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ARTICLE

EWSR1-WT1 gene fusions in neoplasms other than desmoplastic small round cell tumor: a report of three unusual tumors involving the female genital tract and review of the literature

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Abstract

Desmoplastic small round cell tumor (DSRCT) is a high-grade round cell sarcoma that typically arises in the abdominopelvic cavity of young males, co-expresses keratins and desmin, and carries a pathognomonic *EWSR1-WT1* gene fusion. The *EWSR1-WT1* gene fusion is generally considered specific for DSRCT, although there are two reports of this fusion in tumors otherwise lacking features of DSRCT. We report three female genital tract tumors with *EWSR1-WT1* fusions but showing morphologic and immunohistochemical features incompatible with DSRCT. The tumors occurred in the uterine cervix, uterine corpus/ovaries, and vagina, respectively, of 46, 30, and 20-year-old women. Two tumors consisted of a sheet-like to fascicular proliferation of relatively uniform spindled to occasionally more epithelioid cells arrayed about thick-walled, hyalinized, and capillary-sized vessels, with distinctive areas of pseudovascular change, and absence of desmoplastic stroma. The third tumor resembled a monomorphic spindle cell sarcoma with necrosis. All had diffuse desmin and variable but more limited keratin expression, two of three expressed smooth muscle actin, and all were negative for h-caldesmon, CD10, estrogen receptor, myogenin, N-terminus WT-1, and S100 protein. One patient received neoadjuvant chemotherapy and radiation therapy followed by resection and is disease-free 42 months after diagnosis. Another patient was managed by resection only and is disease-free 9 months after initial diagnosis. The remaining patient recently underwent resection of multifocal pelvic disease. Comprehensive differential gene expression analysis on two tumors compared to two classic DSRCTs with known *EWSR1-WT1* fusions resulted in 1726 genes that were differentially expressed (log2 fold change >2 or < −2) and statistically significant (FDR < 5%). In combination with previous reports, our findings suggest pleiotropy of the *EWSR1-WT1* fusion is possible and not limited to DSRCT. Subsets of non-DSRCT *EWSR1-WT1* positive tumors may represent discrete entities, but further study is necessary.

Introduction

Recurrent gene fusions, first described in soft tissue tumors by Delattre et al. in Ewing sarcoma (*EWSR1-FLI1*) [1], are present in a broad and ever-increasing assortment of neoplasms. Despite early optimism that these genetic events might prove specific and defining, it has become clear many are not. For example, the *ETV6-NTRK3* fusion, identified in infantile fibrosarcoma in 1998 by Knezevich and colleagues [2], is now known to also occur in cellular

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mesoblastic nephroma [3], acute myelogenous leukemia [4] and secretory carcinoma of the breast [5]. Similarly, the *EWSR1-ATF1* fusion, thought particular to clear cell sarcoma of soft parts [6], is also in malignant gastrointestinal neuroectodermal tumors, indolent angiomatoid fibrous histiocytomas, unusual myxoid tumors of the lung and central nervous system, and even clear cell salivary gland carcinomas [7]. Rather than standing in isolation, the presence or absence of gene fusions represents one of many data points integrated by the surgical pathologist in establishing a diagnosis.

Desmoplastic small round cell tumor (DSRCT) is a highly aggressive round cell sarcoma, which typically occurs in the abdominal cavity of male children and adolescents, co-expresses keratins, desmin and vimentin, and is characterized at the genetic level by t(11;22)(p13;q12), with in-frame fusion of the first 7 exons of *EWSR1* (22q12.2) with exons 8–10 of *WT1* (11p13) [8–10]. This *EWSR1-WT1* fusion is considered among the most entity-specific; there is no mention of it in tumors other than DSRCT in either the most recent WHO Classification of Soft Tissue and Bone Tumors [11] or the latest edition of Enzinger and Weiss's Soft Tissue Tumors [12].

However, there are two prior reports of the *EWSR1-WT1* fusion in tumors deviating significantly from DSRCT. The first, by Alaggio and colleagues in 2007, described two leiomyosarcoma-like spindle cell tumors of the abdominal cavity in adolescent males with favorable outcome [13]. Eight years later, Ud Din et al. described an indolent glomoid tumor of the L4 spinal nerve roots sharing this same gene fusion.

We present the clinicopathologic, immunohistochemical, and molecular genetic features of three unusual spindle cell neoplasms with *EWSR1-WT1* fusions that involved the gynecologic tract, and discuss their distinction from DSRCT as well as more common spindle cell neoplasms in these locations.

