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Novel *EWSR1::GFI1B* Gene Fusion in Angiofibroma of Soft Tissue

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Abstract

Aims: Angiofibroma of soft tissue is a benign soft tissue tumor characterized by bland spindle cells and a distinct branching vascular network. The majority of soft tissue angiofibromas harbor *AHRR::NCOA2* gene fusions. Here we present three cases of *EWSR1*::*GFI1B*-fused soft tissue tumors that are morphologically most reminiscent of soft tissue angiofibroma.

Methods and Results: All three cases presented in male patients with an age range of 35 to 78 years old (median 54 years). Two cases presented as subcutaneous nodules on the trunk (posterior neck and chest wall); one was an intramuscular foot mass. The tumors were unencapsulated nodules with infiltrative margins ranging from 2.2-3.4 cm in greatest dimension. Histologically, the tumors contained uniformly bland fibroblastic spindle cells with ovoid to fusiform nuclei and delicate cytoplasmic processes embedded in a myxoid to myxocollagenous stroma. All three cases were characterized by a thin-walled, branching vascular network evenly distributed throughout the tumor. Overt cytologic atypia or conspicuous mitotic activity was absent. The spindle cells had an essentially null immunophenotype. By targeted RNA sequencing, an in-frame gene fusion between *EWSR1* exons 1-7 and *GFI1B* exons 6-11 or 7-11 were detected in all three cases. The tumors were marginally excised. For all three cases, there were no documented local recurrence or distant metastases over a limited follow-up period of 6 to 10 months.

Conclusions: We propose that *EWSR1::GFI1B* may represent a novel fusion variant of soft tissue angiofibroma.

Keywords

EWSR1; GFI1B; soft tissue angiofibroma

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Introduction

Monomorphic fibroblastic spindle cell tumors encompass a wide spectrum of soft tissue tumors with different molecular genetic aberrations, clinicopathologic features and clinical outcome. In recent years, with the advent of modern next generation sequencing methods, several new entities in this morphologic spectrum have been discovered. With the advent of the knowledge of consistent molecular abnormalities and frequent nonspecific immunophenotype, these tumors have been defined by their fusion genes, for example *EWSR1::SMAD3*-positive fibroblastic tumor, *NTRK*-rearranged spindle cell neoplasm, *PRRX1::NCOAx*-rearranged fibroblastic tumor, and *GAB1::ABL1* fusion-positive spindle cell neoplasm.[1-5] Moreover, disease defining gene fusions were detected in other distinct fibroblastic neoplasms that had been recently described earlier, e.g., *AHRR::NCOA2* in angiofibroma of soft tissue. Triggered by the molecular identification of a novel *EWSR1::GFI1B* gene fusion in an angiofibroma of soft tissue occurring in the foot of a 49-year-old male, we searched our files for possible similar cases and an identical *EWSR1::GFI1B* fusion gene was found in two additional spindle cell tumors with similar angiofibroblastic morphology. These three cases are presented in this article, and we propose that they are part of the spectrum of angiofibroma of soft tissue, albeit with a different gene fusion.

Material and Methods

Index Case and Patient Selection

The index case was retrieved from the personal consultation files of one of the authors (A.J.H.S) Two additional cases were found in the consultation files for two of the authors (KJF, SDB) from the Department of Pathology, Cleveland Clinic. The study was approved by the Institutional IRB.

Immunohistochemistry

The antibodies applied in all 3 cases were ERG (Abcam AB92513; 1:100), SMA (Dako M0851; 1:50), Pan-cytokeratin (AE1/AE3) (Cell Marque 313M-18; predilute), EMA (Dako M0613; 1:50), GLUT1 (Biocare CM408A; 1:100), desmin (Dako M0760; 1:10), MUC4 (Cell Marque 406M-18; predilute), STAT6 (Abcam Ab32520; 1:200), CD34 (Cell Marque 134M-18; predilute), and S100 (Dako IR50461; predilute). IHC staining was performed on a Ventana Benchmark (Ventana Medical Systems, Tucson, AZ) automated immunostaining platform using a heat-based antigen retrieval method and high-pH buffer. In the institutional IHC quality assurance system appropriate antibody solutions had been tested; moreover, tissue controls were used in each case.

