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RESEARCH ARTICLE

Clinical and molecular characterization of primary sclerosing epithelioid fibrosarcoma of bone and review of the literature

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Abstract

Sclerosing epithelioid fibrosarcoma (SEF) is a rare sarcoma subtype characterized by monomorphic epithelioid cells embedded in a densely sclerotic collagenous matrix. The overwhelming majority of tumors arise in soft tissues; however, rare cases have been documented to occur primarily in bone. The hallmarks of soft tissue SEF include MUC4 immunoreactivity and the presence of an *EWSR1-CREB3L1* fusion. Rare cases with alternative fusions have also been reported such as *EWSR1-CREB3L2* and *FUS-CREB3L2* transcripts. The molecular alterations of skeletal SEF have not been well-defined, with only rare cases analyzed to date. In this study we investigated the clinicopathologic and molecular features of seven patients presenting with primary osseous SEF. There were 3 males and 4 females, with a mean age at diagnosis of 38 years. All cases had microscopic features within the histologic spectrum of SEF and showed strong and diffuse MUC4 positivity, while lacking SATB2 expression. However, due to its unusual presentation within bone, four cases were initially misinterpreted as either osteosarcoma, Ewing sarcoma or chondroblastoma. Half of the patients with follow-up data developed metastasis. The cases were tested by targeted RNA sequencing, MSK-IMPACT, and/or fluorescence in situ hybridization, showing *EWSR1-CREB3L1* in six cases and *EWSR1-CREB3L2* in one case. The fusion transcripts were composed of *EWSR1* exon 11 to either exon 6 of *CREB3L1* or *CREB3L2*. In summary, due to their rarity in the bone, skeletal SEF are often misdiagnosed, resulting in inadequate treatment modalities. Similar to their soft tissue counterpart, bone SEF follow an aggressive clinical behavior and show similar *EWSR1-CREB3L1/CREB3L2* fusions.

KEYWORDS

CREB3L1, *CREB3L2*, *EWSR1*, fusions, sclerosing epithelioid fibrosarcoma

1 | INTRODUCTION

Sclerosing epithelioid fibrosarcoma (SEF) is an aggressive sarcoma characterized by a distinctive epithelioid phenotype in a densely sclerotic collagenous stroma that shows frequent MUC4

immunoreactivity and recurrent gene fusions, often involving the *EWSR1* gene.¹ A pathogenetic link with low grade fibromyxoid sarcoma (LGFMS) has been suggested, due to cases with hybrid morphology as well as overlapping genetic signature.^{2,3} Most cases occur in the deep soft tissues, but rare cases have been reported to arise in

bone.⁴⁻¹⁰ Only in a handful of these reports, the presence of *EWSR1* or *FUS* gene rearrangements has been documented.^{4,8,10} Triggered by three consecutive cases of primary skeletal SEF which were misdiagnosed as tumors that more often occur in the bone, ranging from “atypical chondroblastoma,” small cell osteosarcoma and Ewing sarcoma, we reviewed our files for cases that fit with this clinical presentation. This study investigates the clinicopathologic features and molecular abnormalities of the largest series to date of genetically confirmed osseous SEF. Moreover, we also compare our findings to the previously reported cases from the literature, as well as with the more common soft tissue SEF subset.

2 | MATERIAL AND METHODS

2.1 | Patients selection and data collection

The pathology and molecular files of two participating institutions (MSKCC, Mt Sinai Toronto), as well as the personal consultation files of the senior author (C.R.A.) were searched. A total of seven genetically confirmed SEF of bone cases were identified.

Hematoxylin and eosin-stained slides and immunohistochemical stains were re-reviewed. All cases were handled in accordance with the ethical rules of the respective institutions. The tumors were assessed for growth pattern, cytomorphology, cellular pleomorphism, nuclear features including nuclear contour, chromatin pattern and presence of nucleoli, mitotic activity, necrosis, and type of extracellular stroma. Immunohistochemical stains were performed or reviewed, including *MUC4* and *SATB2*. However, in most cases a more exhaustive immunohistochemical work-up was performed.

