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BRIEF REPORT

Low-grade endometrial stromal sarcoma with a novel *MEAF6-SUZ12* fusion

Naohiro Makise[1,2] · Masaya Sekimizu[3] · Eisuke Kobayashi[4,5] · Hiroshi Yoshida[1] · Masashi Fukayama[2] · Tomoyasu Kato[5,6] · Akira Kawai[4,5] · Hitoshi Ichikawa[3,7] · Akihiko Yoshida[1,5]

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Abstract

Endometrial stromal sarcoma (ESS) is a rare mesenchymal neoplasm. Herein, we report a low-grade ESS with a novel *MEAF6-SUZ12* fusion gene. A 40-year-old woman presented with a 9.0-cm abdominal wall mass juxtaposed to the postoperative scar of surgeries for uterine "leiomyomas" and cesarean section. Histologically, mostly hypocellular and myxoid nodules were comprised of uniform spindle cells and exhibited tongue-like infiltration. Immunohistochemically, the tumor cells were positive for CD10, estrogen receptor, and CD34 (focal). There were occasional h-caldesmon-positive cohesive nests. RNA sequencing along with reverse transcriptase-polymerase chain reaction and Sanger sequencing identified an in-frame fusion of *MEAF6* (exon 4) and *SUZ12* (exon 2). Upon review of the previous "leiomyomas," we revised their diagnoses as low-grade ESS. The patient is alive without disease 2 years after the surgery. In addition to expanding the molecular landscape of low-grade ESS, this case highlights the challenge of diagnosing low-grade ESS in an uncommon clinicopathological setting.

Keywords Endometrial stromal sarcoma · Histone modification · MEAF6 · Polycomb repressive complex · SUZ12

Introduction

Low-grade endometrial stromal sarcoma (ESS) is a rare mesenchymal tumor accounting for approximately 20% of sarcoma of the uterine corpus. Tumor cells of low-grade ESS resemble endometrial stromal cells in the proliferative phase, with occasional differentiation toward smooth muscle, sex cord, or epithelium [1]. A specific gene rearrangement t(7;17)(p15;q11) and the resulting fusion transcript *JAZF1-SUZ12* (also known as *JAZF1-JJAZ1*) are observed in approximately 50% of low-grade ESS [2, 3]. Less common fusions include *JAZF1-PHF1*, *EPC1-PHF1*, *MBTD1-CXorf67*, *MEAF6-PHF1*, *JAZF1-BCORL1*, and *BRD8-PHF1*. Here, we report a case of low-grade ESS with a novel *MEAF6-SUZ12* fusion, which was successfully diagnosed only after careful clinicopathological correlation.

✉ Akihiko Yoshida
akyoshid@ncc.go.jp

[1] Department of Pathology and Clinical Laboratories, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

[2] Department of Pathology, the University of Tokyo, Tokyo, Japan

[3] Department of Clinical Genomics, National Cancer Center Research Institute, Tokyo, Japan

[4] Department of Musculoskeletal Oncology, National Cancer Center Hospital, Tokyo, Japan

[5] Rare Cancer Center, National Cancer Center Hospital, Tokyo, Japan

[6] Department of Gynecologic Oncology, National Cancer Center Hospital, Tokyo, Japan

[7] Division of Translational Genomics, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Tokyo, Japan

Case report

A 40-year-old woman presented with an 8-cm mass in the abdominal wall. She had a history of osteosarcoma in her childhood, which was successfully treated with chemotherapy and above knee amputation. She also reported surgeries for "leiomyomas" at an outside institution, including two "myomectomy" at age 29, and total hysterectomy, left salpingo-oophorectomy, and right salpingectomy at age 39. She also had a delivery via caesarian section at the age of 37.