Materials and methods

Case selection

All available slides and blocks from three consultation cases with a known *EWSR1-WT1* fusion and deviating significantly from DSRCT by morphology (see below) were retrieved from our archives. Formalin-fixed paraffin-embedded (FFPE) tissue blocks were processed by standard protocols for hematoxylin and eosin staining, preparation of immunohistochemical studies and next generation sequencing. Patient history and outcome were retrieved from institutional medical records and managing healthcare providers.

Immunohistochemistry

FFPE sections from cases 1 and 2 were stained for keratins (clone OSCAR, 1:100, Covance, Princeton, NJ, and clones AE1/AE3, 1:100, Dako, Carpinteria, CA), estrogen receptor alpha (clone SP1, prediluted, Ventana Medical Systems, Tucson, AZ), desmin (clone DER11, 1:100. Leica Biosystems, Buffalo Grove, IL), smooth muscle actin (clone 1A4, 1;3000, Dako), h-Caldesmon (clone H-CALD, 1:500, ThermoFisher), CD10 (clone 56C6, Leica Biosystems), N-terminus WT-1 (clone WT49, 1:75, Leica Biosystems), myogenin (clone F5D, 1:50, Biocare), myoD1 (clone EP212, prediluted, Ventana Medical Systems) and S100 protein (polyclonal, 1:750, Leica Biosystems), using routine laboratory protocols and the Ultraview DAB, Refine DAB or Optiview DAB detection systems.

FFPE sections from case 3 were stained for keratins (clone CAM5.2, 1:50, BD Biosciences, Woburn, MA), estrogen receptor (clone SP1, 1:40, Fisher Scientific, Hampton, NH), desmin (clone DE-U-10, 1:5000, Sigma, St. Louis, MO), smooth muscle actin (clone 1A4, 1:20 000, Sigma), h-Caldesmon (clone h-CD, 1:300, Dako), CD10 (clone 56C6, 1:20, Cell Marque, Rocklin, CA), N-terminus WT-1 (clone 6F-H2, 1:75, Dako), C-terminus WT-1 (polyclonal, 1:1000, ThermoFisher), myogenin (clone EP162, 1:200, Cell Marque), myoD1 (clone EP212, 1:100, Cell Marque), CD99 (clone O13, 1:150, BioLegend, Dedham, MA), and S100 protein (polyclonal, 1:3000, Dako).

Next generation sequencing

Cases 1 and 2 were evaluated by the Illumina TruSeq RNA Exome assay that targets over 20,000 genes and includes gene fusion targets as well as common *BCOR* internal tandem duplications. Details of this assay have been previously published [14]. RNA integrity number (RIN) and DV200 values were determined using the Agilent Fragment Analyzer and satisfied laboratory quality thresholds. cDNA libraries were prepared using 200–400 ng of total RNA according to the manufacturer's instructions for the TruSeq® RNA Exome Library Prep Kit. The concentration and size distribution of the final libraries were determined on an Agilent Bioanalyzer DNA 1000 chip (Santa Clara, CA). A final quantification, using Qubit fluorometry (Invitrogen, Carlsbad, CA), was performed to confirm sample concentration.

Samples were sequenced on an Illumina HiSeq 4000 instrument using the 200 cycle Rapid v2 Reagent Kit (Illumina). Base-calling was performed using Illumina's RTA version 2.7.7. Raw data were processed through an automated in-house bioinformatics pipeline [15] with clinically validated scripts [16] for annotating gene fusions in sarcomas. Differentially expressed genes were identified

using bioinformatics R package edgeR 2.6.2 [17], and reported with magnitude of change (log2 scale) and level of significance (False Discovery Rate, FDR < 5%). Canonical pathway analysis was performed using the Ingenuity pathway analysis software IPA (Ingenuity® Systems, www.ingenuity.com).

Case 3 was evaluated using an RNA sequencing platform, the details of which have been previously published [18]. Briefly, the Illumina TruSight RNA fusion panel (Illumina) was used to create libraries of 507 target genes, which were then sequenced on an Illumina MiSeq instrument. Raw data were analyzed using an in-house pipeline and both the STAR aligner and Manta fusion caller and JAFFA fusion caller and BOWTIE2 aligner.

Results

Clinical findings

Table 1 summarizes the clinicopathological features of the three cases that comprise our report as well as three previously reported tumors with *EWSR1-WT1* fusions lacking characteristic features of DSRCT.