Retrospective chart review was conducted to collect clinical information such as maximum tumor size, tumor location, stage at diagnosis (primary vs distant metastasis at diagnosis), modality of initial therapy, local recurrence or metastasis, vital status at last follow-up, and survival time. One case with an *EWSR1-CREB3L1* fusion has been previously reported.¹⁰

2.2 | Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was conducted for *EWSR1/FUS* and *CREB3L1/CREB3L2* in six cases (Case# 2-# 7). FISH for break-apart assay was applied on formalin-fixed and paraffin-embedded 4- μ m sections as previously described.¹¹ Custom probes using bacterial artificial chromosomes (BACs) covering and flanking the *EWSR1*, *FUS*, *CREB3L1*, and *CREB3L2* genes were utilized.¹¹ The BAC clones were selected according to the UCSC genome browser (<http://genome.ucsc.edu>) and obtained from the BACPAC sources of Children's Hospital of Oakland Research Institute (Oakland, CA) (<https://bacpacresources.org>). DNA from individual BACs was isolated in line with 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.

Two hundred tumor nuclei were evaluated using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany), controlled by Isis 5 software (Metasystems, Newton, MA). A cut-off of >20% nuclei showing a break-apart signal was considered positive for rearrangement. Nuclei with incomplete set of signals were omitted from the score.

2.3 | Targeted sequencing

One case (Case# 1) was investigated with the MSK-IMPACT assay, a hybridization capture-based next-generation sequencing assay for targeted deep sequencing.^{12,13} Two cases (Case# 3, 4) were analyzed by targeted RNA sequencing using the TruSight RNA Fusion Panel (Illumina, San Diego, CA),¹⁴ as previously described. RNA was extracted from formalin fixed paraffin embedded (FFPE) tissue using Amsbio's ExpressArt FFPE Clear RNA Ready kit (Amsbio LLC, Cambridge, MA). Targeted RNA sequencing was performed on an Illumina MiSeq platform. Reads were independently aligned with STAR (version 2.3) against the human reference genome (hg19) and analyzed by STAR-Fusion. Transcript IDs used in this study were ENST00000397938.6 (*EWSR1*), ENST00000621158.4 (*CREB3L1*), and ENST00000330387.11 (*CREB3L2*).

3 | RESULTS

3.1 | Clinical presentation

The clinical features of SEF of bone are summarized in Table 1. There were 3 males and 4 females, with a mean age at diagnosis of 38 years (range, 8 to 55). The tumor location was femur in three patients, mandible in two, and one each in the skull and thoracic vertebrae. The tumor greatest dimension obtained from either imaging studies at presentation or gross descriptions had a mean of 5.0 cm (range, 3.0 to 6.0 cm).

Follow-up data was available in six of our patients, summarized in Table 1, which also summarizes the clinical data from the cases previously reported in the literature. All our patients presented with localized disease at diagnosis. Case# 1 was diagnosed at the outside institution as an “atypical chondroblastoma” arising in the femoral diaphysis, where it was resected. Two years later, local recurrence and pulmonary metastases developed. Case# 2 presented with a pathologic fracture in the femur and a diagnosis of Ewing sarcoma was rendered on the biopsy material due to a FISH positive result for *EWSR1* gene rearrangement. The patient was then treated with neoadjuvant chemotherapy with vincristine/doxorubicin/cyclophosphamide and ifosfamide/etoposide per Ewing sarcoma regimen. Diagnosis was revised on the surgical resection specimen as a likely small cell osteosarcoma, showing 50% chemotherapy-related necrosis. Patient received adjuvant chemotherapy with high-dose methotrexate, doxorubicin, and cisplatin with addition of ifosfamide/etoposide per osteosarcoma protocol. However, 12 months later, the patient developed

TABLE 1 Clinicopathologic, molecular, and follow-up information of patients with SEF of bone from current study and published literature

