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BRIEF REPORT

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Detection of *MEAF6-PHF1* translocation in an endometrial stromal nodule

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Abstract

Endometrial stromal nodule (ESN) and low-grade endometrial stromal sarcoma (LG-ESS) are rare uterine tumors known as endometrial stromal tumors (ESTs). In addition to their similarity in morphological features, recent studies have shown that these two tumors share common genetic alterations. In particular, *JAZF1-SUZ12* fusion is found with high frequency in both ESN and LG-ESS. In LG-ESS, some minor fusions have also been described, which include rearrangements involving *PHF1* and its partner genes, such as *JAZF1*, *EPC1*, *MEAF6*, *BRD8*, *EPC2*, and *MBTD1*. Because of the rarity of ESN, genetic alterations other than *JAZF1* fusion have not been investigated in detail. In this study, we performed a next-generation sequencing-based analysis in a case of ESN with peripheral metaplastic bone formation and detected *MEAF6-PHF1* fusion, which has been reported in a small subset of uterine LG-ESSs and soft tissue ossifying fibromyxoid tumors. The finding that *MEAF6-PHF1* fusion is a background genetic abnormality detected both in ESN and LG-ESS, along with *JAZF1-SUZ12*, provides further support for the similarity and continuum between these two types of ESTs. Furthermore, the association between metaplastic bone formation and *MEAF6-PHF1* fusion may not be limited to soft tissue tumors.

KEYWORDS

endometrial stromal nodule, fusion, MEAF6, next generation sequencing, PHF1

1 | INTRODUCTION

Endometrial stromal tumors (ESTs) are rare mesenchymal tumors of the uterus. They are typically composed of neoplastic cells that resemble the endometrial stromal cells of the proliferative endometrium.[1] The current World Health Organization classification subclassifies endometrial stromal and related tumors into five categories: endometrial stromal nodule (ESN), low-grade endometrial stromal sarcoma (LG-ESS), high-grade endometrial stromal sarcoma (HG-ESS), undifferentiated uterine sarcoma (UUS), and uterine tumors resembling ovarian sex cord tumors (UTROSCTs).[2] These categories are defined by both tumor morphology and the presence of translocations that lead to fusion genes. Specifically, a large proportion of LG-ESSs are characterized by *JAZF1-SUZ12* fusion or minor *PHF1* rearrangements, whereas HG-ESSs are characterized by *YWHAE-NTUM2B* fusion.[2] Furthermore, UTROSCTs are now considered a distinct entity characterized by recurrent *NCOA2/3* gene fusions.[3]

ESN, which is considered a clinically benign tumor, consists of tumor cells that are morphologically similar to those of LG-ESS. Histologically, ESN and LG-ESS are distinguished by the presence of stromal invasion. LG-ESS shows invasive patterns, whereas ESN does not. ESN has an expansile but noninfiltrative border that often compresses the surrounding myometrium and endometrium.[4] Recent studies have shown that ESN and LG-ESS share common genetic alterations.[5-18] As in LG-ESS, *JAZF1-SUZ12* fusion is the most common rearrangement in ESN, estimated to be present in ~65% of ESN cases.[18] However, this estimate is based on a small number of cases owing to the rarity of this condition, and genetic alterations other than *JAZF1* fusion have not been investigated in detail.

In this study, we developed an NGS fusion gene panel that covers uterine sarcoma-associated genes and applied it to a case of ESN with focal peripheral ossification to elucidate its background genetic alterations. *MEAF6-PHF1* fusion was detected, which has previously been reported in a small subset of LG-ESS,[19,20] further supporting the genetic similarity between ESN and LG-ESS. The clinicopathological features of the first reported case of ESN with *MEAF6-PHF1* fusion are described in detail below. Since the same fusion is reported in a subset of ossifying fibromyxoid tumors (OFMTs),[21] the significance of peripheral bone formation is also discussed.