Case 1: A 46-year-old woman was referred for biopsy after identification of a cervical mass by physical examination and diagnosis of atypical glandular cells by cervical Papanicolaou smear. This biopsy showed a spindle cell tumor thought to possibly represent a leiomyoma with bizarre nuclei, and she was referred for further surgery. A total hysterectomy, bilateral salpingo-oophorectomy, and bilateral pelvic sentinel lymph node biopsies were performed. A $2.2 \times 1.6 \times 1.6$ cm indurated, yellow-tan to orange-brown mass was confined to the cervix. The remaining specimens were negative for disease. The tumor was diagnosed as an unusual low grade spindle cell sarcoma with *EWSR1-WT1* gene fusion. Four months after resection, physical examination and CT scans of the chest, abdomen, and pelvis showed no recurrent or metastatic disease. A second series of postoperative surveillance CT scans was negative 9 months after diagnosis.

Case 2: A 30-year-old woman experienced sudden onset left-sided abdominal pain and was found by CT imaging to have large ovarian and uterine masses, numerous retroperitoneal cystic and solid masses, and multiple indeterminate pulmonary nodules. She underwent total hysterectomy, bilateral salpingo-oophorectomy, left obturator lymph node dissection and resection of the retroperitoneal, and peritoneal masses. At the time of surgery, she was noted to have multifocal disease involving the left ovary ($9.5 \times 9.2 \times 0.8$ cm), myometrium ($7.1 \times 7 \times 5.8$ cm), retroperitoneum ($18.5 \times 14.5 \times 11$ cm), peritoneum ($21 \times 12 \times 10$ cm), uterine serosa (1.7×1.4 cm), and small soft tissue deposit in the region of left obturator lymph nodes. The tumors were described as solid and multicystic, variably hemorrhagic and necrotic, and brown-tan (Fig. 2A). The tumor was diagnosed as an unusual low grade spindle cell sarcoma with *EWSR1-WT1* gene fusion. Seven months following diagnosis, CT abdomen and pelvis identified two sites of subcentimeter lymph node metastases, one biopsy-proven, in the regions of the right anterior kidney and anterior to the inferior vena cava at the level of the pancreatic head. The patient is considering stereotactic body radiotherapy.

Case 3: A 20-year-old woman presented for evaluation of a vaginal mass. Her clinical history was significant for a remote history of Ewing sarcoma of the toe metastatic to the pleura with membranous CD99 expression and *EWSR1-ERG* gene fusion for which she received adjuvant chemo/radiotherapy. She also had a germline heterozygous *BRIP1* c.2098-3 T > C variant of uncertain significance. At the time of surgery, a 5.4 cm mass was centered in the left vaginal fornix, abutting the cervix, bladder, and rectum. Biopsies from the vaginal mass showed a malignant neoplasm with spindle cell and epithelioid features that were difficult to classify. This patient received neoadjuvant chemotherapy followed by radiation therapy, with partial vaginectomy, total hysterectomy, and left salpingectomy 8 months after initial diagnosis. Residual tumor grossly measured $2.8 \times 2.1 \times 1.4$ cm. The tumor was diagnosed as an unclassifiable spindled and epithelioid sarcoma, morphologically high grade. Nearly three and a half years after diagnosis, the patient is alive without evidence of disease.

Morphologic findings

The morphologic features of Cases 1 (Figs. 1A–F) and 2 (Fig. 2B–F) were similar. At low power magnification, the tumors permeated surrounding cervical stroma and myometrium reminiscent of endometrial stromal sarcoma with multiple tongues and nests of tumor cells percolating fibroconnective tissue and smooth muscle (Figs. 1A and 2B). The neoplastic cells generally formed sheets and fascicles with areas of nested and corded architecture and occasional cystic change (Figs. 1B and 2C). The tumor cells were for the most part spindled with a modest amount of eosinophilic cytoplasm, indistinct cell borders, and elongated nuclei with blunt or tapered ends (Fig. 1C). In some portions, the tumor cells were smaller and more epithelioid (Figs. 1D and 2D). Scattered pleomorphic cells were present. Numerous blood vessels were intermixed, but vessels resembling spiral arterioles were not. The mitotic index ranged from 1 to 4 figures per 10 high power fields. Cystic change was seen in two tumors and necrosis was identified in one tumor. In one tumor, a combination of cystic change and pseudopapillary growth produced in one area a somewhat "cotyledonoid" pattern. Classic features of DSRCT

Table 1 Clinicopathologic features of non-desmoplastic small round cell tumors with *EWSR1-WT1* Fusions.

