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Spindle and round cell sarcoma with *EWSR1-PATZ1* gene fusion: A sarcoma with polyphenotypic differentiation

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Abstract

The evolving classification of round cell sarcomas is driven by molecular alterations. ***EWSR1-PATZ1* fusion positive spindle and round cell sarcoma is one such new tumor entity**. Herein, we report two *EWSR1-PATZ1* fusion positive spindle and round cell sarcomas with overlapping histological features and polyphenotypic differentiation. The intraabdominal tumors affected female patients, 31 and 53-year-old. Both tumors showed sheets and nests of round to spindle cells, fine chromatin, tiny conspicuous nucleoli, moderate cytoplasm and thick bands of intratumoral fibrosis. On immunohistochemistry, both tumors showed positivity for CD99, desmin, myogenin, myoD1, S100, Sox10, CD34 and GFAP and were negative for keratin. Fluorescence in-situ hybridization revealed rearrangement at *EWSR1* locus. Next generation sequencing based RNA fusion assay revealed *EWSR1-PATZ1* fusion in both cases. *EWSR1-PATZ1* fusion positive spindle and round cell sarcomas show abundant intratumoral fibrosis and polyphenotypic differentiation, thus mimicking a range of tumors including desmoplastic small round cell tumor. The precise classification of this spindle and round cell sarcoma and its relationship to the Ewing sarcoma family of tumors remains to be determined.

Keywords

EWSR1; PATZ1; fusions; sarcoma; polyphenotypic differentiation

Introduction:

Round cell sarcomas form a distinct class of sarcomas characterized by unique cytogenetic and molecular alterations. Traditional molecular techniques used in diagnostic soft tissue pathology include cytogenetics, fluorescence in-situ hybridization (FISH) and detection of fusion transcripts by multiplex reverse transcription-polymerase chain reaction (RT-PCR).

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More recently, the broad range of novel gene fusions identified on next-generation sequencing are expanding the spectrum of soft tissue tumors, particularly round cell sarcomas.[1]

Ewing sarcoma, the prototypic round cell sarcoma, harbors pathognomonic fusions involving *EWSR1* and members of *ETS* family of transcription factors. A group of round cell neoplasms, often referred to as Ewing-like sarcoma, harbor *CIC* and *BCOR* rearrangements, and are now categorized as *CIC*-rearranged and *BCOR*-rearranged sarcomas, respectively.[2,3] A few patients with Ewing-like sarcoma show *EWSR1* rearrangements involving fusion partners other than *ETS* family of transcription factors.[4,5] A recent comprehensive study of round cell sarcomas using high-throughput RNA sequencing identified 5 tumors with *EWSR1-PATZ1* (a non-ETS gene) fusion, which on expression profiling clustered away from other *EWSR1*-fusion positive cases, suggesting a novel tumor entity, one genetically unrelated to other *EWRS1* fusion positive round cell sarcomas.[6]

In this study, we report 2 *EWSR1-PATZ1* fusion positive spindle and round cell sarcomas showing unique morphologic and polyphenotypic differentiation on immunohistochemistry.

Patients and methods:

Both cases were seen in consultation by one of the authors (V.D.). The hematoxylin and eosin stained slides were reviewed.

Immunohistochemistry

Immunohistochemistry was performed by the referring as well as at this institution. The source of the antibodies is indicated in supplementary table 1, Supplemental Digital Content 1, http://links.lww.com/PAS/A698.

Fluorescence in-situ hybridization (FISH)

Interphase fluorescence in situ hybridization (FISH) was performed on 5-micron sections of formalin-fixed paraffin-embedded tumor material. A *EWSR1* break-apart probe (Vysis LSI EWSR1 Dual Color, Break Apart Rearrangement Probe) was hybridized. A total of 50 cells were counted and the number of cells containing a rearrangement was evaluated. A *EWSR1* gene rearrangement was considered positive if more than 15% of cells showed separation of the red (5’ probe) and the green (3’ probe) signals by a distance at least two times greater than the size of one hybridization signal. Isolated red-only signals in addition to a non-rearranged red-green fused signal were also considered positive for rearrangement.[7,8] A paired signal and one isolated green signal, was also considered to likely indicate a EWSR1 rearrangement.[8]

Solid fusion assay

We used a clinically validated laboratory-developed assay based on Anchored Multiplex PCR (AMP) for targeted fusion transcript detection involving 29 genes (supplementary table 2, Supplemental Digital Content 1, http://links.lww.com/PAS/A698) as one of the fusion partners, using next generation sequencing.[9] cDNA was prepared from the total nucleic acid

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extracted from formalin fixed paraffin embedded tumor tissue. ArcherDx FusionPlex Solid Tumor Kit primers were used in 2 hemi-nested PCR reactions and the library so prepared was sequenced on an Illumina NextSeq (2 × 150 base paired-end sequencing). A laboratory-developed algorithm was used for fusion transcript detection and annotation (version 2.1.0).

Results:

Case 1:

A 31-year-old female presented with back pain. The 6.5cm retroperitoneal mass was resected along with the left adrenal and kidney. Prior to the availability of molecular data, the diagnosis from an academic center was malignant peripheral nerve sheath tumor. Post-operative chemotherapy included vincristine, doxorubicin and cyclophosphamide. The disease progressed 5 months after surgery with innumerable pulmonary nodules and hepatic metastases. She was initiated on trabectedin but rapidly progressed and passed away from her disease.