Case#	Age	Sex	Location	Size (cm)	Initial diagnosis	Fusion transcript	Mets Y/N	Metastatic site	Vital status	Follow-up (mo)
1	23	F	Femur	5.7	Atypical chondroblastoma	<i>EWSR1</i> exon 11/ <i>CREB3L1</i> exon6 ^α	Y	Lung	AWD	52
2	8	M	Femur	NA	Ewing sarcoma, osteosarcoma	<i>EWSR1/CREB3L1</i> ^β	Y	Skull	AWD	24
3 ¹⁰	55	F	Mandible	4.9	NA	<i>EWSR1</i> exon 11/ <i>CREB3L1</i> exon 6 ^{β,γ}	N	NA	NED	36
4	47	M	Thoracic vertebrae	NA	NA	<i>EWSR1</i> exon 11/ <i>CREB3L2</i> exon 6 ^{β,γ}	NA	NA	NA	NA
5	52	F	Skull	6.0	SEF	<i>EWSR1/CREB3L1</i> ^β	Y	Rib, Spine, Skull	DOD	58
6	28	M	Mandible	3.0	Small round cell tumor	<i>EWSR1/CREB3L1</i> ^β	N	NA	NED	2
7	51	F	Femur	5.5	Osteosarcoma	<i>EWSR1/CREB3L1</i> ^β	N	NA	NED	60
8 ⁴	58	M	Femur	5.2	NA	NA	Y	NA	NA	NA
9 ⁴	51	F	Femur	9.6	NA	NA	Y	NA	NA	NA
10 ⁴	73	F	Ulna	4.0	NA	NA	N	NA	NA	NA
11 ⁴	53	M	Femur	2.8	NA	<i>EWSR1</i> rearrangement	N	NA	NA	NA
12 ⁴	25	M	Humerus	11.5	NA	<i>EWSR1</i> rearrangement	Y	NA	NA	NA
13 ⁴	66	M	Cervical spine	4.2	NA	<i>FUS</i> rearrangement	N	NA	NA	NA
14 ⁴	31	F	Ulna	3.0	NA	NA	N	NA	NA	NA
15 ⁴	43	M	second rib	8.0	NA	NA	N	NA	NA	NA
16 ⁴	48	M	Metacarpal	4.4	NA	NA	N	NA	NA	NA
17 ⁵	16	F	Tibia	4.0	Ossifying fibroma	NA	N	NA	NED	22
18 ⁶	57	F	Pelvis	NA	Metastatic cancer	NA	Y	Bone, Liver	DOD	7
19 ⁷	48	F	Sacrum	8.0	SEF	NA	Y	Lung	DOD	72
20 ⁸	43	M	Ilium	NA	Reactive change	NA	Y	Lung	AWD	24
21 ⁹	24	M	Skull	5.0	SEF	NA	Y	Lung	DOD	24

Abbreviations: AWD, alive with disease; DOD, dead of disease; F, female; M, male; mo, months; NA, not available, Cases confirmed by MSK-IMPACT^α, FISH^β or targeted RNA sequencing^γ; NED, no evidence of disease; SEF, sclerosing epithelioid fibrosarcoma.

skull metastasis. Similarly, case# 7, also presenting with a femur lesion, was diagnosed as a high grade osteosarcoma on the biopsy; patient receiving neoadjuvant chemotherapy per osteosarcoma regimen. The patient has no evidence of disease, 60 months follow-up from diagnosis. Case# 5 presented with a skull mass and underwent resection without neo/adjuvant chemotherapy. She developed multiple rib, spine, and skull metastases, succumbing of disease after 58 months. Cases # 3 and 6, both with mandible tumors, underwent en-bloc resection without neo/adjuvant chemotherapy. Both patients are alive with no evidence of disease at last follow-up. The initial diagnosis for Case #3 was undifferentiated sarcoma and for Case #6 was a small blue round cell tumor.

In total, four cases were initially misinterpreted as either osteosarcoma, Ewing sarcoma or other small blue round cell tumors, and atypical chondroblastoma. Three of six (50%) patients with available clinical follow-up developed metastasis and one patient (17%) died of disease.

3.2 | Radiologic findings

The imaging studies of primary sites were available in four cases. Case #1 presented with right thigh pain and X-ray showed lytic bone lesion within femoral diaphysis, associated with sclerotic cortex (Figure 1A). Magnetic resonance imaging showed an intraosseous mass surrounded by bone marrow edema (Figure 1B). Case #2 presented with progressive left thigh pain and inability to bear weight for 3 months. The patient developed sudden increase in pain and a pathologic fracture was subsequently diagnosed in the proximal femoral shaft, through a radiolucent lesion (Figure 1C). Computed tomography (CT) of Case #5 showed an osteolytic right skull mass associated with destruction of the cortex and soft tissue extension (Figure 1D). CT of Case# 6 also showed a left mandibular lytic lesion, with outer cortex destruction and soft tissue extension. Calcification/ossification within the tumor was suspected (Figure 1E).