Case	Study	Age, sex	Location	Morphology	Keratins	Desmin	SMA/h-caldesmon	Gene fusion	Metastases	Therapy	Patient outcome
1	Present series	46, F	Uterine cervix	Bland ovoid to spindled cells, hyalinized vessels, pseudovascular spaces, absent desmoplasia	Positive (rare cells)	Positive	Positive/ negative	*EWSR1* exon 9-*WT1* exon 8	None	Resection	ANED, 9 months
2	Present series	30, F	Multifocal (uterus, ovaries, retroperitoneum, peritoneum, pelvis)	Bland ovoid to spindled cells, hyalinized vessels, pseudovascular spaces, absent desmoplasia	Positive	Positive	Positive/ negative	*EWSR1* exon 9-*WT1* exon 8	Extensive intra-abdominal disease at presentation	Resection	AWD, 2 small abdominopelvic lymph node metastases developed after resection, considering stereotactic body radiotherapy, 7 months
3	Present series	36, F	Vagina	Monomorphic spindle cell sarcoma, absent desmoplasia	Positive (rare cells)	Positive	Positive/ negative	*EWSR1* exon 10-*WT1* exon 7	None	Neoadjuvant chemo-radiotherapy	ANED, 3.5 years
4	Ud Din et al [14]	34, M	Cauda equina	Glomoid cells, myxoid and hyalinized stroma, rosette-like structures	Negative	Positive	Positive/ NP	*EWSR1* exon 7-*WT1* exon 8	None	Partial resection, eventual radiotherapy and tumor embolization	AWD, 10 years and 9 months
5	Alaggio et al [13]	11, M	Pelvis	Spindled to focally epithelioid tumor resembling leiomyosarcoma, absent desmoplasia	Positive	Positive	Positive/ rare cells	*EWSR1-WT1*	None	Resection	ANED, 40 months
6	Alaggio et al [13]	9, M	Small intestine	Spindled to focally epithelioid tumor resembling leiomyosarcoma, absent desmoplasia	Positive	Positive	Positive/ rare cells	*EWSR1-WT1*	Liver, lymph nodes, omentum	Resection of metastases, multiagent chemotherapy	ANED, 13 years

SMA smooth muscle actin, *F* female, *M* male, *ANED* alive with no evidence of disease, *AWD* alive with disease, *NP* not performed.

