Case Report
Uterine tumor resembling high-grade endometrial mesenchymal sarcoma with GATAD2B-MMRN1 fusion

Mengdie Yue1,2, Junbo Hu2, Xiaohong Min2, Hui Xu2

1The Postgraduate Training Base of Hubei Maternal and Child Health Hospital, Hubei University of Medicine, Wuhan, Hubei, China; 2Department of Pathology, Hubei Maternal and Child Health Hospital, Wuhan, Hubei, China

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Abstract: Uterine sarcomas are a group of rare malignant tumors of mesenchymal tissue of the uterus, and their diagnosis is often difficult because they have variable morphologies and no typical immunophenotype. This report describes a 48-year-old woman who underwent laparoscopic myomectomy and relapsed within 5 years with a large mass in the pelvic cavity. Morphologically, the tumor was composed of oval cells and small arteries, and the cells showed moderate to severe atypia. Immunohistochemical results showed that the tumor cells expressed desmin, smooth muscle actin, and h-caldesmon, which supported myogenic differentiation. They were strongly positive for Cyclin D1, estrogen receptors (ER), and estrogen receptors (PR), supporting their origin from uterine mesenchymal cells. Next-generation sequencing (NGS) revealed a GATAD2B::MMRN1 rearrangement. The patient was diagnosed with uterine sarcoma resembling high-grade endometrial mesenchymal sarcoma with a GATAD2B-MMRN1 fusion. We review the relevant literature and discuss the diagnostic and differential diagnostic points for this disease.

Keywords: Uterine sarcomas, GATAD2B-MMRN1 fusion, gene rearrangement, next-generation sequencing

Introduction
Uterine sarcomas are rare malignant tumors of mesenchymal origin, with an incidence of 3%-7% of uterine malignancies [1]. They mainly include uterine smooth muscle sarcoma, endometrial mesenchymal sarcoma, and undifferentiated sarcoma and rarely include adenosarcoma or malignant perivascular epithelioid cell tumors [2, 3]. Molecular detection techniques have identified several groups of unrecognized uterine sarcomas with specific genetic alterations [4], such as SMARCA4-deficient undifferentiated sarcoma of the uterus [5], NTRK gene rearrangement in uterine sarcoma, and COL1A1-PDGFB fusion in uterine sarcoma [6]. We identified a single case of a new subtype of uterine sarcoma with GATAD2B-MMRN1 gene fusion by using immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and Next-Generation Sequencing (NGS). Immunophenotypically, this tumor expressed the myogenic markers smooth muscle agonist protein (SMA), desmin, and h-caldesmon and diffusely and consistently expressed the cell cycle protein Cyclin D1. The diagnosis of epithelioid leiomyoma (EL) and high-grade endometrial mesenchymal sarcoma (HGESS) overlapped. Histologically, the tumor was similar to other small round cell-type tumors. We report the morphologic, immunohistochemical, and molecular features to facilitate diagnosis.

Case report
In October 2018, the patient underwent laparoscopic myomectomy for “uterine fibroids”. Histopathologic examination revealed a cellular leiomyoma. In February 2022, ultrasonography showed two fibroids measuring 1.6 cm × 2.0 cm × 1.8 cm and 0.9 cm × 0.9 cm × 0.6 cm in the posterior wall of the uterus. The patient did not report any significant discomfort and did not receive special treatment.

In March 2023, 3 d after a pelvic mass was found, the patient was readmitted to the hospital. The pelvic ultrasound examination revealed a solid uterine mass (approximately 4.4 cm × 4.8 cm × 3.6 cm) hypoechoic mass with rich
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Figure 1. Magnetic resonance imaging scanning of the pelvis: coronal view, an enhancing mass is visible in the anterior cervix (A); sagittal view, an enhancing mass is visible in the right front of the cervix (B).

blood flow signals in the posterior wall of the uterus) and a solid dominant mass in the right adnexal region (approximately 8.0 cm × 5.1 cm × 5.4 cm hypoechoic mass with rich blood flow signals in the right adnexal region, possibly originating from the ovaries). The patient was admitted to the hospital with “Ovarian tumor?”.

The patient was cooperative, with a blood pressure of 115/75 mmHg and no history of cancer, infectious diseases, family history of heredity, or significant abnormalities. Next, the relevant laboratory functional tests were conducted. The tests for liver and kidney function and blood and urine analysis, the ECG, and the chest X-ray were normal. The results of tumor markers, such as cancer antigen 125 (CA125), carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), human epididymis protein 4 (HE4), and carbohydrate antigen 19-9 (CA19-9), were within normal range.

