed an identical *SS18-POU5F1* fusion gene; the transcript involved *SS18* exon 10 and *POU5F1* exon 2 (Figure 3). These findings were further validated by FISH using custom BAC probes, confirming both *SS18* and *POU5F1* gene rearrangement in both tumors.

Case 2, which was tested on the TruSight RNA Fusion Panel, was further compared with the expression signature of >200 other tumors tested on the same platform, including mostly translocation-associated subtypes, such as solitary fibrous tumors, dermatofibrosarcoma protuberans, mesenchymal chondrosarcoma, a large spectrum of kinase-fusion mesenchymal tumors, clear cell sarcoma, round cell sarcomas with *CIC-DUX4* and *BCOR* genetic alterations. Unsupervised clustering showed that the *SS18-POU5F1* index case tightly clustered with three other tumors harboring *POU5F1* gene fusions (Figure 3). These tumors represented soft tissue myoepithelial tumors with either *EWSR1-POU5F1* or *FUS-POU5F1* fusions.⁹ The two myoepithelial tumors with *EWSR1-POU5F1* fusion are notable for their morphologic appearance, which consisted of sheets of undifferentiated round to epithelioid cells with scant clear cytoplasm (Figure S1). The third case, harboring a *FUS-POU5F1* fusion, showed a more classic morphologic appearance with epithelioid cells with clear to eosinophilic cytoplasm, arranged in nests and clusters within a fibrotic stromal component (Figure S1). The *POU5F1* genomic cluster did not approximate the three other myoepithelial tumors with alternative fusion non-*POU5F1* partners (*EWSR1-ZNF444*, *FUS-KLF17*), or the two synovial sarcoma cases available on the same platform (Figure 3).

4 | DISCUSSION

Despite significant advances in the molecular classification of undifferentiated round cell tumors, largely through the introduction of next generation sequencing into clinical practice, their diagnosis remains challenging. This is due to considerable morphologic overlap, and complicated by a frequently nonspecific immunoprofile. The promiscuity of certain gene partners, such as *EWSR1* and *FUS* genes, is known to result in a wide spectrum of neoplastic processes and represents a potential diagnostic pitfall. Other genes, however, are increasingly recognized for their ability to participate in alternate fusion products. This not only suggests the potential for misclassification—it emphasizes the need to characterize the breadth of these events, and the mechanisms governing them. To this end, we report two cases of an undifferentiated round cell sarcoma associated with a novel *SS18-POU5F1* fusion gene.

Both patients in this cohort had relatively similar clinical presentations and histopathologic features. The neoplasms occurred in the abdominal cavity and abdominal wall of young adults. Microscopically, the tumors were composed of sheets and trabeculae of round to epithelioid cells with scant amphophilic cytoplasm and round uniform nuclei, exhibiting a high mitotic activity and necrosis. Both cases variably expressed S100 and EMA, and one lesion (case 1) showed focal reactivity for CK AE1:AE3 and TLE1. This immunophenotype raised the possibility of myoepithelial carcinoma, or perhaps a poorly differentiated synovial sarcoma. Some support for the former was suggested by transcriptional analysis of one case (case 2), which showed clustering close to three malignant myoepithelial tumors harboring *EWSR1/FUS-POU5F1* fusions tested on the same targeted RNA sequencing platform; this was distinct from three other myoepithelial tumors with alternative fusions (*EWSR1-ZNF444*, *FUS-KLF17*), and two synovial sarcomas.