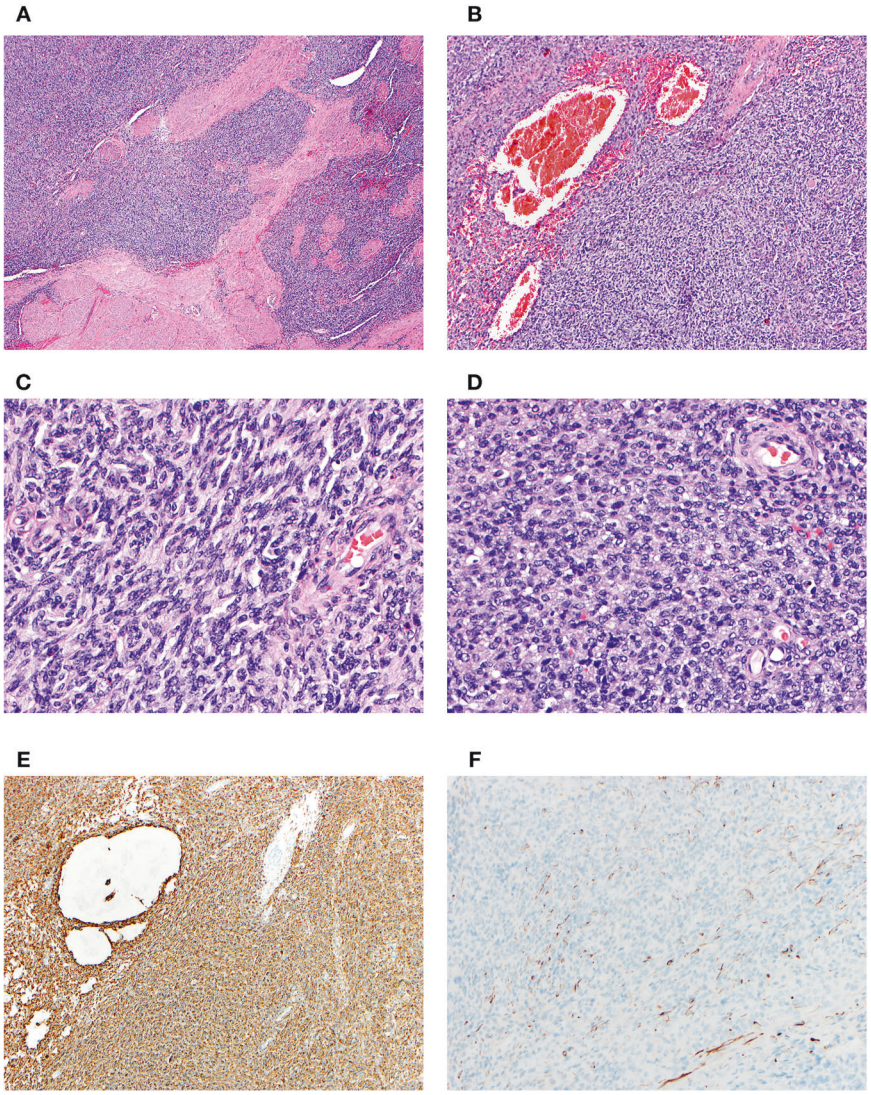

Fig. 1 Morphologic and immunohistochemical findings in Case 1. The tumor in Case 1 permeated cervical stroma in multiple tongues and nests (**A**) and had a mixture of sheet-like and fascicular architecture with scattered cystic change (**B**). The tumor cells were mostly spindled with a modest amount of eosinophilic cytoplasm, indistinct cell borders and elongated nuclei with blunt or tapered ends (**C**). Some portions of tumor had more epithelioid cells (**D**). Desmin expression was diffuse (**E**) whereas keratin AE1/AE3 was limited to a few clusters of cells (**F**).

including primitive round cells in a desmoplastic stroma were absent in both tumors.

In contrast, Case 3 was a fascicular tumor composed of uniform spindle cells with scant lightly eosinophilic cytoplasm, hyperchromatic nuclei, up to 13 mitotic figures/10 high power fields, and scattered foci of necrosis (Fig. 3A–D). The tumor resembled monophasic synovial sarcoma or malignant peripheral nerve sheath tumor, but alternating zones of hyper- and hypocellularity, accentuated perivascular cellularity, staghorn blood vessels and wiry collagen were lacking. Desmoplastic stroma was also not seen.

Immunohistochemical findings

Table 1 summarizes immunohistochemical results. Diffuse expression of desmin was seen in all tumors (Figs. 1E and 2E). Keratin was expressed by all tumors, but it was more limited and varied by antibody (Figs. 1E and 2E). Smooth muscle actin was positive in 2 of 3 tumors; all were h-Caldesmon negative. CD10, estrogen receptor, N-terminus WT-1, myogenin, myoD1 and S100 protein were negative in all tumors. Case 3 was negative for CD99 and showed nuclear positivity for WT-1 directed against the C-terminus.

Next generation sequencing findings

An *EWSR1-WT1* gene fusion was identified in each tumor. The fusion transcript in Cases 1 and 2 had a 5' gene partner breakpoint at exon 9 and 3' gene partner breakpoint at exon 8. For Case 3, the *EWSR1* breakpoint was at exon 10 (of 18) and the *WT1* breakpoint at exon 7; in addition, *WT1* had a 12 bp in-frame deletion.

Comprehensive differential gene expression analysis between Cases 1 and 2 and two cases of classic DSRCTs with known *EWSR1-WT1* fusions in adult males resulted in