2 | MATERIALS AND METHODS

2.1 | Case history

A 57-year-old female, gravida 2, para 2, presented with abdominal distension. She underwent transvaginal ultrasonography, which revealed a solid and cystic pelvic mass measuring 10 cm in diameter. Magnetic resonance imaging revealed low T1 and high T2 signals in the lesion located in the uterus, which suggested the possibility of uterine sarcoma. A computed tomography scan revealed calcification measuring 1.1 cm in the periphery of the tumor. No distant metastases were detected. The patient underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy. The pathological diagnosis of the uterine mass was ESN. The postoperative course was unremarkable, and the patient is free of disease 3 years after the surgery.

2.2 | Immunohistochemistry

Immunohistochemistry was performed on representative formalin-fixed paraffin-embedded (FFPE) sections (4 μm thick) of ESN using antibodies for vimentin, cytokeratin (AE1/3), epithelial membrane antigen (EMA), CD10, smooth muscle actin, desmin, S-100, estrogen receptor (ER), progesterone receptor (PgR), CD34, c-kit, cyclin D1, WT-1, p53, β-catenin, and Ki-67. Immunostaining was performed using standard techniques on a Ventana Discovery XT Autostainer (Ventana Medical Systems Inc, Tucson, AZ). Appropriate controls were included, and immunoreactivity in >5% of the tumor cells was considered as positive.

2.3 | Analyses by next-generation sequencing gene fusion panel

A novel next-generation sequencing (NGS)-based gene fusion panel, SMART Fusion-Seq (Takara Bio Inc., Kusatsu, Japan), which the authors (DM and MS) helped to develop, was applied. The protocol is designed based on SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian. [22-24] The panel is capable of detecting rearrangements of the following genes using FFPE tissues, even when the fusion partner is unknown: *JAZF1*, *YWHAE*, *PHF1*, *ALK*, *ROS1*, *RET*, *NTRK1*, *NTRK2*, *NTRK3*, *FGFR1*, *FGFR2*, and *FGFR3*. Exon skipping mutation of *MET* and 3'UTR deletion of *PD-L1* are also assessable.

First, RNA was extracted from 10 μm thick sections of the representative FFPE block using the AllPrep DNA/RNA FFPE Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's protocol. cDNA was synthesized from 50 ng total RNA using random primers with attached universal sequence tags and template-switching reverse transcription (RT), which is known as SMART technology.[22] Target driver genes and their partners were enriched by semi-nested polymerase chain reaction (PCR). In the 5'RACE protocol, target enrichment was carried out with the universal sequence on the template, switching oligo and gene specific primers on the driver genes. The 3'RACE protocol was performed with the universal sequence on the random primers used in cDNA synthesis and gene-specific primers on the driver genes. The gene-specific primers were designed to be close to target breakpoint positions to directly read the exact sequence of the breakpoint and the partner gene. Illumina sequencing adapters and index barcodes were added to the fusion amplicons. Sequencing was performed on the MiSeq instrument using paired 150 bp reads with MiSeq v3 reagent (Illumina, San Diego, CA).

Alignment and fusion call were performed using STAR-Fusion. Sequence reads were aligned to GRCh38. The detected fusion was validated by Fusion Inspector and the sequence reads were visualized

using Integrative Genomics Viewer. Circos plots and schematic translocation diagrams were generated using Arriba (version 1.2.0).

2.4 | Validation of *MEAF6-PFH1* fusion by RT-PCR and Sanger sequencing

Total RNA was extracted from the representative 10 μm thick section of the FFPE sample using AllPrep DNA/RNA FFPE Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's protocol. For cDNA synthesis, 1 μg total RNA was reverse-transcribed using the PrimeScript RT-PCR Kit (Takara Bio Inc.). To obtain high-yield cDNA, we used a mixture of random hexamers and two *PHF1* gene-specific primers (*PHF1*-380R:5′-GGACCAGACACACCTCCCTAGCACTG-3′ and *PHF1*-327R:5′- AGCCCATCAGTCCATCTGGCCAG-3′).

