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Case report

Protracted clinical course of an AFF1 fusion positive uterine smooth muscle tumor causing diagnostic confusion over a course of 15 years

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1. Introduction

Several morphologic features of uterine smooth muscle tumors contribute to their classification as benign or malignant. The Stanford Criteria were proposed for pathologic criteria for diagnosis of leiomyosarcoma (LMS), with two or more of: 1) moderate or marked cytologic atypia, 2) coagulative tumor cell necrosis, and 3) $\geq$ 10 mitoses per 10 HPF (Bell et al., 1994). Benign leiomyomas lack these features, while tumors with some features are referred to as smooth muscle tumors of uncertain malignant potential (STUMP). The recurrence risk of STUMPs are estimated to be 7–20% (Travaglino et al., 2021), and some have recurred as LMS. We describe a patient with an unusual clinical course whereby over the span of 15 years from hysterectomy for leiomyomas, she had multiple recurrences of the same smooth muscle tumor, which subsequently recurred showing frankly malignant LMS. This clinically and histologically unusual tumor harbored a novel *PTP4A2-AFF1* gene fusion, demonstrated in the original uterine leiomyoma as well as the most recent frankly malignant recurrence.

2. Case presentation

A 38-year-old woman presented to gynecologic oncology with a large pelvic mass four years after a total abdominal hysterectomy for benign leiomyomas (see Table 1 for surgical and pathology details). Surgical resection revealed cellular and atypical leiomyomas with focal marked cytologic atypia, and mitotic rate 5 per 10 high power field (HPF). There was infarct-type necrosis but no coagulative tumor cell necrosis. Two months later, two additional concerning lesions at the common iliac bifurcation and right external iliac were biopsied, showing a smooth muscle neoplasm with moderate cytologic atypia. After a second surgical resection, pathology was reported again as cellular and atypical leiomyomas (no lymph node tissue identified), and she was started on anastrozole.

Four months later, further progressive disease was demonstrated, and despite a trial of switching from anastrozole to leuprolide, subsequent imaging was suggestive of leiomyomatosis peritonealis disseminata. After a third surgical resection all specimens were classified as STUMP due to focal nuclear atypia and increased mitotic activity, though none meet the criteria for malignancy, along with all prior recurrence specimens. Given the unusual disease persistence and progression, the patient received systemic chemotherapy (4 cycles of Doxorubicin 50 mg/m^2 every 4 weeks, followed by Ifosfamide 5000 mg/m^2 Day 1 and Day 2, every 4 weeks). Completion imaging demonstrated no residual disease.

Eight years later, she re-presented with right lower abdominal pain, and a CT scan showing multiple large pelvic and abdominal masses. At a fourth surgery, multiple pelvic/abdominal masses were removed and the majority had frankly malignant histologic features of LMS with tumor cell necrosis, severe nuclear atypia and a mitotic count greater than 10 per 10 HPF. P53 immunohistochemistry analysis consistently demonstrated wild-type expression, and hormone receptor expression was diffuse and strong in all except a relative loss of PR expression in the latest recurrence. She subsequently received additional systemic chemotherapy (Gemcitabine 800 mg/m^2 day 1 and 8, and Docetaxel 80

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mg/m^2 day 1, for 6 cycles, every 21 days). Due to the unusual nature and clinical course, next generation sequencing-based RNA fusion analysis using the TruSight RNA Fusion panel (Illumina, San Diego, CA, USA) was performed on the latest recurrence (LMS) and identified a novel *PTP4A2-AFF1* fusion; there was no evidence of genetic fusion involving HMGA2, PLAG1 or any of the receptor tyrosine kinases (i.e. *NTRK1/2/3, ALK* and *ROS1*). The novel *PTP4A2-AFF1* fusion was orthogonally validated by qPCR and Sanger sequencing in the latest recurrence (LMS) as well as in the original uterine tumor, and joins the end of exon 3 of *PTP4A2 to the start of exon 2 of AFF1*. The putative chimeric protein product is predicted to contain AF4/FMR2 interaction domain of AFF1 but not the tyrosine phosphatase domain of PTP4A2.