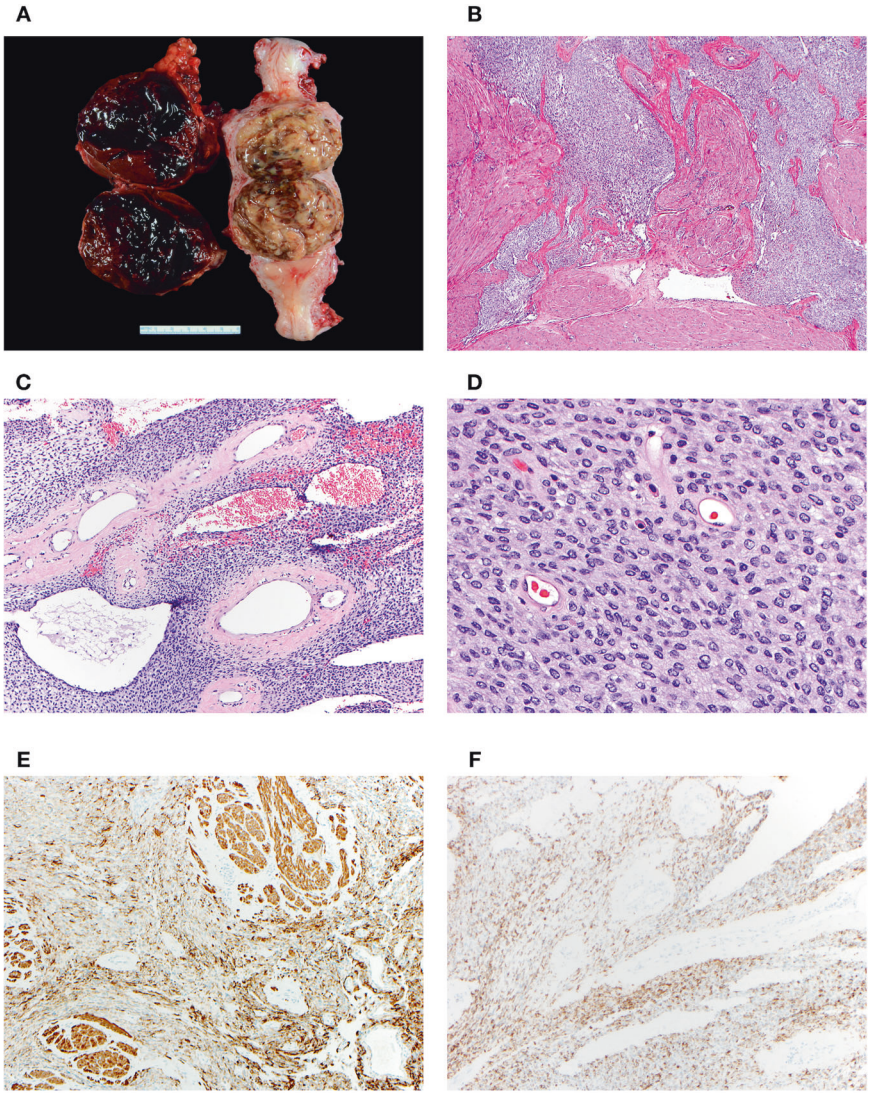

Fig. 2 Morphologic and immunohistochemical findings in Case 2. Case 2's patient presented with multifocal disease that partly included the ovary and uterus as solid brown-tan masses that were variably hemorrhagic and necrotic (**A**). Like Case 1, Case 2 permeated myometrium in a manner resembling an endometrial stromal sarcoma (**B**), and was composed of sheets and fascicles of cells with cystic change (**C**, **D**). Thick and thin-walled blood vessels, occasionally associated with perivascular hyaline, were present (**C**). Desmin was diffusely positive (**E**). Keratin AE1/AE3 was more extensively expressed by tumor cells (**F**, correlative of **C**).

1,726 genes that were differentially expressed (log2 fold change >2 or < −2) and statistically significant (FDR < 5%). About 40% of these genes were upregulated in Cases 1 and 2 (Fig. 4). Canonical pathway analysis indicated activated immune response and negative regulation of cell cycle signaling pathways in Cases 1 and 2.

Discussion

Inclusive of the 3 tumors in our report, we are aware of 6 tumors with *EWSR1-WT1* fusions that are something other than DSRCT (Table 1). Although these non-DSRCT *EWSR1-WT1* tumors share some clinical and immunohistochemical features, they are morphologically heterogeneous, and are unlikely to represent a single discrete entity.

Perhaps most critical is whether these unusual *EWSR1-WT1* tumors are variants of DSRCT. We interpret the morphologic, immunohistochemical and clinical evidence to suggest they are not. DSRCT may occasionally show minor morphologic variation such as glands, papillae, or slightly spindled cells, but the majority of these tumors have nests of malignant primitive round cells embedded in a richly vascular desmoplastic background [19, 20]. The morphologic features of our 3 cases are different, with none having desmoplastic stroma, two consisting of bland ovoid to spindle cells in association with hyalinized blood vessels and pseudovascular spaces, and one resembling a monomorphic spindle cell sarcoma. Furthermore, while our cases co-express keratins and desmin, similar to DSRCT, they also express other markers of smooth muscle differentiation, unlike DSRCT [10]. Co-expression of keratins and desmin is not specific to DSRCT and may be found in conventional