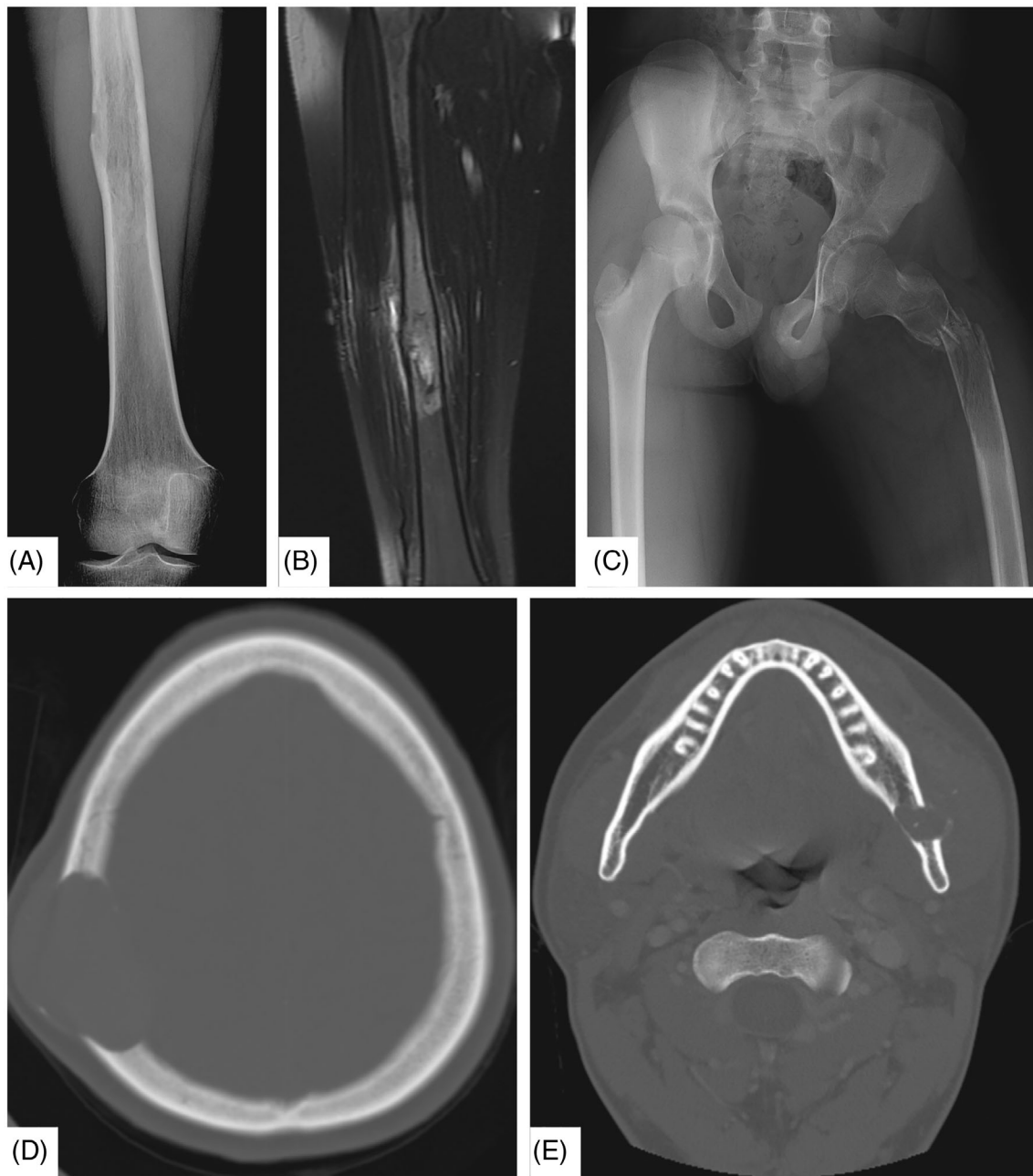


FIGURE 1 Imaging studies demonstrating a primary bone lesion. A, X-ray showing a destructive right femur lesion (Case# 1). B, Magnetic resonance imaging (MRI) T2 weighted image showing a mass in the right femur with bone marrow edema (Case# 1). C, X-ray showing a left proximal femur lytic lesion associated with pathologic fracture (Case# 2). D, Computed tomography (CT) showing lytic skull mass with bone destruction and extra-osseous extension (Case# 5). E, CT showing a left mandibular destructive mass with extra-osseous extension (Case# 6)