3. Discussion

This case demonstrates an unusual progression/histologic evolution from a benign leiomyoma to atypical leiomyoma (further re-classified as STUMP), to STUMP, and finally LMS over the course of 15 years. While the presence of nuclear atypia, mitotic activity and tumor cell necrosis evolved time, the overall microscopic phenotype was still remarkably similar, with frankly malignant histologic features seen only in latest recurrence (Fig. 1). Notably, the latest recurrence exhibited intratumoral heterogeneity with varying degree of atypia and mitotic activity. The initial hysterectomy specimen under-sampled by today's standards, which further enhanced the challenges around the intermediate diagnostic categories of atypical leiomyoma (no longer recommended) and STUMP.

Generally, benign leiomyomas do not represent precursor lesions to STUMP or LMS. However, a small number of cases reporting LMS arising in a pre-existing leiomyoma have been reported, supported by the finding that *MED12* exon 2 more commonly found in uterine leiomyomas is also found in a small subset of uterine LMS as well as STUMP (Markowski et al., 2014). In addition to MED12 mutation, a subset of uterine LMS was recently reported to harbor PLAG1 fusion (Arias-Stella et al., 2019) and KAT6B-KANSL1 fusion (Choi et al., 2021), also seen in a subset of uterine leiomyoma (Moore et al., 2004; Ainsworth et al., 2019) (Table 2). We identified a novel *PTP4A2-AFF1* fusion, which to our knowledge has not been reported previously (Fig. 2). PTP4A2 is a ubiquitously expressed tyrosine phosphatase and the putative fusion protein does not include the tyrosine phosphatase domain; the activity of the putative PTP4A2-AFF1 fusion protein is therefore unrelated to the enzymatic activity of PTP4A2. AFF1 is a member of the AF4/FMR2 (AFF) family, a group of nuclear transcriptional activators that is involved in RNA elongation, splicing and transcriptional regulation (Melko et al., 2011 May 15). Nearly the entire AFF1 protein (starting from the start of exon 2) appears to be maintained in the putative fusion protein, including the AF4/FMR2 domain. It is likely that the putative PTP4A2-AFF1 fusion exerts tumorigenic effect(s) through either qualitatively or quantitatively aberrant AFF1 activity. Genetic fusion involving AFF1 is also known to occur in the form of KMT2A-AFF1 fusions, reported in less than 1% of B-cell lymphoblastic leukemia/lymphoma, lung adenocarcinoma, acute myeloid leukemia, urothelial and breast carcinoma (AACR Project GENIE Consortium, 2017). LMS are characterized by the presence of *TP53* mutations in the majority of cases (Mohammad et al., 2021). The current case showed consistently no evidence of a *TP53* mutation even in the latest recurrence in areas of marked nuclear atypia. Given the wild-type p53 immunohistochemistry finding, we subjected the tumor to RNA sequencing analysis

Table 1
Detailed operative and pathological findings.

Timeline	Operative Procedure	Operative Findings	Pathology	Ancillary testing
Initial surgery	Total abdominal hysterectomy	Multi-fibroid uterus	*Leiomyomas*, largest 7 cm, sampled in only 2 blocks • Infarct type necrosis • No cytologic atypia • Mitotic activity low: 3/10 HPF	• Immunohistochemistry - ER and PR strong and diffuse - p53 wild type - p16 normal heterogeneous • Genetics: *PTP4A2-AFF1* fusion confirmed by RT-PCR*
4 years 5 months later	Laparotomy Myomectomy Resection of retroperitoneal mass (left *peri*-ureteric tumor) Omentectomy Partial cystectomy with repair	Microscopic residual	*Cellular and atypical leiomyomas → STUMP* • Infarct type necrosis Focal marked nuclear atypia of degenerative type • Mitotic count: 5/ 10HPF (hot spot)	• Immunohistochemistry - ER and PR strong and diffuse - p53 wild type - p16 normal heterogeneous • Genetics: Not analysed
6 months later	Para-aortic lymph node debulking Right pelvic lymph node debulking Appendectomy Bilateral salpingectomy	Microscopic residual	*Cellular leiomyomas → STUMP (no lymph node tissue identified)* • No necrosis • No cytological atypia • Mitotic count: 9 /10 HPF (hot spot)	• Immunohistochemistry - ER and PR strong and diffuse - p53 wild type - p16 normal heterogeneous • Genetics: Not analyzed
2 years , 5 months later	Bilateral oophorectomy Resection of intrabdominal and retroperitoneal smooth muscle tumor Omentectomy Paraaortic retroperitoneal tumor debulking	Microscopic residual	*STUMP* • Infarct type necrosis Focal and interspersed single cell marked nuclear atypia • Mitotic count: >10 /10 HPF (hot spot)	• Immunohistochemistry - ER and PR strong and diffuse - p53 wild type - p16 normal heterogeneous - Bcl2 strong diffuse • Genetics: Not analyzed
8 years later	Extensive lysis of adhesions Resection right retroperitoneal mass perirectal tumor, right *peri*-urethral tumor, transverse mesentery tumor Rigid sigmoidoscopy, Ligation of right abdominal vein,	Microscopic residual	*Metastatic leiomyosarcoma* • Coagulative tumor cell necrosis • Marked diffuse nuclear atypia, in a background of less severe atypia • Mitotic count: >10 /10 HPF (hot spot)	• Immunohistochemistry - ER strong and diffuse - PR moderate and focal (5%) - p53 wild type - p16 diffuse strong - FH negative - ALK negative • Genetics: *PTP4A2-AFF1* fusion identified by RNA-sequencing and confirmed by RT-PCR*