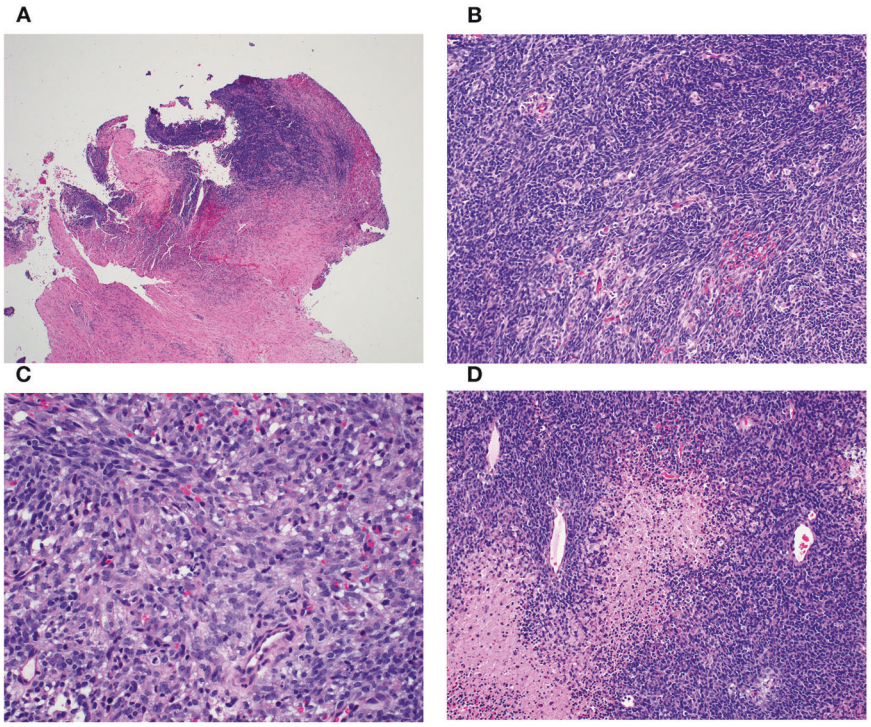

Fig. 3 Morphologic and immunohistochemical findings in Case 3. The tumor in Case 3 was predominantly fascicular (**A**, **B**) and composed of a uniform population of spindle cells with scant pale eosinophilic cytoplasm and hyperchromatic nuclei. In contrast to Cases 1 and 2, mitotic activity in Case 3 was more frequent at up to 13 mitotic figures/10 high power fields and there were foci of necrosis (**C**, **D**).

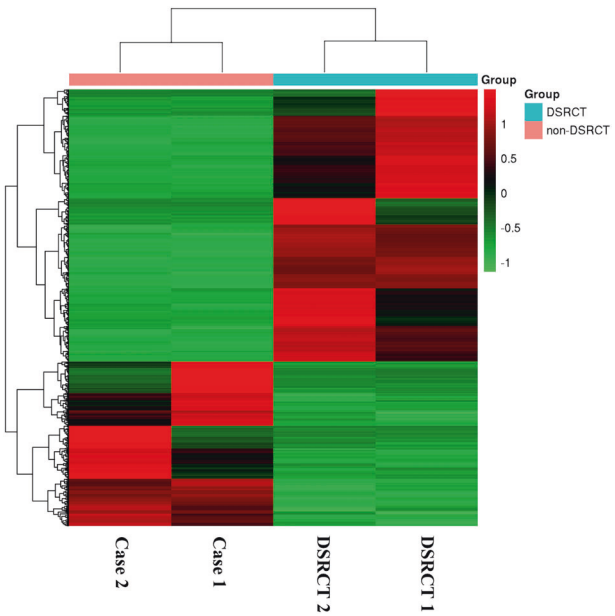

Fig. 4 Differential gene expression analysis of tumors with EWSR1-WT1 fusions. Unsupervised clustering 1,726 differentially expressed genes shows separation of the two study cases (Cases 1 and 2) and two classic DSRCTs with known *EWSR1-WT1* gene fusions, represented as a heatmap.

leiomyosarcomas and rhabdomyosarcomas, among others [12]. In addition, that these unusual *EWSR1-WT1* tumors affect both males and females and are relatively indolent significantly differs from the male predilection and markedly aggressive behavior of DSRCT [21–23]. On a transcriptomic level, profiling of two *EWSR1-WT1* tumors and two classic DSRCTs with *EWSR1-WT1* fusions demonstrated significant differences in gene expression. Finally, DSRCT of the female genital tract is exceptionally rare, with only two reported examples: a genetically confirmed case of the uterine corpus in a 46-year-old woman [21] and a morphologically and immunohistochemically typical example in the cervicovaginal region of a 19-year-old woman [24].

Data are insufficient to definitively address whether non-DSRCTs with *EWSR1-WT1* fusions represent discrete entities—as in tumors with *EWSR1/FUS-ATF1/CREB1* fusions -- or are an assortment of unrelated tumors that share a single molecular genetic event. The distinctive morphologic and immunohistochemical features of Cases 1 and 2 suggest these two tumors may represent a discrete entity within the family of *EWSR1-WT1* neoplasia. Likewise, the tumors reported by Alaggio et al (Table 1, Cases 5 and 6) appear homogenous and do not clearly correspond to another well-defined soft tissue entity [13]. No conclusions can yet be formed about Cases 3 and 4 (Table 1) which have singular features.