3.3 | Pathologic findings

The tumors were composed of solid sheets of monomorphic epithelioid cells with light eosinophilic to vacuolated cytoplasm and round nuclei with fine chromatin. A dense hyalinized stromal component was noted in all cases, although it varied in extent from case to case, or from area to area within the same case (Figure 2A). The tumor cells were divided in columns or nests by wiry collagen, in areas simulating lace-like osteoid. However, no mineralization or chondroid matrix was noted within the fibrous stroma. Occasionally the tumors showed

hypocellular regions, with confluent areas of stromal hyalinization (Figure 2B-D). Some cases showed areas of increased cellularity, reminiscent of a round to undifferentiated neoplasm, in which the stroma was scant (Figure 2E). A few cases showed a more plasmacytoid phenotype or a nested pattern (Figure 2F). No areas of low grade fibromyxoid stroma were detected to suggest a hybrid morphology. However, Case #3 was found to have had a prior biopsy 20 years earlier that, on re-review, was morphology compatible with LGFMS, thereby raising the possibility of progression to SEF. All cases lacked SATB2 expression including in areas resembling osteoid matrix

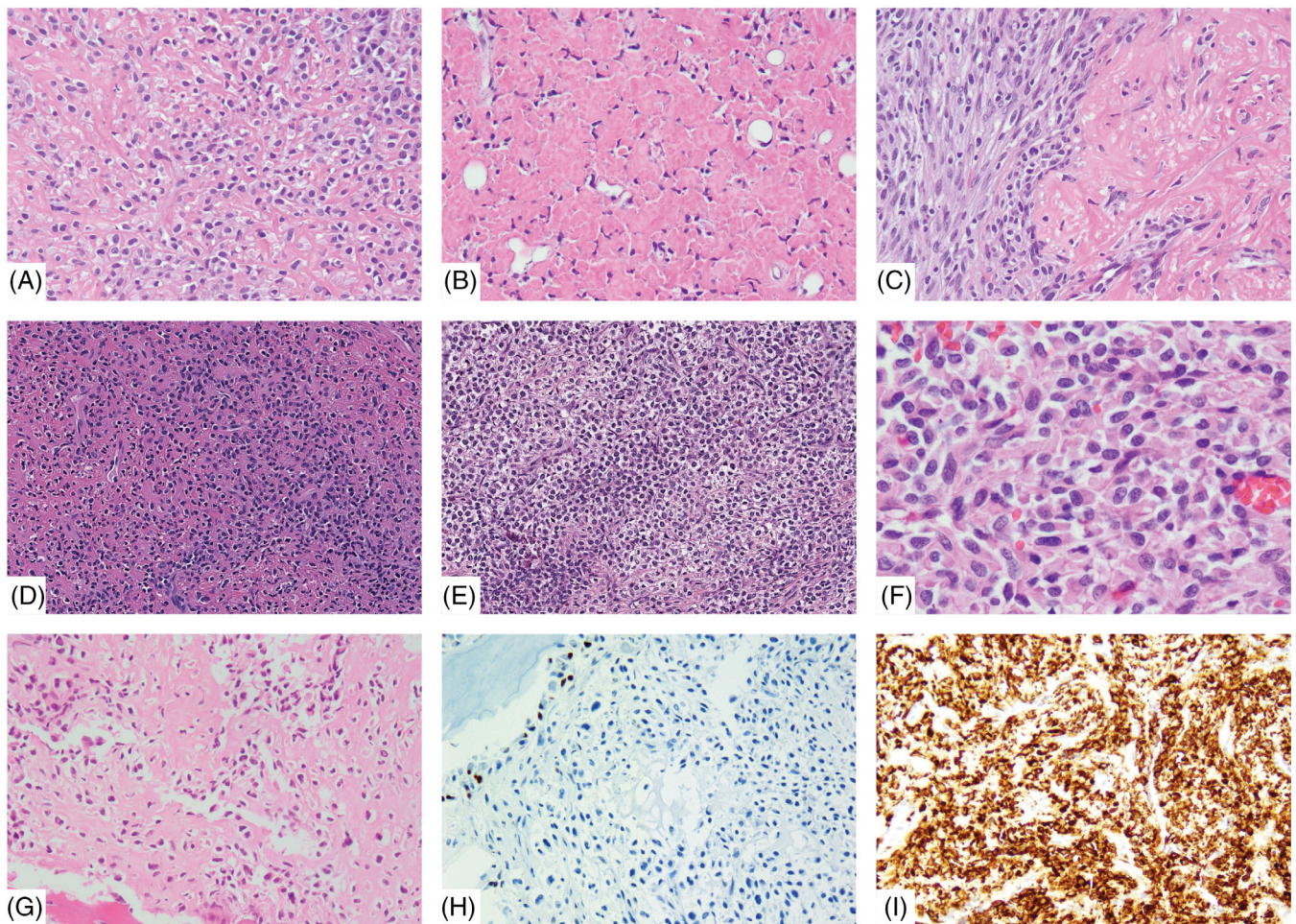


FIGURE 2 Histologic features of skeletal sclerosing epithelioid fibrosarcoma (SEF). Classic morphologic appearance with uniform epithelioid cells separated by wiry collagen bundles in cord and columns (A); alternating markedly hyalinized hypocellular areas suggesting a benign/reactive process is noted (B). An abrupt transition between the more cellular, sometimes with a spindling phenotype, and hypocellular areas can be noted (C). Some tumors show highly cellular/primitive areas, almost completely devoid of fibrotic stroma, arranged