Computed tomography (CT) with contrast identified an increased uterine size with a 7.6 cm × 3.5 cm irregular mixed-density region in front of the uterus. The pelvic magnetic resonance imaging (MRI) showed two mixed-signal masses, approximately 5.6 cm × 4.8 cm and 1.6 cm × 3.9 cm in size, in the left posterior and anterior walls of the uterus (Figure 1A), the majority of which showed slightly short T1 and slightly long T2 signals. One other mixed-signal mass, approximately 5.0 cm × 8.4 cm × 7.4 cm in size, was in the right front of the uterus, with an equal T1 and slightly longer T2 signal (Figure 1B), which showed significant enhancement on imaging and was poorly demarcated from the right ovary.

The patient underwent a total laparoscopic hysterectomy with bilateral adnexal resection. Intraoperative exploration revealed a soft texture and active bleeding mass approximately 8 cm × 7.5 cm × 5 cm in size in the right front of the uterus, attached to the right wall of the peritoneum. The posterior wall of the uterus was densely adhered to the surrounding bowel and bilateral adnexa. Two myoma-like tissues were observed on the left posterior and anterior walls of the uterus, approximately 5.5 cm × 5.0 cm and 1.5 cm × 4.0 cm in size, respectively. Some of the masses were sent for intraoperative frozen section. The remaining masses were sent for routine pathologic examination (intraoperative diagnosis: benign tumor of mesenchymal origin).

The routine pathological examination proceeded in two steps. The first step was (uterus + bilateral adnexa). Thus, the uterus was incised. The incision was 9 cm × 7 cm × 6 cm in size and 6.5 cm deep. Endometrial thickness was approximately 0.1 cm, and myometrium thickness was approximately 1.5 cm-3.5 cm. A grayish-white mass, approximately 4.8 cm × 3.7 cm × 3 cm, was observed between the myometrium. The right tube attached to the ovary and left adnexa were normal in the second step, pelvic peritoneal masses were assessed. A complex cystic and solid gray-yellow mass mea-
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Sasuring 9 cm × 3 cm × 3 cm was observed. Moreover, there was a partially solid area, yellow with a soft texture.

Next, immunohistochemistry was performed on sections 4 µm thick cut from 10% neutral formalin-fixed paraffin-embedded (FFPE) tissue. The antibodies used were purchased from DAKO and we strictly followed the manufacturer's instructions.

The routine paraffin-sections showed that the masses had the same morphologic features as the myometrium and pelvic peritoneum. Microscopically, the tumor was a small round cell tumor with features of round to oval small nucleoli (Figure 2A). Cells in the tumor area were monomorphous and closely spaced with typical small spiral arteries (Figure 2B). Thick-walled vessels were observed, with lamellar infiltration into the myometrium, and had a clear border in some areas of the tumor. The focal area had a high mitotic index (approximately 8-9/10 HPF, Figure 2C) without obvious vascular or lymphatic vessel invasion or tumor necrosis.

Immunohistochemistry revealed diffuse staining for Cyclin D1 (Figure 3A), estrogen receptors (ER; Figure 3B) and progesterone receptors (PR) in the round tumor cells; and positivity for smooth muscle-derived markers such as SMA (Figure 3C), desmin and h-Caldesmon (Figure 3D), FH, INI-1, and Brg-1 were all positive, but CD10 was weakly and focally positive (Figure 3E). By contrast, BCOR, PanTRK, gastrointestinal mesenchymal tumor-1 (DOG-1), cell differentiation (CD117), and CD34 were negative. P53 was wild type, and the cell proliferation marker Ki67 was positive in approximately 20% of tumors (Figure 3F). SMARCB1/SMARCA4-deficient undifferentiated uterine sarcoma [7, 8] was tentatively excluded based on the positive expression of INI-1 and Brg-1 [9], and gastrointestinal stromal tumors (GIST) were excluded based on the negative expression of DOG-1 and CD117 [10, 11]. However, because of diffuse and consistently strong positivity for Cyclin D1, ER, and PR, CD10 was focally positive. Endometrial mesenchymal sarcoma could not be excluded, and FISH showed that gene fusion was not detected in JAZF1 or YWHAE [4, 12, 13]. Combined with the above immunohistochemical results and molecular pathology techniques, the patient was diagnosed with a smooth muscle tumor of uncertain malignant potential [14] (STUMP), and NGS was suggested, except for sarcoma [15]. Furthermore, NGS revealed a GATAD2B::MMRN1 rearrangement that fused GATAD2B exon 1 to MMRN1 exon 2 (Supplementary Table 1). Subsequently, the patient was diagnosed with a uterine sarcoma resembling HGESS with a GATAD2B-MMRN1 fusion.