One patient in our series (Case 3) had a history of Ewing sarcoma (*EWSR1-ERG* positive) and was known to carry a germline single nucleotide variant in intron 14 of *BRIP1*. Although her particular VUS is not present in population databases and has not, to the best of our knowledge, been reported in patients with known *BRIP1*-related neoplasms, hereditary mutations of homologous recombination repair

genes such as *BRIP1*, both pathogenic and likely pathogenic variants, have been linked to Ewing sarcoma predisposition and may be related to development of other *EWSR1* rearranged neoplasms [25]. One might attribute the occurrence of two different *EWSR1* tumors in a young patient as somehow related to her underlying *BRIP1* VUS. However, the morphologic, immunohistochemical, and molecular genetic features of her vaginal mass were not those of recurrent Ewing sarcoma.

The differential diagnosis for these non-DSRCT *EWSR1-WT1* tumors includes endometrial stromal neoplasms, smooth muscle tumors and, for Case 3, other monomorphic spindle cell sarcomas such as synovial sarcoma. Low grade endometrial stromal sarcomas classically permeate as separate nests of monotonous spindle cells resembling endometrial stroma with intermixed spiral arterioles and occasional hyaline plaques [26, 27]. They often express CD10 and hormone receptors, but can be positive for smooth muscle markers, particularly in tumors with smooth muscle differentiation [28]. Several gene rearrangements have been described in low grade endometrial stromal sarcomas, most commonly involving *JAZF1* and *PHF1* [26]. Some low grade endometrial stromal sarcomas are accompanied by a high grade component that has a solid, permeative, or frankly infiltrative growth of small round cells [29]. The high grade round cell component typically diffusely expresses cyclin D1 and BCOR, often expresses KIT, and is negative for CD10 and estrogen and progesterone receptors [30, 31]. Genetically defined high grade endometrial stromal sarcomas can also develop without a low grade component as pure *YWHAE-NUTM2A/B* fused tumors or as myxoid spindled to epithelioid neoplasms resembling myxoid smooth muscle tumors that have *ZC3H7B-BCOR* fusions or internal tandem duplications of *BCOR* [32, 33]. *BCOR*-altered high grade endometrial stromal sarcomas are often positive for CD10, cyclin D1, BCOR, and SATB2 while variably positive for muscle markers and hormone receptors [32–34]. Cases 1 and 2 from our series had some morphologic features of low grade endometrial stromal tumors, but lacked CD10 and hormone receptor expression as well as endometrial stromal tumor-related molecular genetic alterations.

The morphologic and immunohistochemical findings of Cases 1 and 2 also prompted consideration of a gynecologic smooth muscle tumor. However, these tumors lacked characteristic eosinophilic cytoplasm, elongated nuclei and perinuclear vacuoles often seen in smooth muscle neoplasms, as well as the cleft-like spaces and hydropic change found in some gynecologic smooth muscle tumors [35–37]. The immunohistochemical features of Cases 1 and 2 overlap with gynecologic smooth muscle tumors, but absent expression of hormone receptors and h-Caldesmon is unusual for the latter group.

In addition to leiomyosarcoma, the differential diagnosis for Case 3 included other monomorphic spindle cell sarcomas: synovial sarcoma, spindle cell rhabdomyosarcoma, malignant peripheral nerve sheath tumor, and others. Careful morphologic study, a panel of immunostains to include keratins, S100 protein, SOX10, H3K27me3, myogenin, myoD1 and molecular genetic evaluation for events such as synovial sarcoma-specific *SS18/SS18L1-SSX1/2/4* gene fusion should allow differentiation of these considerations from rare *EWSR1-WT1* spindle cell sarcomas.

In summary, we present the clinicopathologic, immunohistochemical, and molecular genetic findings of three unusual neoplasms involving the female genital tract with *EWSR1-WT1* fusions. The features of these tumors deviate from those of DSRCT, and, in combination with prior reports, strongly suggest this fusion is not specific to a single entity. Whether the presence of *EWSR1-WT1* fusions defines other discrete entities remains to be established.

Data availability

Data generated or analyzed during this study are included in the manuscript.

Author contributions The study was conducted with approval by institutional review boards. All authors contributed and approved submission and resubmission of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethics approval The study was conducted in compliance with the authors' institutional ethics committees.

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