Case 2:

This 53-year-old female presented with pain in the lower abdomen. Computed tomography showed a 3.5 ×3.0 cm solid-cystic mass in the right iliac fossa. The mass was resected and adjuvant chemotherapy (doxorubicin, ifosfamide and mesna) was initiated. Prior to the referral at our center, the diagnosis from an academic center was rhabdomyosarcoma. FISH was negative for *FOXO1* alteration. The patient is currently free of disease (follow-up period 3 months).

Histological examination:

Both cases showed similar histological features and were characterized by dense intratumoral fibrocollagenous stroma (Fig 1A, B), although the intratumoral fibrosis was more prominent in case 1, which showed tumor islands surrounded by thick fibrocollagenous bands, an appearance mimicking desmoplastic small round cell tumor. Case 2 showed prominent pseudoalveolar architecture with focal microcystic and macrocystic change (Fig. 1C); an appearance also seen in case 1, albeit less prominent (Fig. 1D). The tumors were composed of an admixture of round and spindled cells (Fig. 2A); the round cell component dominated in case 2 (Fig. 2B) while spindled cells were prominent in case 1 (Fig. 2C). The chromatin was fine with tiny conspicuous nucleoli and a moderate amount of pale eosinophilic cytoplasm. Mitoses were infrequent in both cases (less than 1 per 10 high-power fields). Case 1 showed focal areas of necrosis associated with cholesterol clefts and calcification; case 2 did not show necrosis. In addition, both cases showed a prominent intratumoral proliferation of capillary sized vessels with hyalinized walls (Fig. 2D) and focal collections of lymphocytes and plasma cells.

Both tumors were positive for CD99 (membranous), desmin, myogenin, MyoD1, S100, SOX10, CD34 and GFAP (Fig. 3). Estrogen receptor and progesterone receptor showed focal weak to moderate intensity staining in both cases. The tumor cells were negative for epithelial markers including keratin (AE1.3/Cam 5.2) and epithelial membrane antigen

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(EMA). H3K27me3 was intact in both tumors. Additional immunohistochemical findings are summarized in table 1.

The previously reported *EWSR1-PATZ1* fusion sarcomas positive cases are summarized in table 2.

FISH for *EWRS1*

Interphase FISH performed using a break-apart probe to the *EWSR1* revealed rearrangement of this locus in both cases. For case 1, the results showed isolated red signals in 43 of 50 nuclei. In case 2, three different patterns of abnormal signals were observed: either split red 5' and green 3' EWSR1 signals, isolated red 5' signal, or isolated green 3' signals in, respectively, 9, 11 and 3 nuclei out of the 50 scored. Collectively, *EWRS1* was interpreted as abnormal in both cases (Fig. 4).

Solid fusion assay

The solid fusion assay in both cases revealed fusion transcripts involving *EWSR1* Exon9 (ENST00000414183) and *PATZ1* Exon1 (ENST00000215919) with 530 supporting unique fusion reads for case 1 and 431 for case 2 (Fig. 5). The similar chimeric proteins contain the transcriptional activation domain of *EWSR1* (exons 1–9) fused to the DNA binding domain of PATZ1 (breakpoints within exon 1) (Fig. 5).

Discussion:

Ewing sarcoma, Ewing-like tumors and related tumors involving the bone and soft tissue constitute a heterogenous group of neoplasms with 4 recognized subclasses: 1) classic Ewing sarcoma family of tumors with fusion involving *EWSR1* or *FUS* to members of the *ETS* family of transcription factors that include *FLI1*, *ERG*, *ETV1*/*4*, and *FEV*, 2) Ewing-like sarcomas associated with *CIC* and *BCOR* rearrangements, 3) sarcomas with *EWSR1* fusions to non-*ETS* family genes, a class that includes *EWSR1-PATZ1* and *EWSR1-NFATc2* (other non-*ETS* fusion partners described in isolated reports include *SP3* [10] and *SMARCA5),* [11] and 4) unclassified round cell sarcomas, a group that lacks known fusions. [12,13] The precise classification of tumors showing rearrangements involving *EWSR1* and non-*ETS* family of transcription factors (class #3 from above) remains uncertain.[12,13] Although these tumors share *EWSR1* rearrangements, it remains to be seen whether they account for a biologically homogeneous group, as the fusion partners are biologically and functionally unrelated. Other morphologically distinctive tumors with *EWSR1* rearrangements not considered related to the Ewing family of tumors include desmoplastic small round cell tumor (DSRCT), myoepithelioma of soft tissues, myxoid liposarcoma, extraskeletal myxoid chondrosarcoma and clear cell sarcoma.

Herein, we describe 2 spindle and round cell sarcomas with *EWSR1* fusion to a non-*ETS* gene (*PATZ1*) with overlapping histological features: monotonous proliferation of round to spindled cells, pseudoalveolar architecture, infrequent mitoses, abundant intratumoral stromal bands, and polyphenotypic differentiation. Both tumors share an unusual immunohistochemical profile: expression of CD99, muscle markers (desmin, myogenin, MyoD1), melanocyte/neural markers (S100 and Sox10) and CD34. Of note, prior to the

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availability of the results of the fusion assay, case 1 and case 2 were characterized as malignant peripheral nerve sheath tumor and rhabdomyosarcoma, respectively.