The *MEAF6-PHF1* fusion segment was amplified by PCR to obtain two differently sized amplicons. The primer sequences for the 164 bp amplicon were *MEAF6*-164F:5′-TCTGTGCAGGGAGTGAAACCTCAGA-3′ and *PHF1*-164R:5′-TCCCAAAGTGAGGAGGCACCAGAG-3′; for the 121 bp amplicon they were *MEAF6*-121F:5′- GTCACCACAGCAGCCAT AAAAAGCG-3′ and *PHF1*-121R:5′- GAAGCTGGGTCCCAAAGTGA GGAGG-3′.

The PCR procedures were run on the TaKaRa PCR Thermal Cycler Dice System (Takara Bio Inc.). Amplified products were cleaned up using AMPure XP beads (× 2.5 by volume) (Beckman Coulter, Brea, CA) and subjected to Sanger sequencing to verify the fusion junction with BigDye Terminator v3.1 Cycle sequencing kits (Thermo Fisher Scientific, Waltham, MA), on the Applied Biosystems Model 3730xl DNA Sequencing System (Thermo Fisher Scientific).

2.5 | Fluorescence in situ hybridization (FISH) analyses

FISH analyses were performed using the Zyto*Light* SPEC *PHF1* dual color break apart probe (Zyto Vision, Bremerhaven, Germany) and *MEAF6* Split Dual Color FISH Probe (GSP Laboratory, Kobe, Japan). The latter is a custom-designed BAC probe flanking the *MEAF6* gene. Pretreated FFPE samples were hybridized with each FISH probe at 37°C for 24 hours. Fluorescent signals were captured and analyzed using all-in-one fluorescence microscope (BZ-X700, KEYENCE, Osaka, Japan).

3 | RESULTS

3.1 | Pathological findings

Grossly, a well-demarcated nodular lesion 10 cm in a diameter was observed in the myometrium of the uterine body. The lesion was not connected to the endometrium. The cut surface of the mass was solid and mostly yellowish in color. However, cystic degeneration was also seen. In the periphery of the tumor, focal areas of calcification measuring 1 to 2 cm was observed. A total of 10 tissue sections containing the tumor were made, and the tumor border was evaluated in nine of them.

Histologically, the tumor was a well-demarcated lesion with a clear border between the myometrium (Figure 1A). Collagenous pseudocapsule was present in some parts. No "tongue-like" permeative patterns of invasion were observed. Lobular architecture was not evident. The tumor cells had monotonous ovoid to short spindle-shaped nuclei. They had scant to moderate amount of cytoplasm, and some showed epithelioid appearance. The tumor cells grew diffusely in fascicles or in a patternless pattern. The background stoma was generally fibrous (Figure 1B). The cellularity was variable from area to area. In some areas, spindled cells were arranged densely resembling endometrial stroma (Figure 1C). In other areas, epithelioid tumor cells grew in trabeculae or in cord-like pattern with moderate degree of cellularity. Numerous capillaries were identified in the tumor, and the tumor cells were focally arranged in a spiral pattern around the vessels (Figure 1D). Sex cord-like morphology was not observed. Stromal hyalinization and edematous or myxoid degeneration were predominant in some areas (Figure 1E). Of note, mature bone trabeculae considered as a result of metaplastic ossification was present focally in the periphery of the tumor (Figure 1F). Few mitotic cells were identified (2/50 within a high-power field). No apparent lymphovascular invasion was found.

Immunohistochemical analyses showed positivity for CD10 (Figure 1G), ER (Figure 1H), CD56, smooth muscle actin, and vimentin. PgR, cytokeratin (AE1/3), and desmin were focally positive. The tumor cells showed no immunoreactivity for CD34, c-kit, cyclin D1, EMA, S-100, or WT-1. Aberrant localization of β-catenin was not observed. p53 immunostaining revealed a wild-type pattern with scattered weakly positive cells. The Ki-67 labeling index was 5.1%.

3.2 | Fusion gene analyses

NGS-based gene fusion panel testing revealed the fusion transcript *MEAF6-PHF1* as a product of the translocation t(1;6)(p34;p21), which has been reported in three cases of LG-ESS.[19,20] The sequence analyses showed that exon 5 of the *MEAF6* gene was fused inframe to exon 2 of *PHF1* (Figure 2A). Subsequent RT-PCR and direct sequencing validated the presence of the chimeric transcript with the previously reported breakpoints in intron 5 of *MEAF6* and intron 1 of *PHF1* in both primer pairs (Figure 2B).