All surgical specimens were reviewed by a gynecologic pathologist at multi-disciplinary Tumor Board rounds, arrow indicates change in diagnosis.

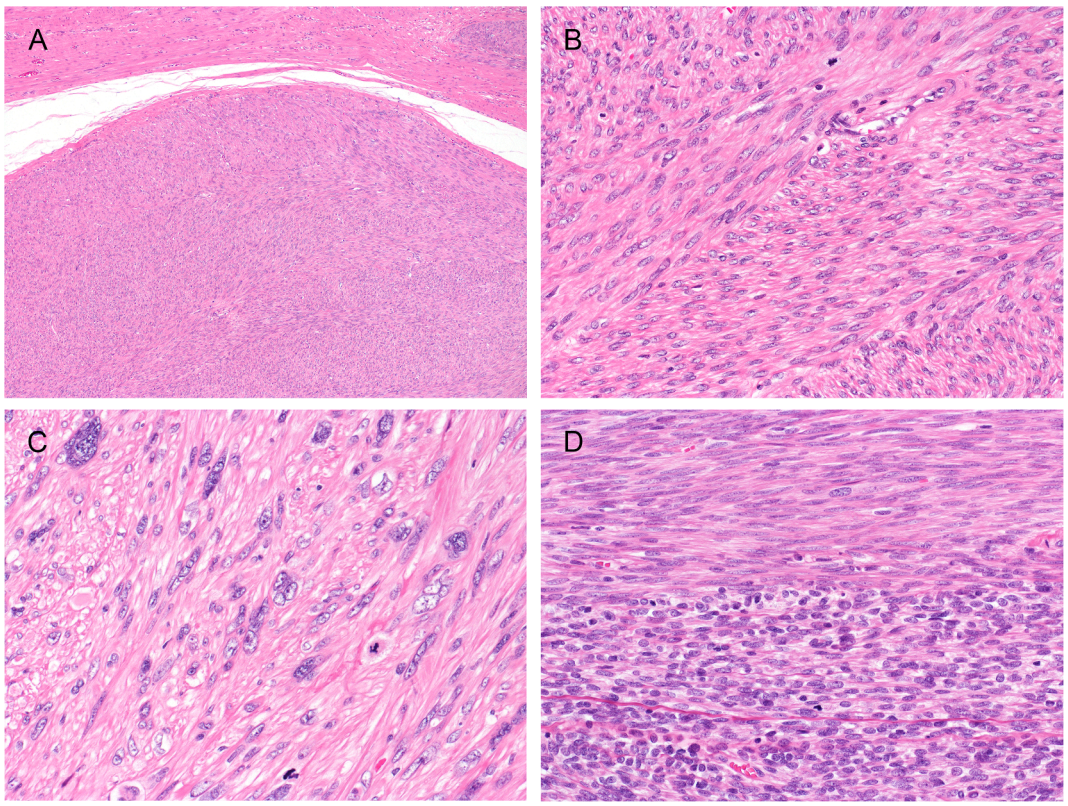

Fig. 1. A, Leiomyoma from primary hysterectomy (H&E, 40x) showing a well circumscribed smooth muscle neoplasm (lower) demarcated from myometrium (upper) by a cleft-like space. B, Leiomyoma from primary hysterectomy (H&E, 200x) showing spindle cells with eosinophilic cytoplasm in a fascicular pattern. Occasional mitoses are noted. Low-grade atypia. C. Latest leiomyosarcoma recurrence (H&E, 200x) showing pleomorphic cells with significant atypia and increased mitotic rate. D. Latest leiomyosarcoma recurrence (H&E, 200x) showing other areas more reminiscent of the primary tumor (upper) as well as more cellular areas (lower).