Discussion

As access to modern genomic technologies increases the routine molecular characterization of tumors, new gene fusion-associated malignancies are found. In this article, we describe the novel rearrangement of GATAD2B-MMRN1 and report a case of uterine sarcoma harboring a GATAD2B-MMRN1 fusion, demon-
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Figure 3. Immunohistochemical results of uterine sarcoma with GATAD2B-MMRN1 fusion: small round cell tumor with diffuse and strong expression of cyclin D1 (A), positivity for ER (B), positivity for desmin (C), positivity for h-caldesmon (D), and with focal expression of CD10 (E), and cell proliferation marker Ki-67 approximately 20% (F). Magnification ×100.

Stratifying the diversity of genetic alterations in uterine tumors. A review of the patient’s medical history revealed recurrence within 5 years after myomectomy with implantation metastasis in the pelvic peritoneum; the tumor was considered to have aggressive biologic behavior.

Morphologically, the tumor was composed of oval cells and small arteries, and the cells showed moderate to severe atypia. Immunohistochemical results showed that the tumor cells expressed desmin, SMA, and h-caldesmon, which supported myogenic differentiation. Although strongly positive for Cyclin D1, ER, and PR, supporting an origin from uterine mesenchymal cells, diffuse cyclin D1 nuclear immunostaining was considered a diagnostic immunomarker for HGESS. Furthermore, the GATAD2B-MMRN1 rearrangement was detected using NGS. Uterine leiomyosarcoma (uLMS) is the most common type of uterine sarcoma. SMA, desmin, and h-caldesmon are positive, while some cases are also positive for ER, PR, or CD10. Mutations and deletions in retinoblastoma protein 1 (RB1) and TP53 have been confirmed by TCGA and whole exome sequencing, which are the common types of genetic alterations and are considered potential biomarkers for uLMS, and diffuse positivity for P16 and P53 is crucial for the diagnosis of uterine leiomyosarcoma [16]. The types of HGESS are divided into two main categories based on the molecular abnormalities associated with YWHAE and BCOR fusion [12]. In YWHAE-NUTM2 fusion HGESS, cyclin D1 is diffusely and consistently expressed, and; in some cases, CD117 is positive but negative in Dog-1, ER, PR, or CD10; by contrast, in BCOR internal tandem repeat (ITD) HGESS, cyclin D1 and BCOR are usually diffusely positive, CD10 is focally positive or negative, desmin may be positive, and SMA and h-Caldesmon are often negative; however, neither ER nor PR are negative [4].

In this study, we reviewed histologic features and performed genetic testing using FISH and NGS. This case is similar to HGESS with aggressive behavior but has none of the abnormal
genes associated with uLMS and HGESS based on immunophenotypic and molecular features. Rather, it is considered a new type of uterine sarcoma independent of uterine leiomyosarcoma and HGESS. Combining morphologic, immunohistochemical, and molecular genetic findings, uterine sarcomas resembling high-grade endometrial mesenchymal sarcomas with GATAD2B-MMRN1 fusion are a priority diagnosis, and clinical postoperative complementary treatment with reference to HGESS is recommended [17]. Because this case of gene fusion in uterine tumors is novel, additional functional studies are necessary to assess the relationship between the fusion of specific genes and the clinicopathologic features.

Uterine sarcomas resembling HGESSs are rare, and studies have not defined GATAD2B-MMRN1 fusions as a genetic variant that aids in sarcoma diagnosis. Based on the rarity of this sarcoma, our conclusion is that uterine sarcomas harboring a GATAD2B-MMRN1 gene fusion need to be distinguished from uterine epithelioid leiomyomas, high grade endometrial stromal sarcoma (HGESS), endometrial sarcoma (ES), and CIC rearrangement sarcomas (CRS).

Uterine epithelioid leiomyosarcoma is a malignant tumor that grows around blood vessels; it consists of round to polygonal cells with visible pink cytoplasm, visible nuclear atypia (grade 2 or 3 nuclei), mitotic activity (usually at least 3-4/10 HPF), and tumor cell necrosis. Immunohistochemistry plays an important role in sarcoma diagnosis, owing to tumor cells losing their typical smooth muscle histology. There are no specific antibody markers for epithelioid leiomyosarcoma. Immunohistochemistry is usually positive for SMA, h-caldesmon, and vimentin, and sometimes fordesmin, CK, and EMA but not consistently. ER and PR are not always expressed, and sometimes positive [18, 19].