Finally, *MEAF6* and *PHF1* break-apart FISH probes showed split signal patterns, suggesting the presence of gene rearrangements involving *PHF1* and *MEAF6* (Figure 2C,D).

4 | DISCUSSION

ESTs are a genetically heterogeneous group of tumors, and their variant histologic appearances sometimes cause diagnostic difficulties. A number of cytogenic studies have been conducted to gain insight into

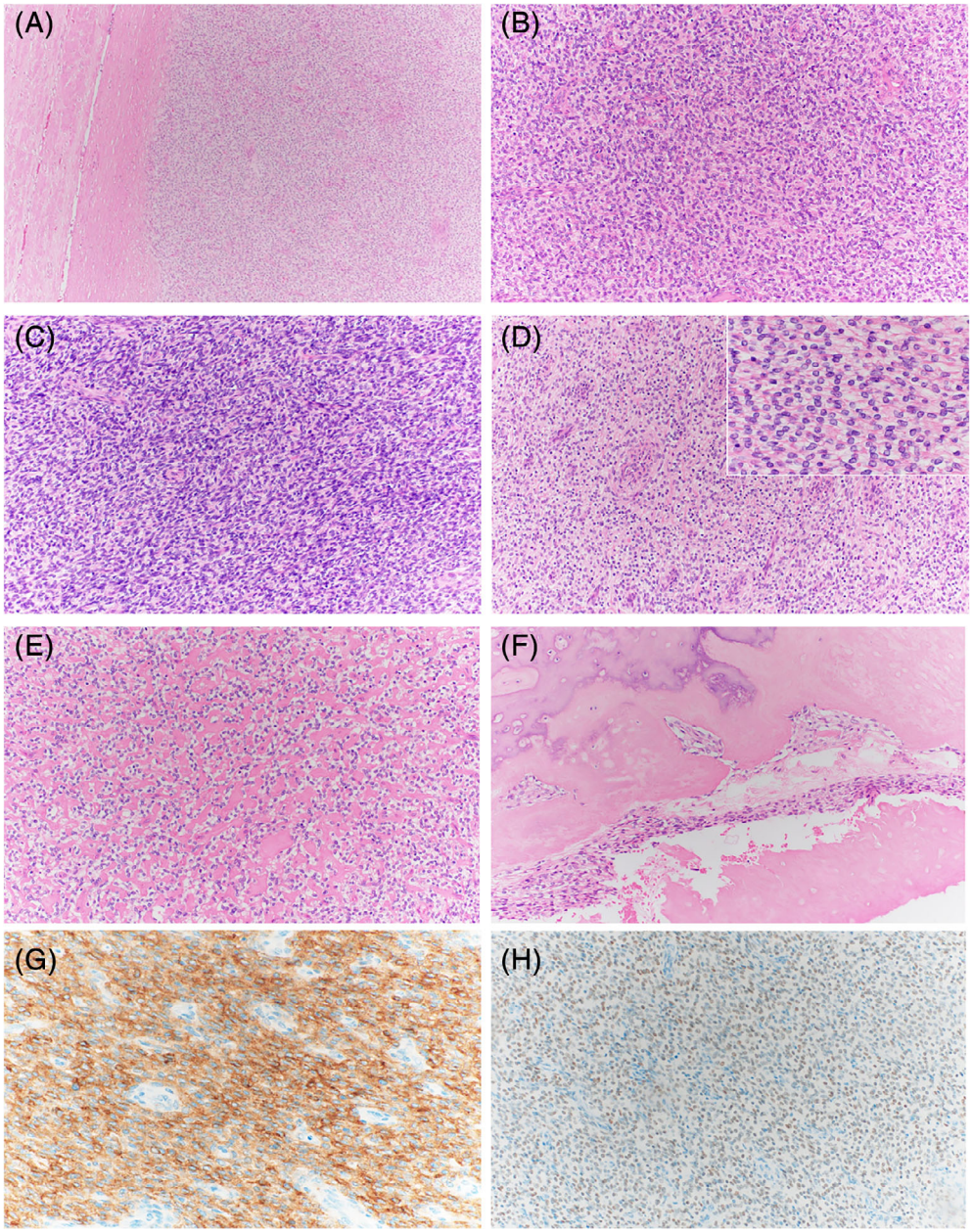

FIGURE 1 Histopathological and immunohistochemical features of the ESN A, The tumor is a nodular lesion that shows no permeative patterns of invasion into the myometrium. B, The tumor cells with ovoid nuclei exhibit a diffuse pattern of growth. C, The tumor cells with spindled nuclei are densely distributed resembling the endometrial stroma. D, Focal areas where the tumor cells with mildly epitheliod morphology are arranged in a whorl-like pattern around the capillary vessels. E, Stromal fibrosis and hyalinization is prominent in some areas. F, Metaplastic bone formation is identified in the periphery of the tumor. G, The tumor cells are diffusely immunopositive for CD10. H, ER-positivity is observed in most tumor cells. ESN, endometrial stromal nodule [Color figure can be viewed at wileyonlinelibrary.com]

their pathogenesis and aid in diagnosis. The discovery of specific fusion genes has helped to characterize each EST category.

JAZF1-SUZ12 fusions, resulting from t(7;17)(p15;q21) chromosome translocation, were first reported in ESTs in 2001.[5] Evidence suggests that *JAZF1-SUZ12* fusion is detected specifically in ESN and LG-ESS with conventional morphology, and not in HG-ESS.