The *EWSR1-PATZ1* fusion was initially described in a 16-year old male with a chest wall mass.[4] The tumor was referred to as a peripheral neuroectodermal tumor, possibly Askin-Rosai type, alluding to a round cell neoplasm. The tumor cells were positive for keratin, desmin, synaptophysin and NSE, although negative for CD99. More recently, whole transcriptome sequencing of 184 small round cell sarcomas identified 5 additional cases with *EWSR1-PATZ1* fusion (table 2).[6] The tumors affected patients with a wide age range (mean 41 years; range 1 – 68 years), a male preponderance (4 male, 1 female) and a predilection for the abdomen (4 of 5 patients). The histology, evaluated in 3 cases, showed varied morphologic features, although the presence of fibrous stroma and a focal spindle cell component were the most consistent features observed. The tumors were negative for EMA and keratin and positive for S100 and CD99. The *EWRS1-PATZ1* fusion has also been detected in cerebral ganglioglioma and pediatric high-grade gliomas and gangliogliomas.[14,15,16]

The morphologic differential diagnoses for the two cases presented herein is broad and includes several mesenchymal neoplasms including malignant peripheral nerve sheath tumor, rhabdomyosarcoma, DSRCT and myoepithelial carcinoma. Other diagnoses that could be considered include conventional Ewing sarcoma, *CIC*-rearranged sarcomas, *BCOR*-rearranged sarcomas and poorly differentiated synovial sarcoma. Alveolar rhabdomyosarcoma is characterized by fibrovascular septa surrounding discohesive clusters and nests of relatively monomorphic small round blue cells, an appearance focally present in the current tumors.[17] The tumors showed reactivity for desmin, myogenin and myoD1. However, the tumors lacked *FOXO1* rearrangements on the fusion assay and/or FISH assay. The polyphenotypic nature also argues against rhabdomyosarcoma as well as a malignant peripheral nerve sheath tumor. To the best of our knowledge, *EWSR1* translocation has not been reported in any histological subtype of rhabdomyosarcoma or malignant peripheral nerve sheath tumor.

The neoplasms with the greatest degree of overlap with the tumors presented herein are DSRCT and myoepithelial carcinoma. Intrabdominal location, monomorphic small round cells set in abundant fibrous stroma and polyphenotypic differentiation, make DSRCT a distinct possibility. However, the fusion assay did not detect *EWSR1-WT1* fusion, a near universal finding in DSRCTs.[18] Furthermore, in contrast to DSRCTs, these tumors expressed myoD1 and myogenin and did not express epithelial markers. DSRCTs, occasionally express CD99, although the reactivity is cytoplasmic and not membranous, as noted in the current two cases. A dot-like pattern of desmin reactivity, not seen in the current neoplasm, is also characteristic for DSRCT.[19] Myoepithelial carcinoma also deserves strong consideration given that *EWSR1* alteration has been identified in half of all reported cases. The present tumors notably lacked evidence of epithelial differentiation. The three fusion partners identified thus far in myoepithelial carcinomas were not detected on the solid fusion assay, namely, *PBX1*, *POU5F1*, and *ZNF444*.[20]

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Given the relatively monotonous cellular features, *EWSR1* rearrangement and CD99 positivity, conventional Ewing sarcoma deserves consideration, particularly prior to the identification of the fusion partner. Although uncommon, conventional Ewing sarcoma are known to express desmin and S100; myogenic differentiation with myogenin and/or myo D1 positivity has not been reported.[21] RNA sequencing has demonstrated that tumors with fusions involving *EWSR1* or *FUS* with members of the *ETS* transcription factor genes cluster tightly together in a homogenous group. Tumors with *EWSR1* fusions to non- ETS genes (*PATZ1*, *NFATc2, POU5F1, SMARCA5*) from a separate cluster, unrelated to the former neoplasms suggesting that each of these constitute a distinct entity.[22, 23]

The morphologic features overlap with *CIC and BCOR*-rearranged sarcomas. Microscopically *CIC*-rearranged sarcomas show solid sheets of round cells with variable nuclear atypia, coarse chromatin, conspicuous nucleoli and focal areas of spindling and myxoid change. *BCOR*-rearranged sarcomas are characterized by sheets of medium sized round to spindle shaped cells with focal whorling. Of note *CIC-DUX4* tumors may show focal expression with cytokeratin, EMA, S100 protein and desmin. Although the tumors illustrated here show immunohistochemical overlap with *CIC*-rearranged sarcomas, the identification of *EWRS1* fusion would exclude both *CIC*- and *BCOR*-rearranged sarcomas.[24–26]