An open biopsy of the mass showed spindle cell proliferation within fibromyxoid stroma. The tumor cells harbored uniform slightly hyperchromatic nuclei and scant cytoplasm, and they were immunohistochemically positive for estrogen

receptor, CD34 (focal), and smooth muscle actin (focal), while negative for AE1/AE3, epithelial membrane antigen, S100 protein, desmin, h-caldesmon, myogenin, MUC4, β-catenin, and STAT6. No definitive diagnosis was rendered, but benign tumor such as cellular angiofibroma was favored. The patient was closely followed up for 1 year, during which time the tumor enlarged to 9 cm. Re-biopsy obtaining a larger volume of tissue demonstrated a prominent tongue-like infiltrative border of tumor tissue and arteriole-like vessels that were swirled around by tumor cells. Prompted by this histology, clinical information was further obtained, which revealed that the mass was located near the postoperative scar. Due to the combination of the past surgical history of uterine tumors, the anatomical relationship to the scar, and the histological findings, a diagnosis of low-grade ESS was suspected. The tumor was widely resected.

Grossly, the 9.0-cm well-circumscribed intramuscular tumor was juxtaposed to the postoperative scar. A cut section showed aggregates of multiple soft tan nodules within a sclerotic background, each measuring 1 to 15 mm (Fig. 1a).

Histologically, a variably cellular tumor formed numerous nodules within a sclerotic background and exhibited prominent tongue-like projections into the surrounding tissue (Fig. 1b). Many tumor nodules were hypocellular and myxoid, populated by loose fascicles of uniform oval to fusiform tumor cells with slightly hyperchromatic nuclei and scant cytoplasm (Fig. 1c). The tumor cells occasionally swirled around arterioles (Fig. 1d). Other tumor nodules exhibited hypercellular fascicular growth (Fig. 1e). Some tumor cells assumed cohesive structures, forming cords, trabeculae, or nests (Fig. 1f). Vascular invasion was present, and surgical margins were negative. Less than one mitotic figure was seen per 10 high-power fields (2.37 mm^2). Necrosis was absent.

Immunohistochemically, the tumor cells were diffusely positive for estrogen receptor (Fig. 1g), progesterone receptor, WT1 (nucleus), INI1 (retained), and focally positive for CD10 (Fig. 1h), Rb (retained), and CD34 (Fig. 1i), whereas it was negative for AE1/AE3, epithelial membrane antigen, myogenin, MDM2, MUC4, STAT6, HMB45, cyclin D1, BCOR, and inhibin α. Ki-67 was positive in 5–10% of the tumor cells. Some cohesive tumor cell nests suggested sex cord differentiation; however, they were positive for desmin and h-caldesmon (Fig. 1f inset) while negative for inhibin α, consistent with focal smooth muscle differentiation. However, that was a focal phenomenon and the overwhelming majority of the tumor lacked myogenic differentiation. Based on characteristic histology (e.g., tongue-like infiltration and swirling growth around arterioles) and immunoprofile, a final diagnosis of low-grade ESS was made.

By break-apart fluorescence in situ hybridization, the tumor showed no evidence of rearrangements of *JAZF1* or *PHF1* genes. The formalin-fixed paraffin-embedded tumor tissue was then submitted to RNA sequencing (RNA-Seq). Total RNA was extracted using an RNeasy FFPE Kit (Qiagen, Hilden, Germany). The sequencing library was prepared from 0.2 μg of total RNA using a TruSight RNA Pan-Cancer Panel (Illumina, San Diego, CA, USA), and paired-end sequencing (2 × 75 bases) was performed on a NextSeq sequencer (Illumina). Fusion genes were searched using the STAR-Fusion version 1.2.0 and the FusionCatcher version 1.00 algorithms. RNA-Seq identified an in-frame fusion of *MEAF6* (exon 4) and *SUZ12* (exon 2), which was validated by reverse transcriptase-polymerase chain reaction (RT-PCR) and Sanger sequencing (Fig. 2) by using a pair of primers (5′-TGATCGAAGGAACCGGAAGT-3′ and 5′-TGGTGCTATGAGATTCCGAGT-3′, predicted product size of 166 bp). Another variant fusion transcript, joining *MEAF6* (exon 4) to *SUZ12* (exon 5), was also detected by RNA-Seq; however, it was not confirmed by RT-PCR. Additional immunohistochemistry showed retained expression of H3K27me3 and SUZ12.