Table 2
Summary of current known putative driver fusion mutations and other mutations in uterine smooth muscle tumor.

Mutation	Tumor
HMGA2 or *HMGA1* fusion	Leiomyoma
KAT6B-KANSL1 fusion	Leiomyoma
PLAG1 genetic fusion with *TRPS1* or *RAD51B*	Leiomyosarcoma
*PTF4A2-AFF1 fusion**	*Novel gene fusion identified in current case of STUMP and subsequent leiomyosarcoma specimens*
Other mutations: *MED12, FH (fumarate hydratase)*	Leiomyoma
Other mutations: *TP53, BRCA1/2, RB1, ATRX*	Leiomyosarcoma

* *AFF1* genetic fusion in the form of *KMT2A-AFF1* has been reported in B-cell lymphoblastic leukemia/lymphoma, lung adenocarcinoma, acute myeloid leukemia, urothelial, breast carcinoma

(Mohammad et al., 2021). Future studies using molecular tools may refine unsatisfactory diagnostic categories such as recurrent STUMP or "low-grade leiomyosarcoma", helping to avoid misdiagnosis such as ALK driven inflammatory myofibroblastic tumor (IMT), high-grade endometrial stromal sarcomas, PECcoma and NTRK-rearranged spindle cell neoplasm.

The risk of recurrence of STUMPs was described in a review of 14 studies that included 219 patients, at 17.4% (Travaglino et al., 2021). STUMPs defined by high mitotic index alone was not associated with recurrences, suggesting that high mitotic activity alone should not warrant a diagnosis of STUMP, and in cases of either significant atypia or coagulative tumor cell necrosis the risk of recurrence approached 20% (Travaglino et al., 2021). Additional risk factors for recurrence have included abnormal p16 and p53 expression, genomic index, atypical mitotic figures, vascular involvement, irregular margins, subserosal tumor location, and myomectomy (Shim et al., 2020). The recurrence of STUMP does include a risk of progression to LMS, which has been described in a minority of cases. Within 287 cases of STUMP, eight of 33 reported recurrent cases were LMS (Shim et al., 2020). In another large single-center study of 67 cases of STUMP, 10 recurrent cases noted, two of which were LMS (Huo et al., 2020). Recurrence interval in these ten cases of LMS ranged from 6 to 53 months (mean 24.9 months) (Shim et al., 2020; Huo et al., 2020), whereas in our case was significantly longer, at 8 years (96 months).

In our case the pathological diagnostic criteria for malignancy were borderline. Until the latest recurrence, the nuclear atypia was variably present and focal, the mitotic count was also variable and hot spot assessment revealed a rate around the cut-off of 10/10 HPF, and necrosis was present in most specimens, favored to be infarct-type. Immunohistochemistry did not show adverse marker expression: p53 normal/wild type, normal p16, and strong diffuse ER and PR expression in almost all tumor cell nuclei. Only in the last recurrence was the morphological threshold of LMS reached, a change in p16 expression to block diffuse staining and a remarkable reduction of PR staining (~90% reduction) were interpreted as feature of potential progression.

In conclusion, we describe histologic progression of an unusual uterine smooth muscle tumor to a leiomyosarcoma after successive recurrences that are characterized by a novel genetic fusion (*PTP4A2-AFF1*). This case underscores the challenge with diagnostic classification of uterine smooth muscle tumor, as it may not be reasonable to apply the same histologic criteria for malignancy across all different molecular/genetic types. More specifically, certain molecular/genetic types of uterine smooth muscle tumors may follow an unusual course, not clearly benign or rapidly malignant, but rather a gradually progressive category. More studies are needed for further diagnostic and prognostic