Targeted RNA sequencing (Archer)

The detailed procedure for the Archer Anchored Multiplex RNA sequencing assay has been previously described.[6] In short, unidirectional gene-specific primers were designed to target specific exons in 58 genes known to be involved in oncogenic fusions in solid tumors. RNA was extracted from FFPE specimens, followed by cDNA synthesis and library

preparation. Anchored Multiplex polymerase chain reaction amplicons were sequenced on Illumina Miseq, and the data was analyzed using the Archer software.

RESULTS

Case Presentation, Histomorphology and Immunohistochemistry

The index case #1 was that of a 49-year-old man presenting with a 2.2 cm intramuscular tumor in the musculus abductor longus of the left foot. The patient had noticed swelling for several weeks, which was painful while walking. Needle biopsies were performed, which revealed a diagnostically challenging fibroblastic tumor with a non-specific immunophenotype. After Archer RNA sequencing had identified the novel *EWSR1::GFI1B* fusion, of which the tumor biological significance was unclear, complete excision of the tumor was carried out for diagnostic purposes. Grossly, on cross section, the excisional specimen showed a 2.2 cm solid white tumor nodule with extensive myxoid change and relatively sharply delineated white fibrous and glistening gelatinous areas (Figure 1A). Case #2 presented as a mobile subcutaneous painful mass on the chest wall of a 35-year-old male, clinically thought to be a benign lipoma. Grossly, the mass was a glistening and encapsulated lobulated lesion measuring 2.2 cm in greatest dimension. Sectioning revealed a soft gelatinous cut surface. Case #3 was a superficial soft tissue mass in the posterior neck of a 78-year-old male, measuring 3.4 cm in greatest dimension. The contributing pathologists for cases #2 and #3, both previously unbiopsied, favored a low-grade fibromyxoid sarcoma.

Histologically, the tumors were unencapsulated and showed a nodular or multinodular growth pattern (Figures 1A-C). Case #1 contained relatively cellular areas at the periphery and myxoid hypocellular central areas (Figure 1D). In contrast to the index case, cases #2 and #3 did not show a distinctive zonation pattern. The variable myxoid to myxocollagenous stroma contained fine fibrillary collagen and thin collagen bands. Other distinctive stromal features such as areas of keloid-like hyalinized collagen or perivascular collagen deposits were absent. There was a distinct background network of plexiform vasculature composed mostly of thin-walled branching vessels and few arteriolar vessels evenly distributed throughout the lesion (Figure 2A-F). Occasionally, a subset of the vessels demonstrates slight variability in shapes and wall thicknesses with relatively large endothelial cells and a distinct pericytic layer (Figure 2B, Figure 4B). The tumor was composed of uniformly bland fibroblastic spindle cells with ovoid to fusiform nuclei, fine chromatin, small/inconspicuous nucleoli, and tapering to stellate eosinophilic cytoplasmic processes (Figure 3A-C). The tumor cells lacked significant cytologic atypia or nuclear pleomorphism; mitotic figures were inconspicuous (<1 in 10 HPF). All three showed an infiltrative growth into surrounding skeletal muscle or subcutaneous fat (Figure 3D-F).

Immunohistochemical staining results of the three tumors are summarized in Table 1. By immunohistochemistry (IHC), all three cases showed an essentially null immunophenotype. The fibroblastic spindle cells were negative for ERG (Figure 4A), smooth muscle actin (Figure 4B), desmin, caldesmon, S100, SOX10, MUC4 and STAT6. The capillaries were composed of oval, ERG-positive endothelial cells enveloped by a single layer of SMA-positive pericytes (Figure 4B). In addition to ERG, the endothelial cells expressed CD31 and

CD34 (Figure 4C). In one of three cases, the fibroblastic spindle cells showed rare to focal CD34 expression (case #3) and cytokeratin expression (case #1) (Figure 4D).

Novel *EWSR1::GFI1B* fusion

In all three cases, targeted RNAseq (Archer) identified an *EWSR1::GFI1B* fusion, with exon 7 of *EWSR1* (22q12.2; NM_005243.3) fused to exon 6 (cases #1 and #3) or exon 7 (case #2) of *GFI1B* (9q34.13; NM_004188.6). In both fusion variants, the predicted chimeric amino acid sequence was in-frame and contained the N-terminal transcriptional activation domain (TAD) of *EWSR1* fused to the C-terminal C2H2 zinc finger domain of *GFI1B* (Figure 5).