We retrieved and reviewed previous pathology materials, which were originally diagnosed as "leiomyomas" elsewhere, and we found that they all demonstrated histology identical to that of the abdominal wall tumor and revised their diagnoses as low-grade ESS. The patient is alive with no evidence of sarcoma, 2 years after the surgery of the abdominal wall mass and 12 years after the first surgery for the primary uterine tumor.

Discussion

Low-grade ESS is characterized by specific fusion genes, with *JAZF1-SUZ12* being the most common [2]. Other less common types include *JAZF1-PHF1*, *EPC1-PHF1*, *MBTD1-CXorf67*, *MEAF6-PHF1*, *JAZF1-BCORL1*, and *BRD8-PHF1*. Notably, these fusions nearly always involve genes that encode members of histone modification complexes, especially polycomb-group proteins. *SUZ12*, *PHF1*, and *MBTD1* encode polycomb-group proteins that are associated with histone methylation and transcriptional repression [2, 4, 5]. *BCOR* [6] and *BCORL1* [7] encode transcriptional co-repressors, which are reported to interact with histone deacetylases.

Here, we reported the first case of low-grade ESS with a novel fusion that combines *MEAF6* (located on 1p34) and *SUZ12* (located on 17q12), although both partner genes are known to be involved in the creation of fusions in low-grade ESS, namely *JAZF1-SUZ12* and *MEAF6-PHF1*. Because there are no reports documenting a karyotype with a 1;17-rearrangement in low-grade ESSs, to our knowledge [3, 8], the prevalence of this fusion is estimated to be low (< 5%).

SUZ12 encodes a member of the polycomb repressing complex 2 (PRC2), which catalyzes trimethylation of the 27th lysine of histone 3. One of the most critical domains of