[5-18] It is currently considered the most frequently detected gene fusion in both ESN and LG-ESS.[18] The fact that ESN and LG-ESS share this background genetic alteration indicates that their histogenesis is similar, and that they may be within the same spectrum of tumors. In LG-ESS, some minor fusions have also been described, which include rearrangements involving *PHF1* and its partner genes, such as *JAZF1*, *EPC1*, *MEAF6*, *BRD8*, *EPC2*, and *MBTD1*.[9,19,20,25-27] D'Angelo et al showed that LG-ESS with *PHF1* fusion tends to exhibit sex cord differentiation.[15] Because UTROSCTs are characterized by *NCOA* fusion,[3] LG-ESS with sex cord differentiation is now considered distinct from UTROSCT. *MEAF6* rearrangements have been reported in only a few LG-ESS cases (three cases with the aforementioned *MEAF6-PHF1* fusion and one case with *MEAF6-SUZ12* fusion).[19,20,28] As for ESN, genetic alterations other than *JAZF1* fusion have not been investigated in detail, mostly because of the rarity of this condition. There have been no reports of ESN with *PHF1* or *MEAF6* fusion.

In the present study, we report the first case of ESN with *MEAF6-PHF1* fusion. The tumor in our case had pathological features compatible with ESN, such as diffuse growth of monotonous tumor cells with ovoid to spindled nuclei, CD10, and ER immunoreactivity. The previously reported LG-ESS cases with *MEAF6-PHF1* had similar features, except that they were invasive.[19,20] Sex cord differentiation was not observed in our ESN. Because only focal sex cord differentiation was documented in one of the LG-ESSs with *MEAF6-PHF1* fusion,