Follow-up

In all 3 cases, the tumor excisions were intralesional with positive margins. Case #1 received postoperative radiotherapy, because a low-grade sarcoma was considered at the time of initial histopathological diagnosis. Clinical follow-up information was available for all three cases. For cases #1, #2 and #3, over a follow-up period of 6, 9 and 10 months, respectively, there was no documented recurrence or metastasis.

Discussion

Angiofibroma of soft tissue was first described by Marino-Enriquez and Fletcher[7] in 2012 as a distinctive benign fibrovascular soft tissue tumor composed of uniform fibroblastic cells embedded in a variably collagenous or myxoid stroma and showing a prominent vascular network composed of numerous small, branching, thin-walled blood vessels. In five tumors, classic cytogenetics had revealed a simple karyotype with a balanced t(5;8) chromosomal translocation. In the same year it was reported that this chromosomal translocation resulted in an *AHRR::NCOA2* reciprocal gene fusion with upregulation of aryl hydrocarbon receptor target genes.[2] In later years it appeared that these *AHRR::NCOA2* fusions were encountered in 60-80% of all angiofibromas.[8,9] Moreover, incidental cases were found to harbor *GTF2I::NCOA2*, *AHRR:NCOA3*, or *GAB1::ABL1* fusions.[9-11] On the other hand, in a recent case series of 13 fibroblastic spindle cell tumors with *GAB1::ABL1* fusions, it was shown that these tumors show morphologic and immunophenotypic overlap with perineurioma or hybrid schwannoma–perineurioma, often with expression of CD34, EMA, claudin 1 and GLUT1, with EMA immunohistochemical staining highlighting delicate bipolar cytoplasmic processes reminiscent of perineurioma.[5]

In this study, we report novel *EWSR1*::*GFI1B* gene fusions in 3 soft tissue tumors with the characteristic morphologic features of angiofibroma of soft tissue. Microscopically, these *EWSR1::GFI1B*-fused soft tissue angiofibromas contained bland spindle cells with fibroblastic cytomorphology embedded in myxoid to fibromyxoid stroma and is distinguished by an elaborate network of thin-walled branching vessels. By IHC, the neoplastic spindle cells showed an essentially null immunoprofile. In the vascular network, endothelial cells expressing ERG, CD31, and CD34 were enveloped by a single layer of SMA-positive pericytes. It is worth noting that the three cases in this study demonstrated infiltrative borders, which is a feature reported in only a minority of the angiofibroma of soft tissue (4 of 35 cases) in the initial series, the majority of which is tightly circumscribed.[7]

Additionally, although there was slight variation in size and wall thickness in our cases, the "classic" angiofibroma of soft tissue reported in previous studies appeared to display greater variation in vascular sizes, shapes and wall thicknesses.[7,8]

Notably, the zinc finger protein *GFI1B* gene is a novel *EWSR1* fusion partner in translocation-associated neoplasia. Under normal physiologic conditions, *GFI1B* functions as a transcriptional repressor and has been shown to play an essential role in hematopoiesis by regulating the dormancy and proliferation of hematopoietic stem cells and the development of erythroid and megakaryocytic cell lineages.[12,13] In the predicted chimeric fusion protein, GFI1B loses the N-terminal repressive SNAIL/GFI1 (SNAG) domain and retains the C-terminal DNA binding Zinc finger domain, which fuses to the N-terminal DNA transactivation domain (TAD) of EWSR1. This implies that the chimeric protein may no longer functions as a transcriptional repressor but is instead activated by the TAD of EWSR1.