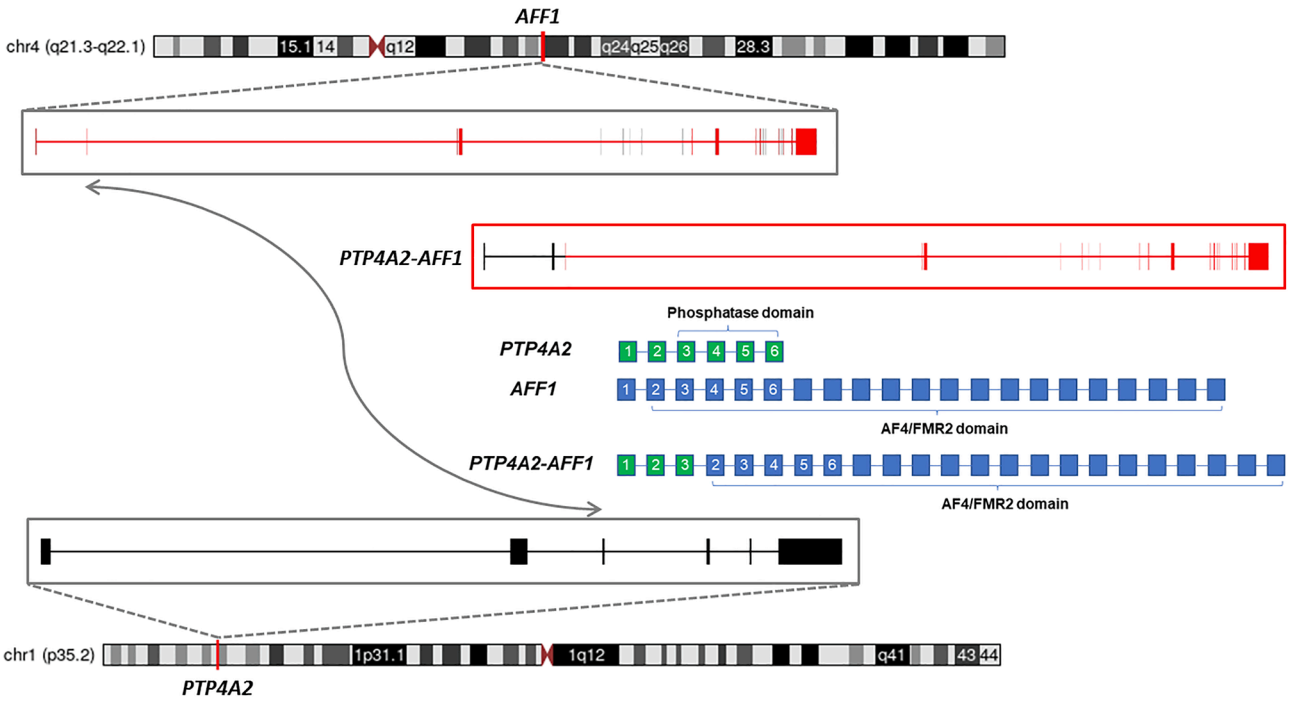

Fig. 2. Schematic illustration of the AFF1 gene at 4q21.3 and the PTP4A2 gene at 1p35.2, with the putative fusion of AFF1 exon 2 (maintaining the entire coding region including the AF4/FMR2 interaction domain) with PTP4A2 exon 3 (encoded region excludes the tyrosine phosphatase domain of this ubiquitously expressed protein tyrosine phosphatase).

insights in relation to *PTP4A2-AFF1* fusion. Moreover, our therapeutic approach for different molecular/genetic types of uterine smooth muscle tumors needs to be refined. Future studies will hopefully provide more robust prediction of prognosis of these tumors and may reveal targetable alteration. There are ongoing clinical trials investigating molecular therapies targeting *AFF1* mutations (NCT04878003), and perhaps the fusion discovered in our case might qualify the patient for future trials.

Consent was obtained prior to preparation of this manuscript from the individual described.

Author Contribution

All authors listed have contributed in accordance with the ICMJE Authorship recommendations. The manuscript was drafted by C. Aubrey, and H. Mal. Figures and Pathology expertise were provided by M. Köbel, CH Lee, G Turashvili, S Yip, and A Yum. G. Nelson provided Gynecologic Oncology expertise, and is the supervising author. All authors were involved in the process of review and editing the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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