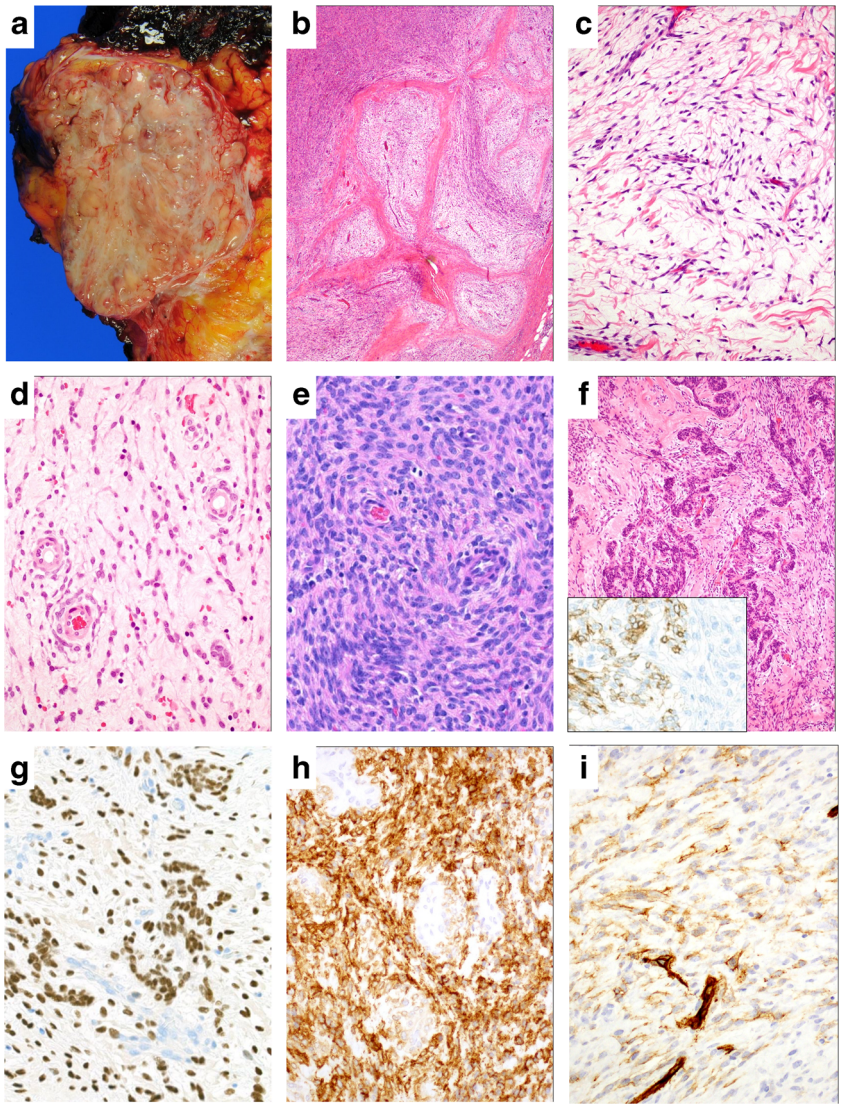

Fig. 1 Morphological and immunohistochemical findings of low-grade endometrial stromal sarcoma with *MEAF6-SUZ12* fusion. Grossly, the well-circumscribed tumor was comprised of aggregates of multiple soft tan nodules within a sclerotic background (**a**). Histologically, the tumor showed multinodular infiltration with tongue-like projections (**b**). Many tumor nodules were composed of hypocellular myxoid tissue populated by loose fascicles of uniform spindle cells, similar to the first biopsy (**c**), but careful observation revealed arterioles swirled around by tumor cells (**d**). Hypercellular conventional histology of low-grade endometrial stromal sarcoma was also observed (**e**). There were small foci of cohesive cells arranged in cord or nest, which were positive for h-caldesmon (**f** inset shows h-caldesmon staining). The tumor cells were positive for estrogen receptor (**g**) and CD10 (**h**). CD34 was focally positive (**i**)

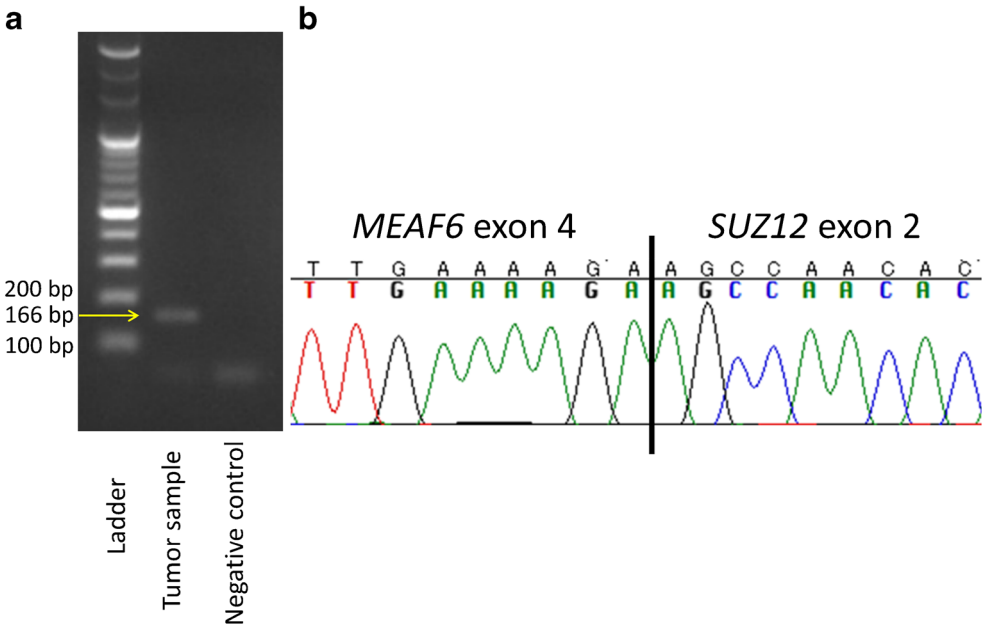

Fig. 2 In-frame fusion transcript of exon 4 of *MEAF6* and exon 2 of *SUZ12* was detected by RT-PCR (**a**) and Sanger sequencing (**b**)