in sheets of nests, reminiscent of an undifferentiated round cell sarcoma or Ewing sarcoma (D, E). In other tumors, the lesional cells have a more plasmacytoid or rhabdoid phenotype, with densely eosinophilic cytoplasm and eccentric round nuclei (F). This tumor showed a densely hyalinized matrix s/p neoadjuvant chemotherapy, highly suggestive of osteoid matrix deposition (G); however, the tumor cells were negative for SATB2 (with good internal positive control in the adjacent osteoblasts), while being strongly positive for MUC4 [Color figure can be viewed at wileyonlinelibrary.com]

(Figure 2G, H) and showed strong and diffuse MUC4 positivity (Figure 2I).

3.4 | Molecular findings

FISH revealed *EWSR1* gene rearrangements in all six cases tested. *CREB3L1* gene break-apart was identified in five patients (Case # 2, 3, 5-7), while a *CREB3L2* gene rearrangement was detected in the remaining patient (Case #4). Representative rearrangements are depicted in Figure S1.

In one patient (Case # 1), MSK-IMPACT identified the presence of an *EWSR1-CREB3L1* fusion, composed of *EWSR1* exon 11 fused to exon 6 of *CREB3L1*. MSK-IMPACT also identified copy number

changes, including amplification of *CHECK2*, and copy number losses of the *NF2*, *RAC2*, *EP300*, and *CRKL* genes.

TruSight RNA Fusion Panel detected two different in frame fusions, including one between *EWSR1* exon 11 and *CREB3L1* exon 6 (Case #3); and the other between *EWSR1* exon 11 and *CREB3L2* exon 6 (Case #4) (Figure 3).

3.5 | Literature review

In addition to our current series, there are 14 patients with primary SEF of bone previously reported, 8 males and 6 females, with a mean age of 45 years (range, 16 to 73 years)(Table 1).⁴⁻⁹ Eight tumors (57%) were located in the extremity; with the most frequent primary site