EWSR1-PATZ1 fusion results from intrachromosomal inversion at 22q12; the two genes are located around 2 Mbp apart.[27] Interestingly, both cases reported here result from similar breakpoints in both genes, consistent with this mechanism. PATZ1 encodes a zinc finger protein and plays an important role in chromatin remodeling and transcriptional regulation. Both heterozygous and homozygous PATZ1 knockout mice developed malignant neoplasms including sarcomas. (reviewed in Ref. no. 28) These findings and the observation that PATZ 1 is downregulated in a range of malignant tumors support its role as a tumor suppressor gene.[29] While the precise oncogenic mechanism of EWRS1/PATZ1 chimeric protein is uncertain, the recently uncovered mechanism behind the chimeric EWS/FLI1 oncoprotein may provide a framework to our understanding of this process.[30] The EWRS1/FLI1 interacts with GGAA repeats present in satellite DNA within the genome, resulting in upregulation of selected genes. The chimeric EWS/FLI1 oncoprotein also downregulates genes by displacing ETS factors bound to canonical ETS-binding sites.[30]

The similarities between this neoplasm and DSRCT are also intriguing. *WT1* is a zinc finger DNA-binding transcription factor which functions predominantly as a tumor suppressor but may also have oncogene-like function in some contexts.[31,32] Notably DSRCT, characterized by *EWSR1-WT1* fusion, also shows polyphenotypic differentiation with expression of keratins, EMA, vimentin, desmin, CD57 and S100.[18] Although the precise mechanism for this phenomenon is unknown, the chimeric EWSR1-WT1 protein transcript activates neural reprogramming factor ASCL1, accounting for neural differentiation.[33] Activation of transcriptional factors along multiple lineages might thus be responsible for polyphenotypic differentiation in DSRCT and, by extension, the *EWSR1-PATZ1* fusion positive sarcoma.

In summary, we report 2 patients with *EWSR1-PATZ1* fusion positive sarcoma showing histological features traditionally associated with ‘atypical Ewing sarcoma’ and

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polyphenotypic differentiation. The precise classification of this neoplasm remains an open question. The unique immunohistochemical profile and data from expression profiling support *EWRS1-PATZ1* spindle and round cell sarcomas as an entity distinct from Ewing sarcoma.[6] Continued refinement of round cell sarcomas could allow for more precise assessment of the risk of progression, response to treatment and help to identify novel therapeutic algorithms. However, as with other uncommon neoplasms, assembling large international cohorts is essential to draw robust conclusions with regards to the clinical, histological, immunohistochemical, biologic and therapeutic aspects of this neoplasm. Regardless of the uncertainties surrounding the precise classification of *EWRS1-PATZ1* spindle and round cell sarcoma, pathologists should consider this entity when confronted with an unusual immunohistochemical phenotype, particularly those that express neural/melanocytic and myogenic markers, and in instances where DSRCT and myoepithelial carcinoma is being considered. Prior to molecular analysis, the immunophenotype could suggest rhabdomyosarcoma or malignant nerve sheath tumor. Advanced molecular techniques such as next generation sequencing or RT-PCR with specific primer set may be needed for this diagnosis, as conventional FISH assay with *EWSR1* break-apart probes may not be able to detect this translocation due to the small size of inversion (although both cases were positive on FISH) [16], and furthermore, would not be able to identify the fusion partner. A multiplexed NGS-based RNA fusion assay is preferred because it lacks bias to the partner gene and minimizes tissue used as compared to sequential testing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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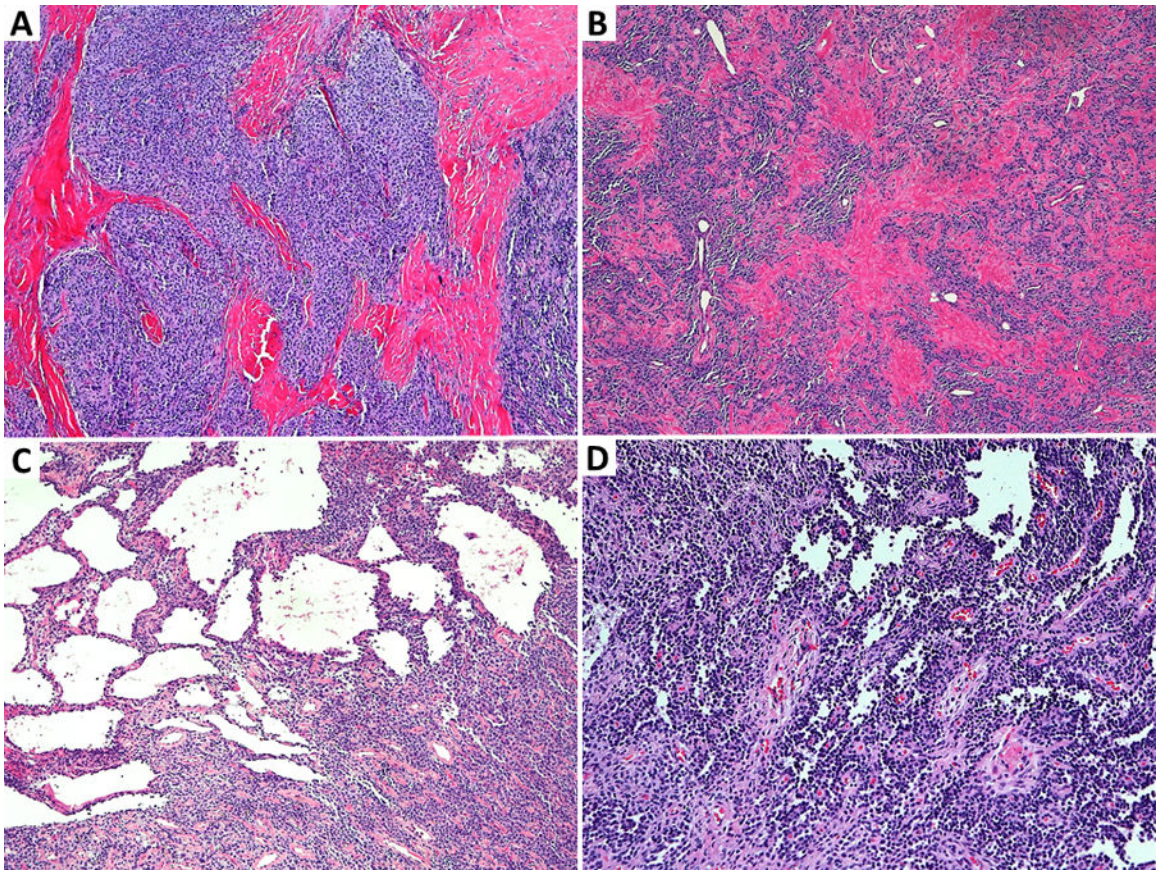