In contrast to *EWSR1* and *FUS* genes, *SS18* and *POU5F1* are not prototypical examples of promiscuous fusion partners. Both are ostensibly limited to the pathogenesis of single

diseases, synovial sarcoma, and myoepithelial tumor, respectively.^{9,12,13} Thus, from a molecular standpoint, the differential diagnosis of a tumor driven by a hybrid *SS18-POU5F1* fusion product is tantalizing—its manifestations, as they relate to histotype and biologic potential, difficult to predict. Given a primitive round cell morphology and nonspecific immunophenotype, the differential would include novel molecular variants of synovial sarcoma, malignant myoepithelial tumor, or perhaps an altogether novel round cell sarcoma. There is recent precedence for alternative *SS18* gene partners in sarcoma.^{3,4} First, an *SS18-NEDD4* fusion was recently described as a case report of a renal tumor occurring in a young adult, with a morphology reminiscent of myxoid synovial sarcoma.³ This gene fusion was predicted to fuse *SS18* exon 10 with *NEDD4* exon 10, which encompasses a similar *SS18* breakpoint to synovial sarcomas with canonical fusions, resulting in retention of all but the C terminal 8 amino acids of the *SS18* transcriptional coactivator. The tumor, notably, clustered with other cases of synovial sarcoma on the same RNA-Seq platform.³ Alternatively, a *CRTC1-SS18* fusion was recently reported in two undifferentiated round cell sarcoma of soft tissue, with an Ewing-like morphology and strong CD99 immunopositivity. These arose in the soft tissue of the lower extremity of young adults. Interestingly, the two tumors did not cluster together with other synovial sarcomas with canonical *SS18-SSX* fusion, and thus, are presumed unlikely to represent molecular variants of poorly differentiated synovial sarcoma.⁴

The *SS18-POU5F1* fusion also raises the possibility of a molecular variant of *POU5F1*-positive myoepithelial tumor. Myoepithelial tumors with *EWSR1-POU5F1* fusions represent the most common molecular subset (28%), being prevalent in children and young adults, often presenting in the deep soft tissues of the extremities.⁹ Morphologically, tumors are often characterized by a nested epithelioid morphology with clear cytoplasm, and thin fibrous septa, with a majority (73%) displaying microscopic features in keeping with malignancy.⁹ Moreover, the myoepithelial tumors arising in soft tissue and bone lack a corresponding cell of origin, compared with the lesions from salivary gland or skin, which appear to relate to the normal myoepithelial cells surrounding tubulo-acinar glandular structures.

In a recent comprehensive molecular analysis of myoepithelial tumors, 10% of cases showed an undifferentiated round cell morphology, which resembled round cell sarcoma, such as EFTs or a desmoplastic round cell tumor.⁹ Cases displaying this primitive phenotype had *EWSR1* or *FUS* fusions with the following partners: *POU5F1*, *ZNF444*, and *KLF15*. The results of that study also highlighted the imperfect concordance between the so-called “myoepithelial immunoprofile,” defined by the co-expression of EMA+/- cytokeratin and S100, which is used currently as the gold standard for diagnosis, and the presence of these fusion gene alterations. In fact, 12 (20%) cases lacked this immunoprofile, including seven with classic morphologic features and characteristic *EWSR1/FUS-POU5F1* or *PBX1/3* related fusion, while the remaining five cases had an atypical phenotype, composed of undifferentiated round cell morphology, and were associated with variable gene fusions, including *EWSR1-POU5F1* and *EWSR1-ZNF444*. In addition, myoepithelial tumors with *EWSR1-KLF15*, which so far have been mainly described in children, are also strongly associated with an undifferentiated round cell morphology and a clinically malignant behavior.^{9,14,15} These data further emphasize the significant challenges in diagnosing

myoepithelial tumors, especially at the malignant/high-grade end of the spectrum, without a comprehensive molecular analysis. In fact, three tumors in our prior study occurring in children were initially misinterpreted as EFTs at the outside institutions—based on demonstration of *EWSR1* gene rearrangement alone— and treated with Ewing sarcoma regimens.⁹ Indeed, Stevens et al used the terminology “malignant Ewing-like neoplasm” for an *EWSR1-KLF15*-positive myoepithelial carcinoma arising in the kidney of a 20-year-old, which did not respond to Ewing sarcoma standard chemotherapy regimen.¹⁵ Of interest, the first reported case of *EWSR1-POU5F1* positive tumor, occurring in the pelvis in a 39-year-old woman, also had an undifferentiated round cell phenotype,¹⁶ suggesting that at least a subset of tumors with *POU5F1* gene abnormalities, regardless of its fusion partner, might be associated with a primitive round cell morphology, mimicking EFTs. Further studies are clearly necessary to establish the relationship of undifferentiated tumors harboring so-called “myoepithelial gene fusions” with other undifferentiated round cell sarcomas in the spectrum of what was formally known as “Ewing sarcoma-like” tumors. It would appear that in such cases, demonstration of the specific gene partners is imperative for definitive classification.