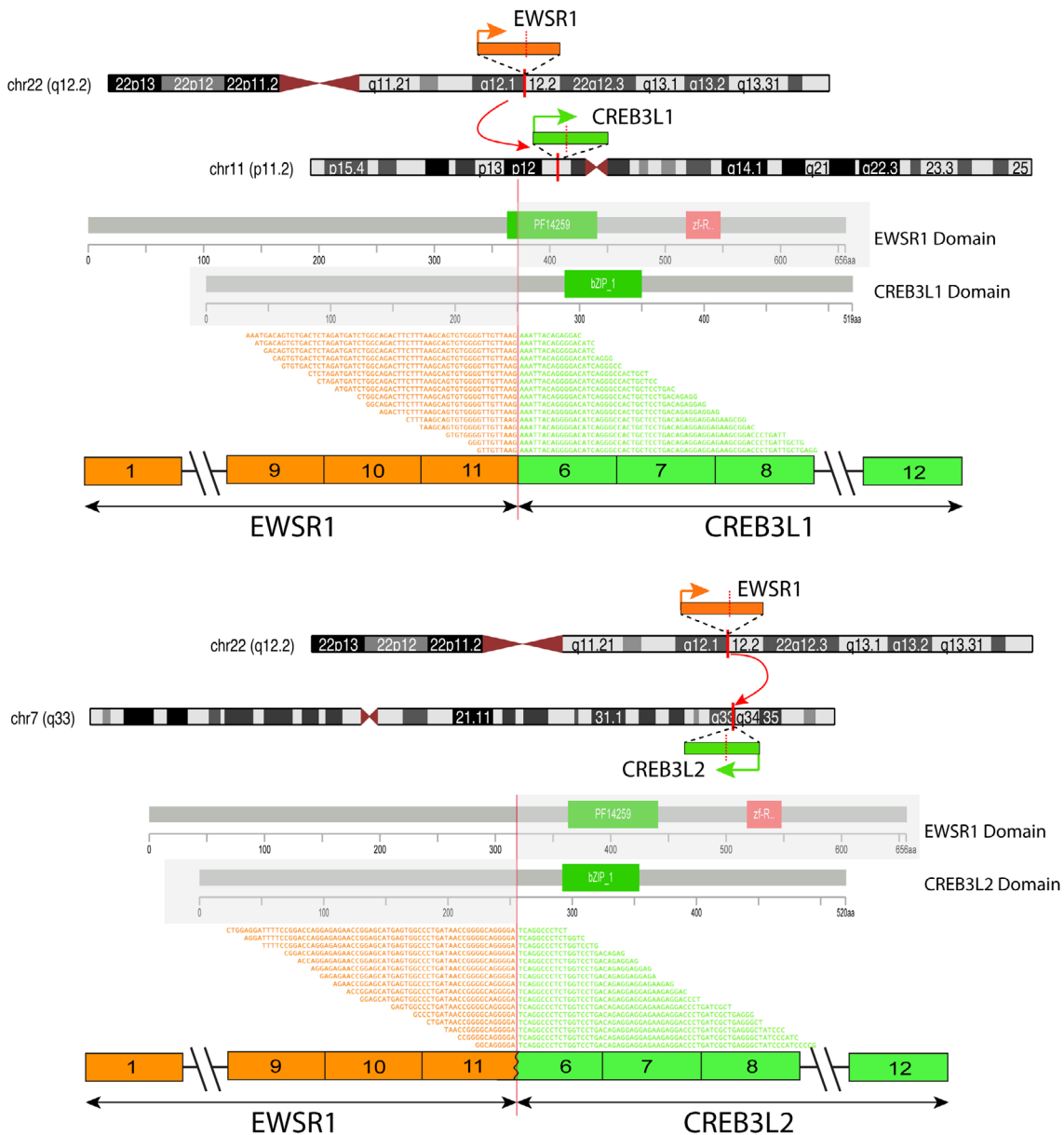


FIGURE 3 Diagrammatic representation of the *EWSR1-CREB3L1* and *EWSR1-CREB3L2* fusions studied by targeted RNA sequencing. Upper and middle panel show chromosomal localization of *EWSR1* on 22q12.2, *CREB3L1* on 11p11.2 and *CREB3L2* on 7q33. Red vertical lines depict the genomic breakpoints. Orange arrows and bars show the direction of transcription of each gene and the exonic breaks, respectively. The protein domains of each gene are shown below. Fusion variants are depicted at the bottom of each panel showing *EWSR1* exon 11 fused to *CREB3L1* exon 6 and *EWSR1* exon 11 to *CREB3L2* exon 6 [Color figure can be viewed at wileyonlinelibrary.com]

being the femur (21%). Three cases were initially misdiagnosed as ossifying fibroma, metastatic cancer, or reactive change. Half of the patients with available clinical follow-up developed metastasis and 60% (3 of 5) patients died of their diseases.

Of the 11 patients with available histologic evaluation, all except 2 (82%) were composed of pure SEF, with small nests and linear arrays of epithelioid cells embedded in a dense collagenous matrix. The remaining two cases contained areas of bland spindle cells embedded in a fibromyxoid stroma, in keeping with a LGFMS component (hybrid SEF-LGFMS type). Of nine cases with available data, seven tumors (78%) were positive for MUC4 immunostain. Three

cases showed *EWSR1* rearrangement and one case showed *FUS* rearrangement. None were tested to determine their gene partner or fusion breakpoints.