SUZ12 is the VEFS domain near the C-terminal, which is necessary for the integrity and catalytic activity of the PRC2 complex [9]. The predicted fusion product MEAF6-SUZ12 in this case retained this domain, similar to *JAZF1-SUZ12*, which usually contains exon 2 through 16 of *SUZ12* [8]. Although the exact oncogenic role of JAZF1-SUZ12 remains unknown, one study [10] showed that JAZF1-SUZ12 destabilizes PRC2 and impairs trimethylation of histone 3, and, as a result, the H3K27me3 is reduced as detected by Western blotting. Another study [11] reported that *JAZF1-SUZ12* fusion and concurrent knockdown of endogenous *SUZ12* reduce the level of H3K27me3, inhibit apoptosis, and accelerate proliferation. MEAF6-SUZ12 fusion might similarly induce epigenomic alteration in the development of low-grade ESS. In the present tumor, immunohistochemical expression of H3K27me3 and SUZ12 were both retained.

MEAF6 encodes a member of the acetyl transferase complexes, which also include NuA4, HBO1, and MOZ/MORF. NuA4 complex contains products of the *EPC1*, *EP400*, and *BRD8* genes [12]. *MEAF6-PHF1* fusion gene is detected in a subset (11%) of low-grade ESS [3, 4], and in these tumors, exon 5 of *MEAF6* is fused to the partner gene, in contrast to exon 4 in the present case. The oncogenic role of *MEAF6* rearrangement is largely unknown; however, a few studies suggested that EPC1-PHF1 and MEAF6-PHF1 fusion proteins may induce abnormal histone acetylation of PHF1 target genes, which are normally maintained in the repressed state [4, 12].

The diagnosis of the present tumor was challenging because of two reasons. First, the tissue was mostly hypocellular and myxoid in the initial biopsy without characteristic patterns. The histological spectrum of low-grade ESS is broad, and variations include fibromyxoid morphology and differentiation into sex cord, smooth muscle, and epithelium [1]. Classic tongue-like infiltration and swirling growth around arterioles were identified only in the re-biopsy specimen, which supported the diagnosis of low-grade ESS. Although the immunophenotype of low-grade ESS is relatively nonspecific, expression of ER and CD10 is highly recurrent. Myogenic differentiation was limited to the focal areas in this case, which was, along with the histomorphology, incompatible with smooth muscle tumor. The present tumor also did not fit with high-grade ESS, because of long clinical course, absence of necrosis, low proliferative activity (reflected by low Ki-67 index), and negative expression for BCOR and cyclin D1. Focal CD34 expression in the present tumor, which partly misdirected the initial assessment, is a rare, but documented feature of low-grade ESS [13].

Second, the tumor was located in the abdominal wall, and with the previous uterine tumors having been reported as leiomyomas, low-grade ESS was not included in the initial differential diagnosis. Low-grade ESS can metastasize to the abdominal wall, but the close proximity of the sarcoma to the postoperative scar in this case might not be incidental, because abdominal scar is a known recipient of implantation of uterine pathology [14].

Molecular testing to detect specific fusions can be helpful in the diagnosis of low-grade ESS, given that they are absent in other mesenchymal neoplasms occurring in the gynecologic organs [2]. However, the specificity depends on the histological context, because ossifying fibromyxoid tumor, which usually occurs in the somatic soft tissue, shares with low-grade ESS, some fusions involving *PHF1* and *BCOR* [15].

In summary, we reported a case of low-grade ESS with a novel *MEAF6-SUZ12* fusion. The case also highlighted the challenge of diagnosing low-grade ESS with an uncommon histological pattern and the critical role of clinical information in the histological assessment.

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Author's contributions NM and AY conceived and designed the study, and wrote, edited, and reviewed the manuscript. NM, HY, and AY did histological assessment. MS and HI did molecular analyses. EK, TK, and AK collected clinical samples. MF supported the study. All authors gave final approval for publication.

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Compliance with ethical standards

The study was approved by the institutional review board (2014–089).

Conflict of interest The authors declare that they have no conflict of interest.

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