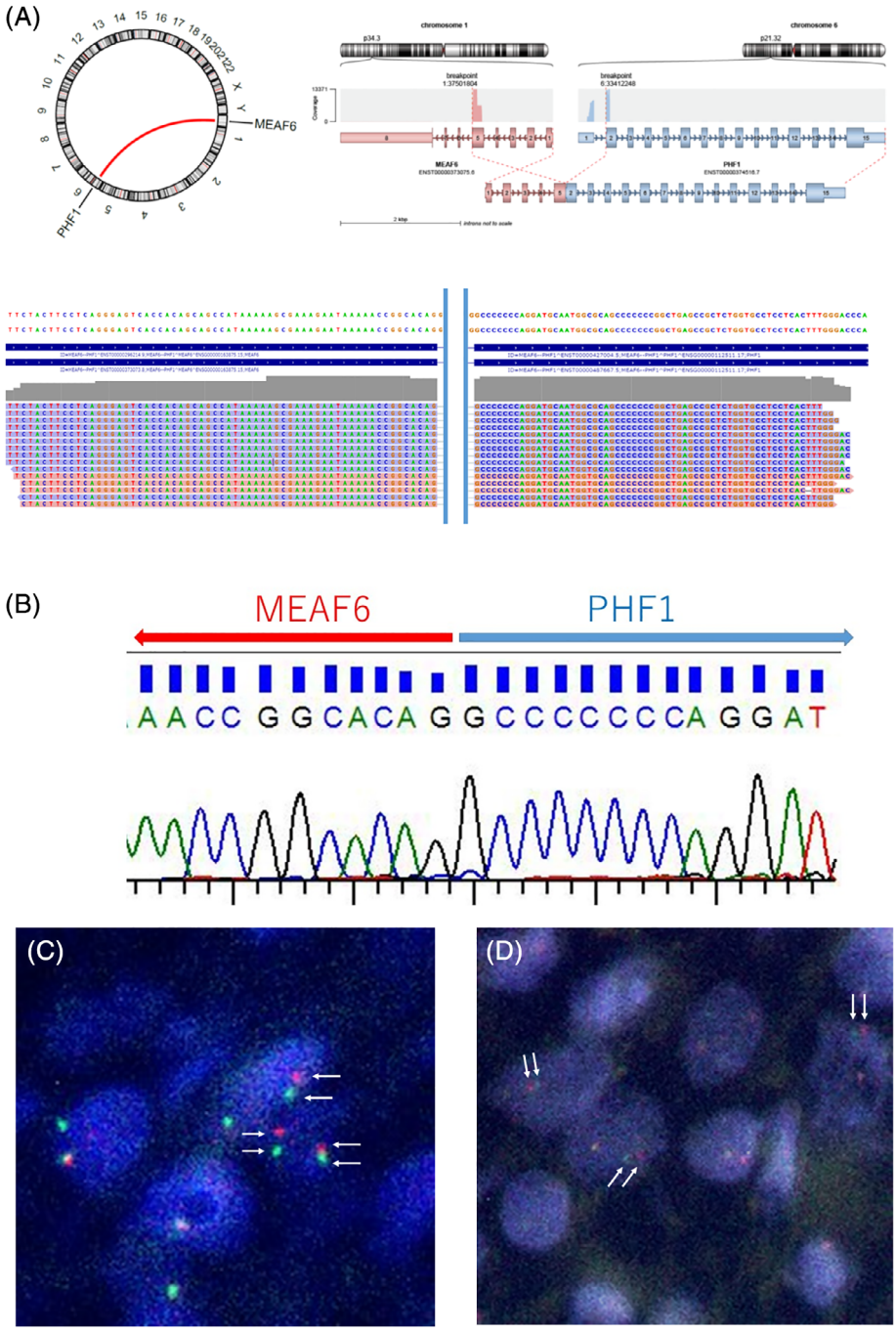

FIGURE 2 *MEAF6-PHF1* fusion in the ESN A, NGS-based fusion gene panel testing revealed a fusion transcript, *MEAF6-PHF1*. Sequence analyses showed that exon 5 of the *MEAF6* gene was fused inframe to exon 2 of *PHF1*. B, Partial sequence chromatogram of the RT-PCR product shows the junctions of the *MEAF6-PHF1* chimeric transcript. C, Break-apart FISH assay for the *PHF1* gene demonstrates the presence of separate green and red signals, indicating rearrangement (arrows). D, Break-apart FISH assay for the *MEAF6* gene demonstrates the presence of separate green and red signals, indicating rearrangement (arrows). ESN, endometrial stromal nodule [Color figure can be viewed at wileyonlinelibrary.com]

we assume that sex cord differentiation is not a frequent manifestation in ESN/LG-ESS with *MEAF6-PHF1* fusion. Other *PHF1* rearrangements, such as *JAZF1-PHF1*, may be involved in the morphological variation.