Many soft tissue tumor entities demonstrate relative bland spindle cell morphology and fibromyxoid stroma, and thus are to be considered in the differential diagnosis of the tumor described in this study. These entities include cellular angiofibroma, intramuscular myxoma, superficial angiomyxoma, myxoid solitary fibrous tumor (SFT), low-grade fibromyxoid sarcoma (LGFMS), soft tissue perineurioma, low-grade myxofibrosarcoma, and myxoid liposarcoma. Hallmark features of these tumors which differentiates them from soft tissue angiofibroma are discussed here. Cellular angiofibroma is a benign superficial circumscribed tumor usually presenting in the vulva and inguinoscrotal regions; the spindle cells have plump, stubby nuclei, and the tumor stroma contains more rounded blood vessels of different caliber. In cellular angiofibroma, loss of 13q14 including loss of the *RB1* gene is typical and the resultant loss of RB1 protein may be demonstrable by IHC.[14] Cellular examples of intramuscular myxoma also do not show the prominent branching vascular pattern and may be diagnosed by *GNAS* mutation analysis. Superficial angiomyxoma typically presents as a hypocellular and hypovascular cutaneous lesion with IHC expression of CD34. The myxoid variant of SFT is hypovascular and may show staghorn vessels with perivascular hyalinization. By IHC, SFT shows diffuse staining for CD34 and nuclear expression of STAT6 (as surrogate marker of its molecular signature, the *NAB2::STAT6* fusion).[15] Akin to soft tissue angiofibroma, LGFMS has alternating fibrous and myxoid areas, but in myxoid areas, spindle cells are arranged in whorling patterns and small vessels may be arranged in arcades. LGFMS shows diffuse expression of MUC4 and typically has *FUS::CREBL2/CREBL1* fusions, with a minority harboring *EWSR1::CREB3L1* fusions.[16] Soft tissue perineurioma may demonstrate myxocollagenous stroma and typically contains bland spindle cells with bipolar, elongated cytoplasmic processes arranged in whorls, which can be highlighted by EMA and GLUT1.[17] Low-grade myxofibrosarcoma may show curvilinear instead of thin-walled branching blood vessels. Moreover, fibroblastic tumor cells have hyperchromatic nuclei with at least focal nuclear atypia. Myxoid liposarcoma usually occurs as a large deep-seated tumor in the lower extremity. The myxoid stroma has a branching arborizing vascular network but the tumor cells have a more immature appearance with small hyperchromatic nuclei and inconspicuous cytoplasm. Signet ring-like lipoblasts may be present but scant. In challenging cases molecular analysis may assist in the diagnosis as *FUS/EWSR1::DDIT3* translocations are pathognomonic for this tumor.[18]

As yet, the clinical behavior of *EWSR1*::*GFI1B*-fused tumors remains unclear, although over a limited median follow-up period of 9 months, there was no local recurrence or metastases in any of the three cases. Given how these tumors are morphologically quite reminiscent of angiofibroma of soft tissue, with bland fibroblastic spindle cells in a myxoid to myxocollagenous stroma, distinct thin-walled branching vascular network, and infiltrative growth pattern, we propose that *EWSR1::GFI1B* may represent a novel fusion variant of soft tissue angiofibroma. Future studies with additional cases may help shed light on the histopathologic spectrum and clinical behavior of this group of tumors.