Figure 1.

Low power appearance of case 1 (A) and case 2 (B) showing sheets and nests of cells with prominent fibrocollagenous stroma, (C) pseudoalveolar architecture with microcystic change in case 2, (D) focal pseudoalveolar pattern in case 1.

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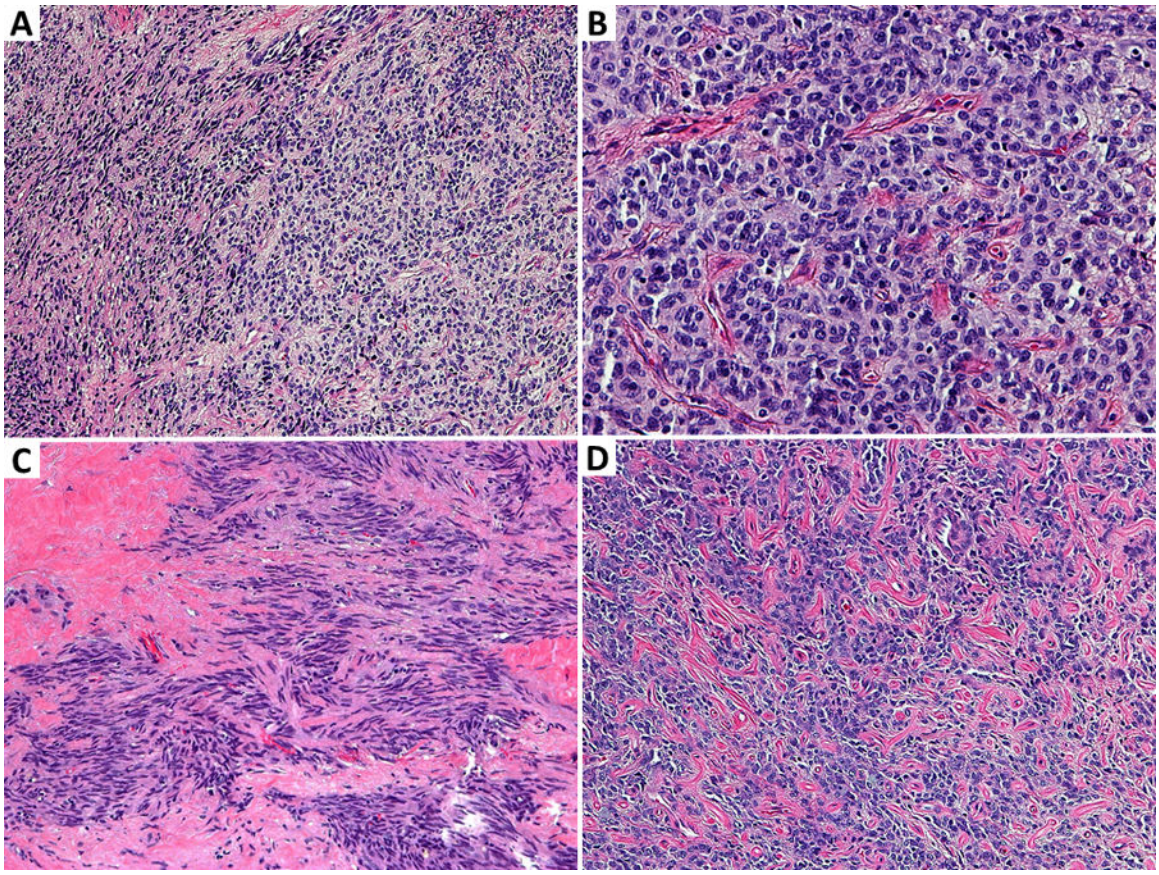

Figure 2.

(A) Sheets of round cells admixed with spindle cells, (B) monomorphic round cells with fine chromatin, tiny conspicuous nucleoli and moderate cytoplasm, (C) prominent spindle cell component was seen in case 1, (D) prominent proliferation of capillary channels with hyalinized walls (case 2).