Outside the gynecological tract, *PHF1* fusion is known to be frequently detected in OFMT of the soft tissue.[21,29-33] *EP400-PHF1* fusion, which has not been reported in ESTs, is the most common genetic alteration of OFMTs.[21,33] However, a small subset of OFMTs harbor EST-associated fusions such as *MEAF6-PHF1*, *ZC3H7B-BCOR*, and *EPC1-PHF1*, which is suggestive of similarity between ESN/LG-ESS and OFMT in terms of histogenesis.[21] Of note, the ESN presented in this report showed focal peripheral ossification, a rare finding in ESN/LG-ESS, but a hallmark feature of OFMT. Although ossification or calcification was not documented in the previously reported three cases of LG-ESS with *MEAF6-PHF1* fusion,[19,20] the finding of our case is indicative of the possibility that *MEAF6-PHF1* fusion is associated with metaplastic bone formation not only in the soft tissue but also in the uterine mesenchymal neoplasm. Our ESN shared some morphological features in common with OFMT, such as predominantly fibrous background stoma and slightly epithelioid appearance of the tumor cells. But typical features of OFMT such as lobulation and S-100-positivity were lacking. In the report by Antonescu et al, three OFMTs with *MEAF6-PHF1* fusion were reported to negative for S-100.[21] Thus, OFMTs with *MEAF6-PHF1* fusion may be distinct in

terms of genotype and immunoreactivity. Accumulation of both OFMT with *MEAF6-PHF1* fusion and ESN/LG-ESSs with *MEAF6-PHF1* fusion is definitely needed to assess their similarities and differences.

The breakpoints of the *MEAF6* and *PHF1* genes in our case of ESN were exactly the same as in the previously reported LG-ESS cases with *MEAF6-PHF1* fusion. In this fusion, the entire coding region of *PHF1* becomes the 3' terminal fusion, and the fusion protein is predicted to contain 750 amino acids. The *PHF1* gene belongs to the polycomb group (PcG) of genes. PcG proteins function as epigenetic regulators of transcription and have key roles in stem cell self-renewal, differentiation, and genome maintenance processes in response to DNA damage.[34] The *MEAF6* gene encodes a protein that belongs to the multi-subunit complexes of the MYST histone acetyl transferase family. These enzymes are involved in a number of key nuclear processes, and play critical roles in gene-specific transcription regulation, DNA damage response and repair, and DNA replication.[35] The expected *MEAF6-PHF1* fusion protein in our ESN contains the histone acetyltransferase subunit NuA4 domain of *MEAF6* and the tudor, PHD zinc finger, and MTF2 domains of *PHF1*. Although the precise oncogenic function of this fusion remains largely unknown, the characteristics of the genes suggest that *MEAF6-PHF1* fusion might alter epigenetic regulation.

In summary, *MEAF6-PHF1* fusion is a shared genetic abnormality in ESN and LG-ESS, along with *JAZF1-SUZ12*. Our results lend further support for the similarity and continuum between ESN and LG-ESS. They may possibly be regarded as one tumor entity. Further studies are warranted to expand the understanding of the pathogenesis of these diseases.

CONFLICT OF INTEREST

The research of DM is partly supported by Takara Bio Inc. MS is an employee of Takara Bio Inc.

AUTHOR CONTRIBUTION

Yusuke Nomura, Masakazu Sato, Shinya Sasaki, Yukitsugu Kudo-Asabe, Kei Koyama, and Daichi Maeda performed the research. Daisuke Tamura, Michinobu Umakoshi, Yohei Yamamoto, Kenichi Makino, Yukihiro Terada, and Akiteru Goto provided essential clinicopathological information. Shinogu Takashima, Kazuhiro Imai, and Yoshihiro Minamiya participated in the preparation of surgical specimens. Masafumi Horie and Masakazu Sato analyzed genetic data. Shinichi Yachida interpreted genomic findings. Yusuke Nomura and Daichi Maeda wrote the paper. All authors approved the final manuscript.

ETHICS STATEMENT

Ethical approval was obtained from the relevant institutional review boards (Reference No. 1211 of Akita University and Reference No. 858 of Osaka University).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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