HGESS is a diverse form of uterine sarcoma that is morphologically, immunohistochemically, and genetically classified into three molecular subtypes: YWHAE-NUTM2A/B fusion, ZC3H7B-BCOR fusion, and BCOR ITD, of which chromosomal rearrangements are thought to be its pathogenesis [12]. Microscopically, the tumor cells are monomorphic and composed of round or spindle cells, usually invading the myometrium in an expansible and penetrative manner. Unique vascular structures (small spiral arteries similar to those of the endometrium), mitosis, and neoplastic necrosis can be observed around the tumor cells. A commonly observed feature of HGESS is the strong expression of cyclin D1, encoded by the Wnt-targeted gene CCND1, which is also positive for BCOR, KIT, and CD99, usually focally positive for CD10, and negative for ER, PR, and smooth muscle markers. The most commonly observed cytogenetic abnormality in HGESS is a recurrent translocation involving the fusion between YWHAE and NUTM2, which can be detected by FISH and GNS [20, 21].

ES is a small cell malignant tumor that occurs in the bone, composed of small round blue cells with “stippled salt and pepper chromatin” in the nuclei [22]. The majority of ES cells express high levels of the cell surface glycoprotein CD99 or MIC2 surface antigen, and membrane-localized MIC2 expression is a sensitive diagnostic marker of ES. Positivity for CD99 strongly suggests ES. The frequent occurrence of specific chromosomal translocations is another important feature of ES; the EWS-FLI1 translocation is observed in 85-90% of cases, in which the ESWR1 rearrangement is a specific molecular genetic feature for ES [23]. Combining immunohistochemistry and molecular tests can improve the diagnostic sensitivity and specificity of ES.

CRS is a group of undifferentiated small round cell sarcomas with unique molecular characteristics prevalent in the extremities and trunk [24]. Microscopic examination revealed that the tumors were composed of nodules or sheets of round cells with map-like necrosis and hemorrhage separated by thin fibrous septa. The tumor cells were relatively homogeneous in morphology, with vesicular chromatin, prominent nucleoli, and frequent mitotic figures. Immunohistochemistry is positive for CD99 in tumor cells: approximately 90% of CRS express ETS translocation variant 4 (ETV4), 70% strongly, and diffuse nuclear positivity for Wilms tumor 1 (WT1), but marker of myoepithelial origin are generally negative. FISH shows the CIC gene is fragmented and translocated in CRS [25]. Therefore, a combination of immunohistochemistry and FISH is crucial for CRS diagnosis.
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Conclusion

The application and development of molecular technologies have substantially facilitated the diagnosis of soft tissue sarcomas [26, 27]. NGS technologies have identified many genetic abnormalities in a wide variety of uterine mesenchymal tumors, and the discovery of gene fusion phenomena has further expanded the current classification and treatment strategies for sarcomas. Specific genetic alterations provide a basis for accurate classification of uterine sarcoma and hold promise for exploring targeted therapies [28]. With the discovery of an increasing number of uterine sarcomas with specific genetic alterations, an accurate diagnosis must combine morphological, immunohistochemical, and molecular genetic findings. In summary, we reported a case of a uterine tumor resembling HGESS and harboring GATAD2B-MMRN1 rearrangement and the discovery of a GATAD2B-MMRN1 rearrangement, facilitating further expansion of the molecular spectrum of new subtypes of uterine sarcoma.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Junbo Hu, Department of Pathology, Hubei Maternal and Child Health Hospital, No. 745, Wu Luo Road, Hongshan District, Wuhan, Hubei, China. Tel: +86-130-06342808; E-mail: cqjbhu@163.com

References

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Supplementary Table 1. Bone and soft tissue tumour fusion gene profile test reports

<table>
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<tr>
<th>Test results</th>
<th>Number of fused reads</th>
<th>Genetic variant-associated sarcoma subtypes</th>
<th>Level of evidence</th>
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<tr>
<td>GATAD2B-MMRN1 Fusion G1:M2 (NM_020699.4/NM_001371403.1)</td>
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<tr>
<td>SEC31A-ARHGAP24 Fusion S21:A4 (NM_001077207.4/NM_001025616.3)</td>
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<td>SEC31A-ARHGAP24 Fusion S22:A4 (NM_001077207.4/NM_001025616.3)</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>LYPD1-CAT Fusion L3:C3 (NM_001077427.4/NM_001752.4)</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>EIF4E3-FOXP1 Fusion E7:F3 (NM_001134649.3/NM_001244810.2)</td>
<td>10</td>
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<td>-</td>
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</tbody>
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This product comprehensively detects 1166 genes using probe hybridization capture technology and high throughput sequencing, with a total of 5 gene rearrangements, of which 0 gene rearrangements are genetic variants mentioned in classifications/guidelines/expert consensus to assist in the diagnosis of sarcoma.