Acknowledgement

AJHS and JKD analyzed the data and wrote the manuscript. AJHS, AHGC, CRA, LAD, KJF, SDB and JKD performed data collection, editing and critical review of the manuscript. All authors have reviewed and approved the final version of this manuscript.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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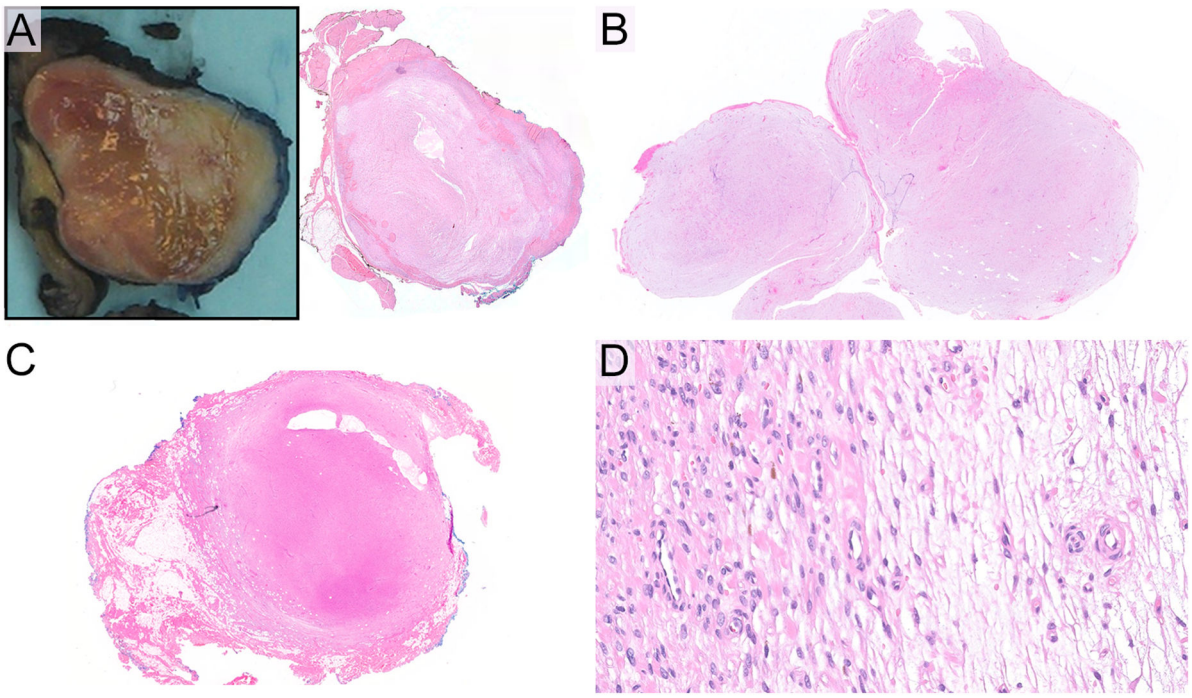

Figure 1.

(A) Gross examination of case #1 shows an unencapsulated nodule with glistening gelatinous cut surface alternating with white fibrous areas. On lower power, the nodule demonstrates myxocollagenous stroma and is surrounded by skeletal muscle. (B) Case #2 is a myxoid and multinodular tumor also surrounded by skeletal muscle. (C) Case #3 is a myxocollagenous nodule surrounded by subcutaneous adipose tissue. (D) Case #1 also contains relatively cellular areas at the periphery and myxoid hypocellular central areas.