4 | DISCUSSION

In this study we investigated the clinicopathologic and molecular features of seven cases of primary SEF of bone. Our study showed the presence of a *EWSR1-CREB3L1* fusion in six cases and *EWSR1-CREB3L2* fusion in one case. Our results also reinforce that

SEF of bone is a sarcoma with a highly aggressive clinical course and that the differential diagnosis is much more challenging than in the soft tissues due to the rarity of this disease in bone and its resemblance to other more common bone sarcomas, particularly with osteosarcoma and Ewing sarcoma. As a result of these misdiagnoses in the initial biopsies or subsequent resections, three patients were subjected to neoadjuvant or adjuvant therapies for osteosarcoma or Ewing sarcoma, with mostly no benefit.

In this series, SEF of bone exhibited an aggressive behavior with a high frequency of metastasis, seen in half of the patients with available follow-up. A literature review of previously reported cases showed a similar incidence of metastatic disease, occurring in 50% of the patients.⁴⁻⁹ Similar to a previous report,⁵ one of our cases showed a poor response to Ewing sarcoma chemotherapy regimen. The aggressive clinical features and chemoresistance noted in the skeletal SEF recapitulate the experience seen with the more common soft-tissue SEF, showing high rates of metastasis (86% to 92%) and death of disease (57% to 77%).^{3,15}

In contrast to soft tissue SEF where the diagnosis appears straightforward, the differential diagnosis of primary SEF of bone appears much more challenging.^{5,6,8} One of the reasons being the rarity of SEF as a primary bone lesion. Additionally, the dense extracellular collagenous stroma can be misinterpreted as osteoid matrix, and thus misdiagnosed as osteosarcoma. Immunostaining for SATB2 might be critical in confirming the osteoblastic nature of the tumor cells, staining which was consistently negative in our cases and a previous study.⁴ Furthermore, close radiographic correlation might refute the presence of mineralized matrix on images. Differentiating the diagnosis from osteosarcoma is clinically important, as skeletal SEF have been resistant to osteosarcoma regimen chemotherapy.^{5,15} Moreover, due to its round and monomorphic appearance, SEF can also be misdiagnosed as an Ewing sarcoma family of round cell tumors or even small cell osteosarcoma. Based on the reported literature, skeletal SEF can also be mistaken as a benign neoplasm, due to its bland histologic appearance. One case in our series was also initially misdiagnosed as an "atypical chondroblastoma."

Morphologically SEF of bone shared similar features to the more common soft tissue SEF, including a monomorphic epithelioid phenotype, arranged in cords and nests, separated by a dense collagenous stroma. Also similar to the soft tissue counterpart, MUC4 was diffusely and strongly positive which can be used to confirm the diagnosis.

At extraskeletal locations, an *EWSR1-CREB3L1* gene fusion is the most common abnormality in pure SEF, while *FUS-CREB3L2* transcript is commonly present in the hybrid SEF/LGFMS.^{16,17} In keeping with these findings, our study composed of pure SEF morphology demonstrated the presence of an *EWSR1-CREB3L1* fusion in all except one case. The only case harboring a *EWSR1-CREB3L2* fusion occurred from the thoracic vertebrae. The predicted structure of the fusion protein is similar to other *EWSR1*-related fusions, including the N-terminal serine-tyrosine-glutamine-glycine transactivation domain of *EWSR1*. *CREB3L1* and *CREB3L2* belong to the *CREB3* family of transcription factors, which encode proteins localized to the endoplasmic

reticulum and act as transcription factors activated by cyclic AMP stimulation.¹⁸ Further studies are warranted to define the oncologic role of fusion protein in SEF.

In conclusion, this is the largest study to date investigating the clinical and molecular abnormalities of primary SEF of bone using a combined approach of targeted RNA/DNA sequencing and/or FISH. Our results stress the diagnostic challenges encountered in establishing a correct diagnosis outside the molecular testing, mainly due to its resemblance to other more common bone sarcomas. As seen with the soft tissue tumors, skeletal SEF emerges as a highly aggressive sarcoma type, with a high rate of distant metastases and resistance to both Ewing sarcoma and osteosarcoma regimens. Adjunct immunohistochemical markers (*MUC4*, *SATB2*) and FISH/next generation sequencing (NGS) molecular methods are critical in reaching the correct diagnosis.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

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

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