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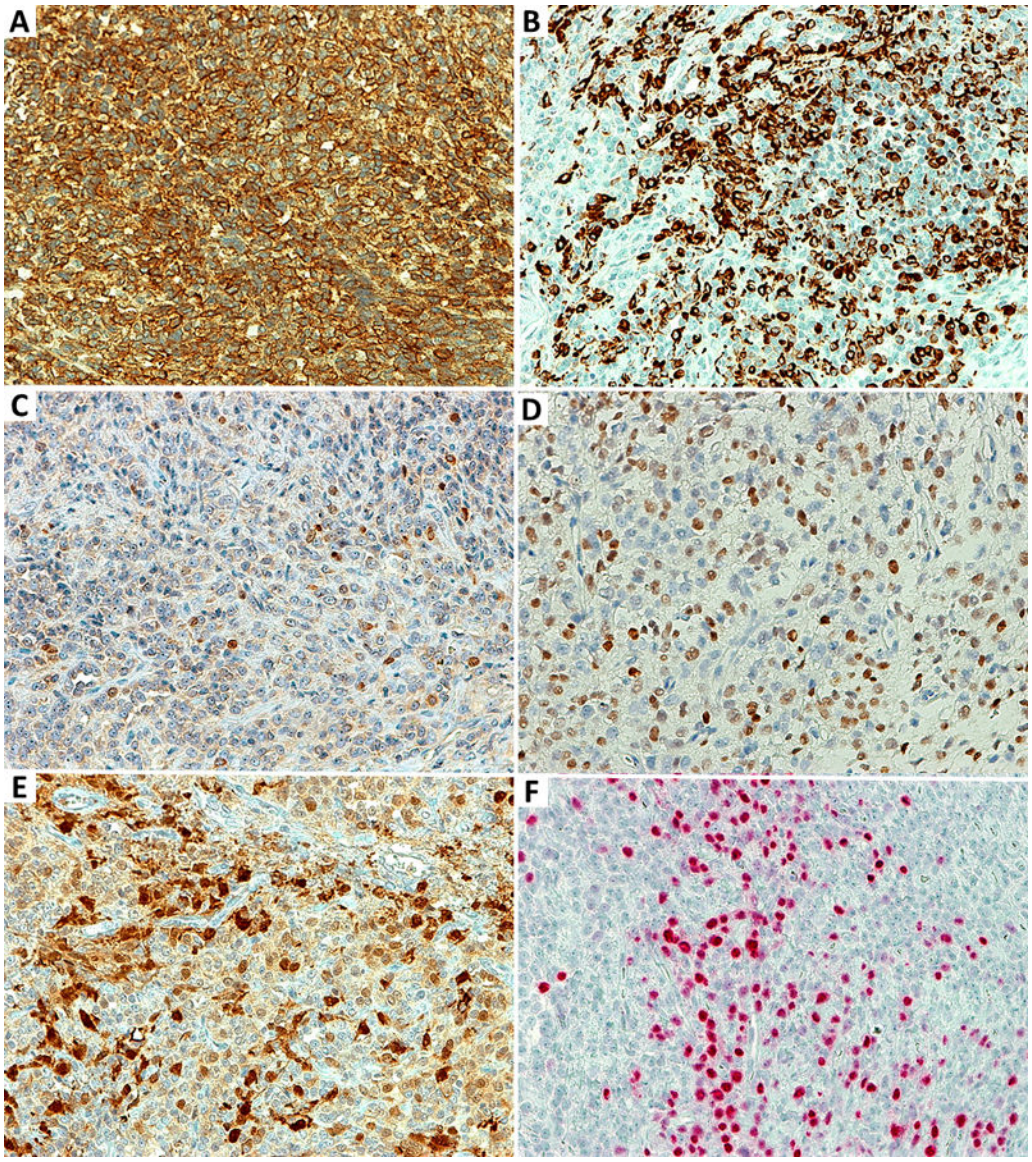

Figure 3.

(A) Strong and diffuse membranous CD99 positivity. The tumors were positive for (B) desmin, (C) myogenin (focal), (D) myo D1 (diffuse), (E) S100 and (F) Sox10.

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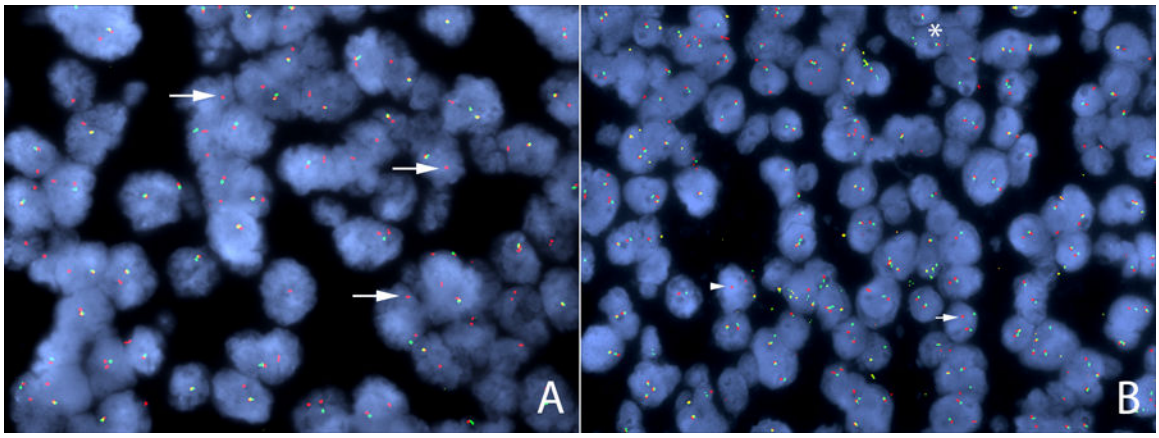

Figure 4.