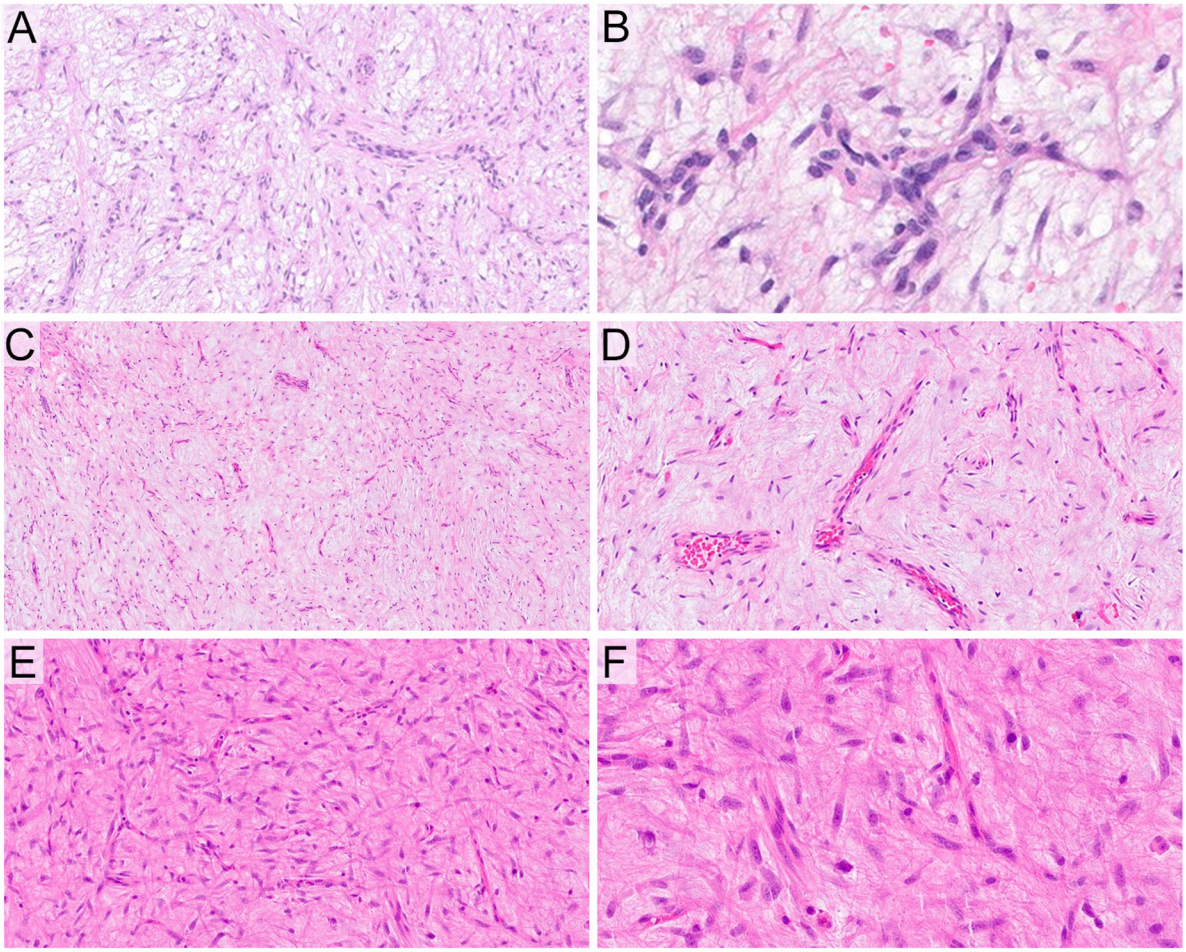

Figure 2.

The tumor contains a distinct branching vascular network throughout, ranging from thin-walled vasculature to occasional vessels with medium wall thickness (A, B: case 1; C, D: case 2; E, F: case 3).