POU5F1 is a transcription factor essential for the self-renewal activity and pluripotency of embryonic stem cells and germ cells.^{17,18} Knockdown of *EWSR1-POU5F1* fusions by siRNA mediated gene silencing in a t(6;22) sarcoma-derived GBS6 cell line resulted in a significant decrease of cell proliferation and G1 cell cycle arrest associated with p27(Kip1) up-regulation.¹⁹ The precise mechanism of action of the *SS18-POU5F1* chimera is unknown; although it is conceivable it may involve SS18-mediated transcriptional coactivation,²⁰ which enhances the transcriptional activity of *POU5F1*.²¹

In summary, this limited study identified a novel gene fusion, *SS18-POU5F1*, associated with an undifferentiated round cell phenotype in two patients with a relatively similar clinical presentation. Although the precise subclassification of these tumors remains challenging at the microscopic level, the fusion product identified by RNA sequencing appears to cluster with myoepithelial tumors harboring *EWSR1/FUS-POU5F1* fusions, thereby suggesting molecular kinship with this group of tumors. This study illustrates the practical utility of RNA sequencing in classifying challenging neoplasms by identifying novel gene fusions. Systematic studies are needed to elucidate the underlying mechanisms governing fusion development, characterize their contribution to tumor histogenesis, and identify novel therapeutic strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Pathologic findings of case 1 with a *SS18-POU5F1* fusion. A,B, Low and intermediate magnification showing cells arranged in ill-defined trabeculae and nests within a fibrotic stroma. C, High magnification showing solid sheets of cells with punctate necrosis. D, The cells have scant amphophilic cytoplasm. The nuclei are round and relatively monomorphic, with fine chromatin, small nucleoli, and brisk mitotic activity. By immunohistochemistry, the tumor was positive for EMA, E, with focal expression of cytokeratin AE1:AE3, F, S100, G, and TLE1 (weak, H)

FIGURE 2.

Pathologic findings of case 2 with a *SS18-POU5F1* fusion. A,B, Low to intermediate magnification showing solid nests and trabeculae separated by a delicate fibrous bands. The cells have scant cytoplasm, round nuclei with clumped chromatin and show a brisk mitotic activity is brisk. C, Focal areas with myxoid stromal changes also present. D, The tumor showed positivity for S100 protein

FIGURE 3.

Genomic variability of tumors harboring *POU5F1*-related fusions. A, Diagrammatic representation of the chromosomal locations of *POU5F1* and its three fusion gene partners. Vertical red bars indicate the exact genomic break, while orange and green arrows indicate the direction of transcription for each gene. B, Unsupervised clustering using the targeted RNA sequencing platform on >200 mesenchymal neoplasms, mostly driven by chromosomal translocations, revealed that one of the index case with *SS18-POU5F1* fusions clustered together with two myoepithelial cases with *EWSR1-POU5F1* and one with *FUS-POU5F1*, forming a tight genomic cluster (red lines). These tumors clustered separately from the two synovial sarcomas available on this platform with *SS18* fusions (purple lines), 2 myoepithelial tumors with *FUS-KLF17* (green lines) and one myoepithelial tumor with *EWSR1-ZNF444* (blue line). C–E, Diagrammatic representations of the *FUS-POU5F1*, *EWSR1-POU5F1* and *SS18-POU5F1*, revealing rather similar breakpoints on *POU5F1*, most of its coding region being retained. Protein domains are also represented