FISH using a break-apart probe to the *EWSR1*. (A) case 1, shows numerous isolated red signals (arrow). (B) In case 2, three different patterns of abnormal signals were observed: split red 5' and green 3' EWSR1 signals (arrow), isolated red 5' signal (arrowhead), or isolated green 3' signals (*). *EWRS1* was interpreted as abnormal in both cases.

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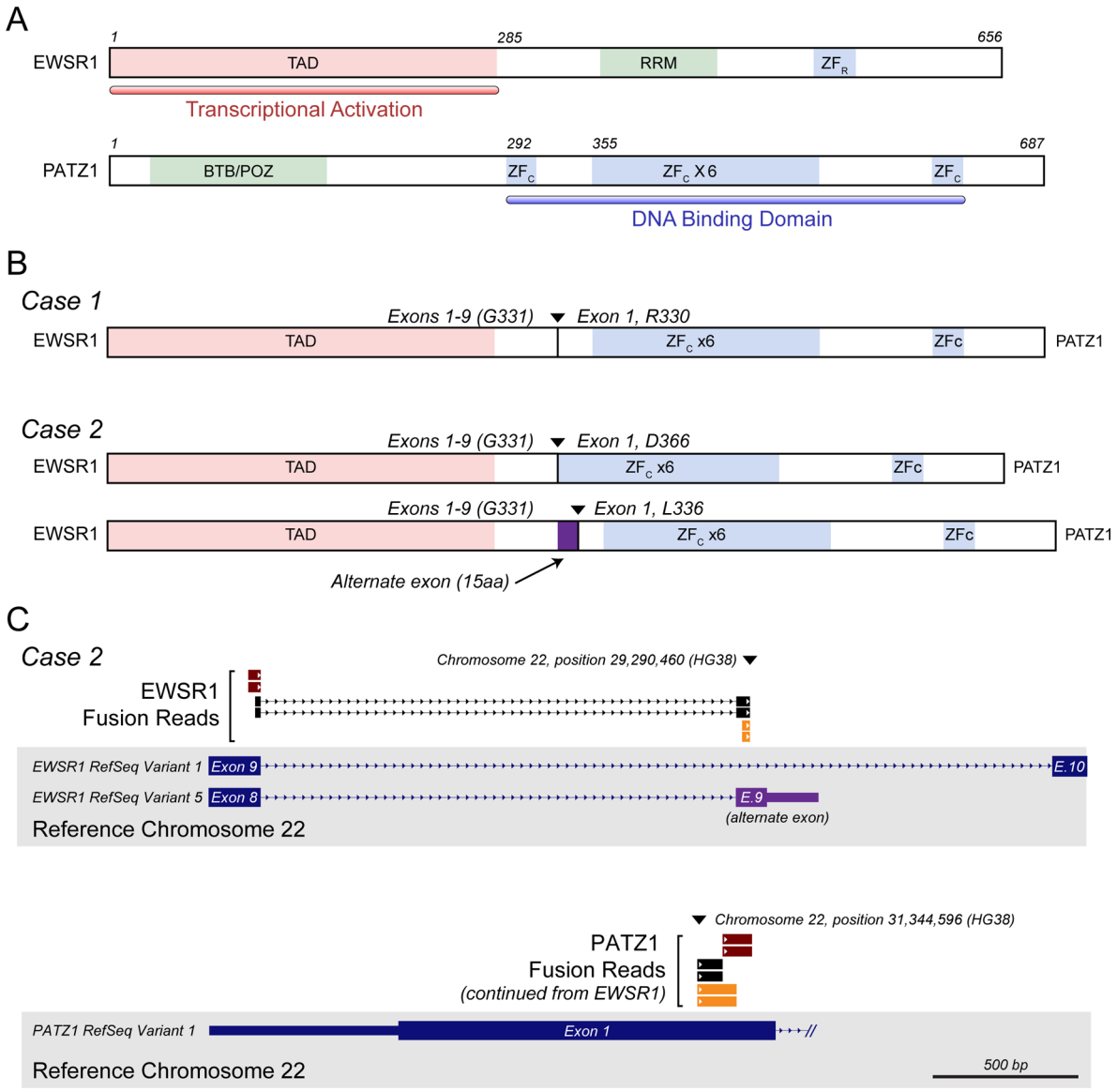

Figure 5.