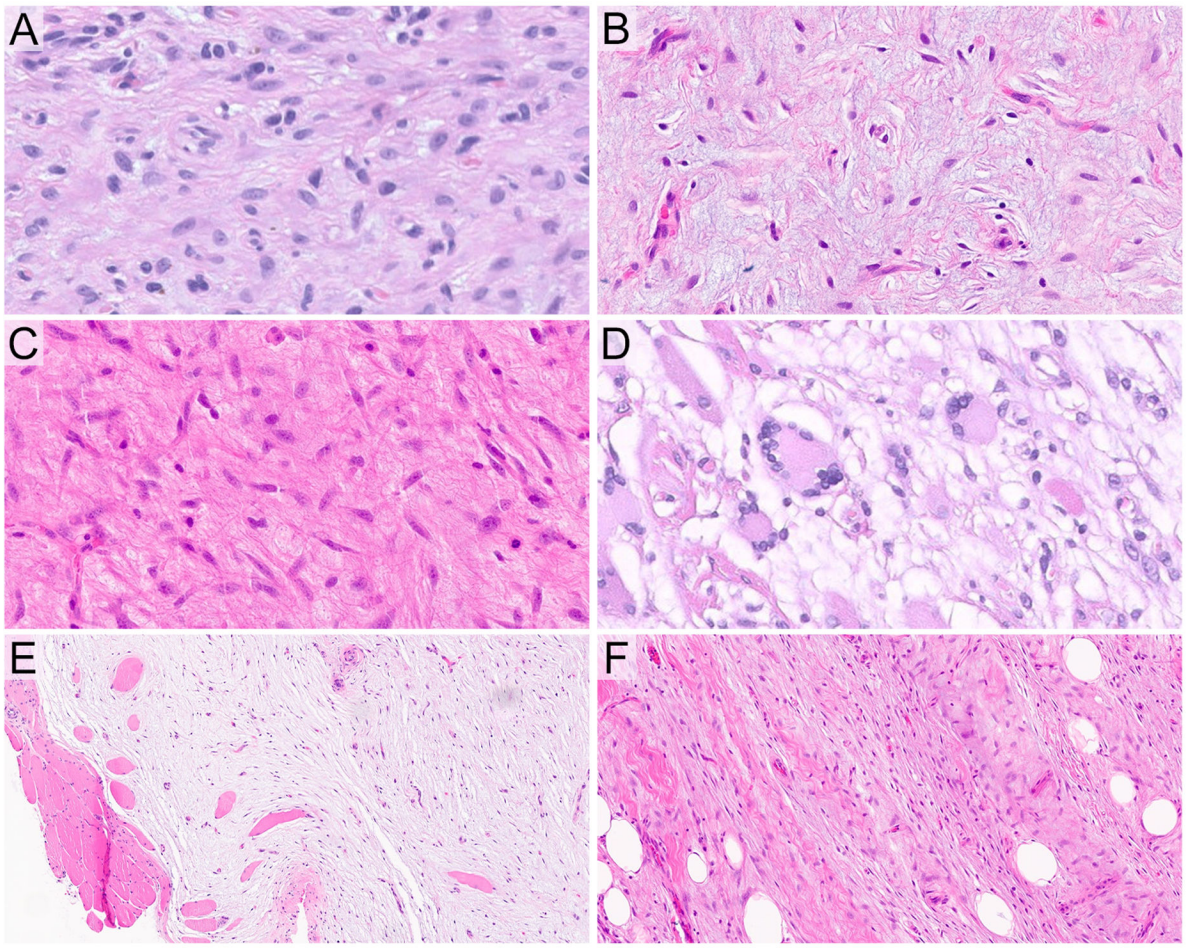

Figure 3.

A-C: The tumors contain fibroblast spindle cells with ovoid to fusiform nuclei, inconspicuous chromatin, and delicate tapering cytoplasmic processes set in myxoid to myxocollagenous stroma (A: case 1; B: case 2; C: case 3). D-F: At the periphery, the tumor infiltrates skeletal muscle (D: case 1; E: case 2) or adipose tissue (F: case 3) at the margins.

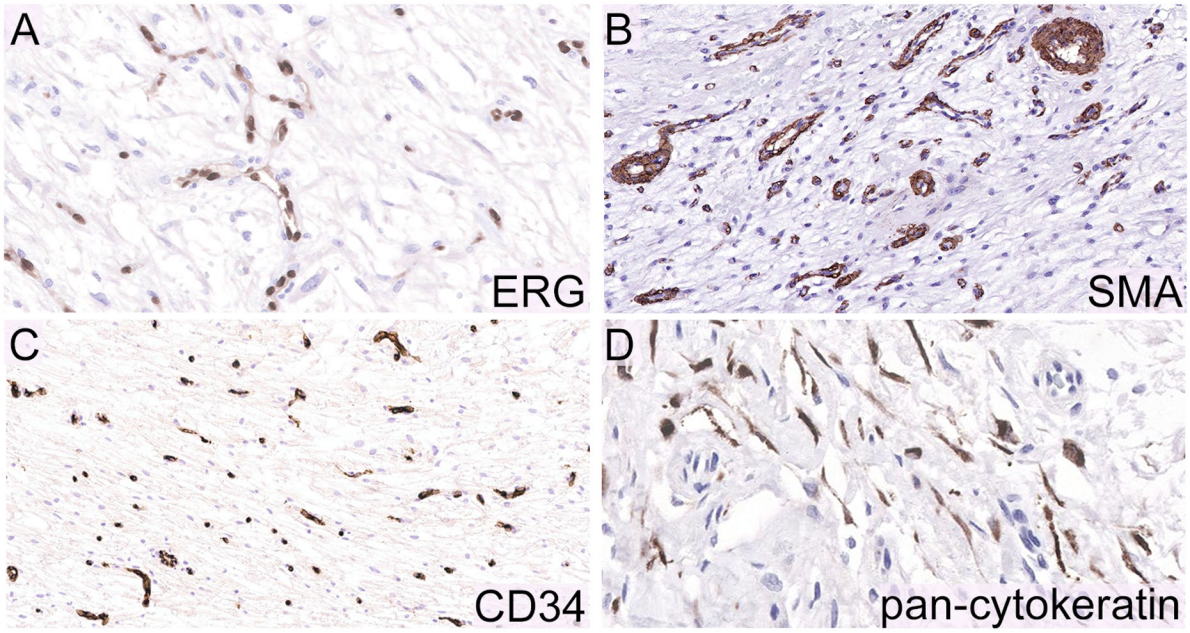

Figure 4.