(A) Schema of EWSR1 and PATZ1 proteins (NP_053733.2 and NP_055138.2, respectively). EWSR1 contains an N-terminal transcriptional activation domain (TAD) commonly fused to DNA binding domains of other proteins. PATZ1 contains a C-terminal DNA binding domain composed of 8 C2H2-type zinc finger domains, along with an N-terminal BTB/POZ homodimerization domain. (B) Schema of EWSR1-PATZ1 fusion proteins. Both cases fuse exons 1–9 of EWSR1 to the majority of the DNA binding domain of PATZ1, with similar breakpoints. In Case 1, the EWSR1 breakpoint is in intron 9, fused to a breakpoint within PATZ1 exon 1. In Case 2, the breakpoint in EWSR1 lies within an alternate exon within intron 9 (see Fig 5C), and the breakpoint in PATZ1 is within exon 1, resulting in an apparent direct exon-exon fusion. The sequencing data also show two alternate splice forms (shown), the second of which contains 15 residues from this alternate exon (purple box). (C) Detailed view of two splice forms of the fusion transcript in Case 2. Representative fusion reads (red,

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black, and orange) are shown aligned to the blue reference sequences (gray shading). The style is similar to the UCSC genome browser: thick bars indicate coding regions, thin bars indicate untranslated regions, blue triangles indicate the direction of an intron, and white triangles indicate breakpoints in fusion transcripts. Red fusion reads support the upper transcript in B, a splice form lacking the alternate exon. Black and orange fusion reads include the EWSR1 alternate exon (purple) and support the lower transcript in (B). Both splice variants are in frame and predicted to be functional. Abbreviations: RRM, RNA recognition motif. ZF_R, zinc finger DNA binding domain, RanBP2-type. RefSeq transcripts: EWSR1 NM_013986.3 (variant 1) and NM_001163287.1 (variant 5); PATZ1 NM_014323.2 (variant 1).

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Table 1:

Immunohistochemistry features of study cases

	Case 1	**Case 2**
CD99	Positive; strong and diffuse	Positive, weak and focal
Desmin	Positive; strong and focal	Positive; diffuse and strong
Myogenin	Positive; focal and weak	Positive; focal and strong
MyoD1	Positive: focal strong	Positive; moderate and focal
Smooth muscle actin	Negative	Positive; strong and focal
Muscle specific actin	Negative	Not done
S100	Positive; multifocal and strong	Positive; diffuse and strong
h-Caldesmon	Negative	Positive: strong and multifocal
Estrogen receptor	Positive; focal and moderate	Positive; diffuse and moderate
Progesterone receptor	Positive; focal and moderate	Positive; weak and focal
CD34	Positive; strong and diffuse	Positive; weak and focal
CD31	Negative	Negative
ERG	Not done	Negative
SOX10	Positive; focal and strong	Positive; focal and strong
HMB45	Negative	Negative
Melan A	Not done	Negative
MITF	Not done	Positive; focal and strong
CAM5.2	Negative	Not done
Keratin (AE1.3/Cam 5.2)	Negative	Negative
EMA	Negative	Negative
GFAP	Positive; strong and diffuse	Positive, focal
CD117	Negative	Not done
WT1	Negative	Negative
Inhibin	Negative	Not done
D240	Not done	Negative
H3K27me3	Intact	Intact

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Table 2:

Brief review of previously reported *EWSR1-PATZ1* fusion positive sarcomas.

Study	Age(years) /Sex	Tumor site	Tumor size	Morphology	Immunohistochemistry	Diagnosis before molecular analysis	Follow up
Mastrangelo et al[4]	16/M	Chest wall	NA	Primitive neuroectodermal tumor possibly Askin-Rosai type	Positive: Synaptophysin, NSE, desmin, keratin Negative: CD99	Peripheral primitive neuroectodermal tumor	Pulmonary metastasis 2 years after diagnosis
Watson et al[6]	0.9/M	Thigh	NA	Malignant monomorphic spindle cell neoplasm	Positive: CD99, S100 Negative: EMA, keratin	Unclassified malignant neuroectodermal tumor	NA
Watson et al[6]	68.5/M	Back (subscapular)	NA	Malignant spindle cells neoplasm Hemangioma-like vasculature	Positive: CD99, S100. Negative for EMA and AE1/AE3	Myxoid liposarcoma	NA
Watson et al[6]	56.2/M	Mediastinum	NA	Malignant epithelioid and spindle cell neoplasm	Positive: CD99, S100 Negative: Keratin, EMA	Unclassified spindle cell sarcoma	NA
Watson et al[6]	46/F	Paravertebral region	NA	NA	NA	Unclassified malignant neuroectodermal tumor	NA
Watson et al[6]	32/M	Flank	NA	NA	NA	Ewing or Ewing-like sarcoma	NA
Present study	31/F	Retroperitoneum	6.5cm	Malignant round and spindle cell neoplasm	Positive: CD99 (membranous), desmin, myogenin, MyoD1, S100, SOX10, CD34, GFAP	Malignant peripheral nerve sheath tumor	Multiple pulmonary and hepatic metastases. Died of disease at 5 months
Present study	53/F	Right iliac fossa	3.5cm	Malignant round and spindle cell neoplasm	Positive: CD99 (membranous), desmin, myogenin, MyoD1, S100, SOX10, CD34, GFAP	Rhabdomyosarcoma	Alive without disease at 3 months follow-up

NA= not available

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