By immunohistochemistry, ERG (A) and CD34 (B) highlights the endothelial cells of the vascular network, while SMA (C) highlights the pericytes and the slight variability in the shapes and wall thickness of the blood vessels (case #1). These stains are negative in the neoplastic spindle cells. (D) In case #1, focally the spindle cells express pancytokeratin.

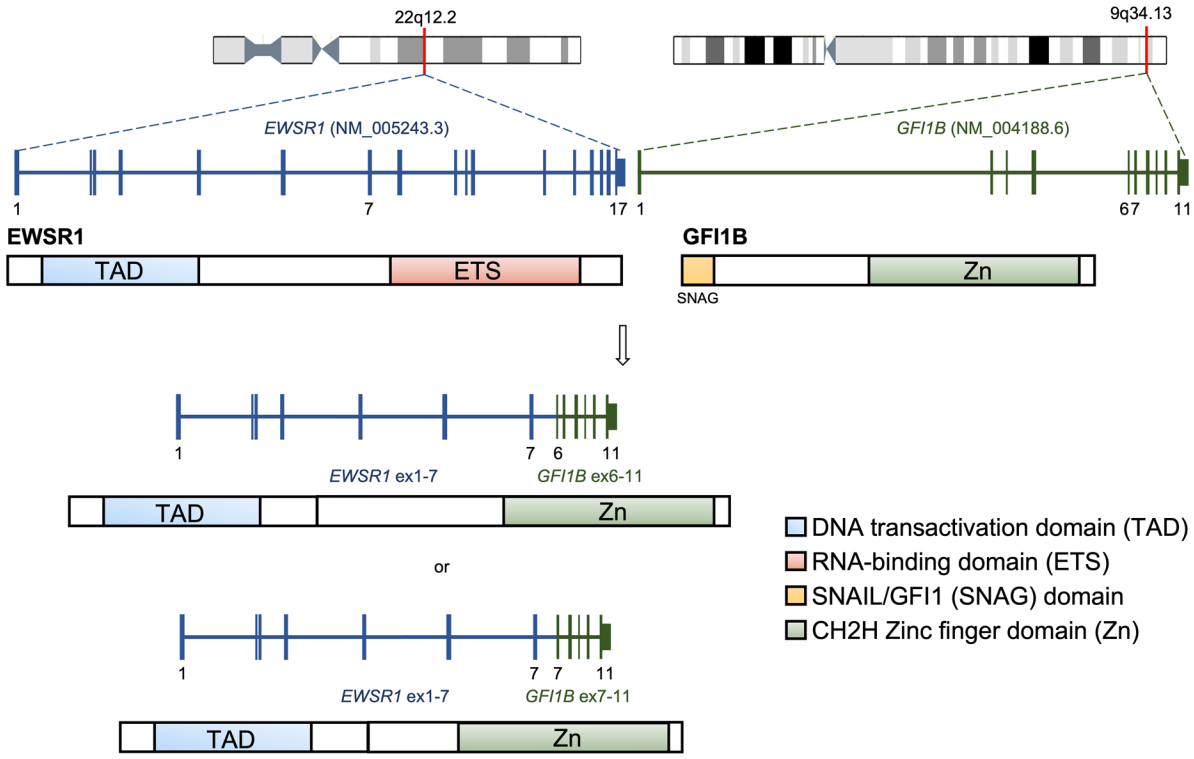

Figure 5.

Schematic of the *EWSR1::GFI1B* fusion. In the fusion transcript, which is in-frame, exons 1-7 of *EWSR1* are fused to exons 6-11 (cases #1 and #3) or exons 7-11 (case #2). In the predicted chimeric protein, the N-terminal transactivation domain (TAD) of EWSR1 is fused to the C-terminal C2H2 Zinc finger domain of GFI1B, which loses the N-terminal repressive SNAG domain in the process.

Table 1.

Immunohistochemistry of *EWSR1::GFI1B*-fused tumors

Case	ERG	SMA	PanCK	EMA	GLUT1	desmin	MUC4	STAT6	CD34	S100
#1	neg	neg	focal	neg	ND	neg	neg	neg	neg	neg
#2	neg	ND	neg	neg	ND	neg	neg	neg	neg	neg
#3	neg	neg	neg	neg	neg	neg	neg	neg	focal	neg

ND: not done