Open Peer Review on Qeios

[Communication] Potential Life Prognostic Marker for Mesenchymal Tumor Resembling Uterine Leiomyosarcoma

Shoko Nishikawa[1], Takuma Hayashi[2], Nobuo Yaegashi[3], Kaoru Abiko[1], Ikuo Konishi[3]

1 National Hospital Organization Kyoto Medical Centre

2 Kyoto Medical Center

3 Japan Agency for Medical Research and Development (AMED)

Funding: This clinical research was performed with research funding from the following: Japan Society for Promoting Science for TH (Grant No. 19K09840), START-program Japan Science and Technology Agency for TH (Grant No. STSC20001), and the National Hospital Organization Multicenter clinical study for TH (Grant No. 2019-Cancer in general-02), and The Japan Agency for Medical Research and Development (AMED) (Grant No. 22ym0126802j0001), Tokyo, Japan.

Potential competing interests: No potential competing interests to declare.

Abstract

Background/Aim: Benign uterine leiomyoma (U.LMA) and malignant uterine leiomyosarcoma (U.LMS), which are both uterine mesenchymal tumors, are distinguished by the number of cells with mitotic activity. However, uterine mesenchymal tumors contain tumor cells with various cell morphologies; therefore, making a diagnosis, including differentiation between benign tumors and malignant tumors, is difficult. For example, cotyledonary dissecting leiomyoma (CDL) or uterine smooth muscle tumors of uncertain malignant potential (STUMPs), etc. are a group of uterine mesenchymal tumors for which performing a differential diagnosis is challenging. A standardized classification system for uterine mesenchymal tumors has not yet been established. Furthermore, definitive preoperative imaging techniques or hematological examinations for the potential inclusion of CDL or STUMP in the differential diagnosis have not been defined. Several clinical studies showed that there is no correlation between biomarker expression and mitotic rate or tumor recurrence. The immunohistochemical biomarkers reported so far cannot effectively help determine the malignant potential of CDL or STUMPs in patients who wish to become pregnant in the future.

Materials and Methods: The establishment of gene expression profiles or detection of pathogenic variants by employing next-generation molecular techniques can aid in disease prediction, diagnosis, treatment, and prognosis. We examined the oncological properties of STUMP in adults using molecular pathological techniques on tissue excised from patients with uterine mesenchymal tumor.

Result: In a clinical study conducted by our medical team, the gene expression profiling results identified factors that may be associated with the malignancy of uterine mesenchymal tumors.

Conclusion: Here, we describe the problems in diagnosing uterine mesenchymal tumors along with the results of the latest clinical studies. It is expected that establishing a diagnostic targeting characteristics of mesenchymal tumor cells will lead to the treatment of malignant tumors with a low risk of recurrence and metastasis.

Shoko Nishikawa[1], **Takuma Hayashi**[2,3,*], **Nobuo Yaegashi**[3,4], **Kaoru Abiko**[1], **Ikuo Konishi**[1,3]

[1] *Department of Obstetrics and Gynecology, National Hospital Organization Kyoto Medical Centre, Kyoto, Japan.*

[2] *Cancer Medicine, National Hospital Organization Kyoto Medical Centre, Kyoto, Japan.*

[3] *Medical R&D Promotion Project, The Japan Agency for Medical Research and Development (AMED), Tokyo, Japan.*

[4] *Department of Obstetrics and Gynecology, Sendai Red Cross Hospital, Miyagi, Japan.*

[*]**Corresponding Author:**

Takuma Hayashi

National Hospital Organization, Kyoto Medical Centre

Cancer precision medicine at Kyoto University Hospital

Mukaihatake-cho, Fushimi-ku, Kyoto Kyoto, Japan.

ORCiD ID: 0000-0002-7525-2048

e-mail: yoyoyo224@hotmail.com

Short Title: Molecular pathological analysis of malignancy of STUMP

Key words: STUMP, uterine mesenchymal tumor, leiomyoma, leiomyosarcoma.

Molecular pathological features of cotyledonary dissecting leiomyoma

Recently other clinical group reported the case of a 49-year-old woman with CDL diagnosed by molecular pathological examination using a paraffin-embedded postoperative tissue section [1]. In routine clinical gynecologic practice, uterine mesenchymal tumors are among the most common gynecologic tumors. Uterine leiomyoma is a benign mesenchymal tumor that develops from the uterine smooth muscle layer and accounts for approximately 75% of all uterine tumor tissues resected from patients by surgical treatment [2]. Uterine mesenchymal tumors occur mainly in women of reproductive age, and the prevalence of uterine leiomyoma in women in their 50s is approximately 70% [2][3]. However, its incidence is low in postmenopausal women (The age at which menopause occurs is generally between 50 and 51 years old.). Since many cases uterine of leiomyoma express hormone receptors, secretion of female hormones affects uterine leiomyoma proliferation, and the size and growth of uterine leiomyomas varies slightly depending on the sexual cycle. In the World Health Organization (WHO) classification of gynecologic tumors, typical uterine leiomyoma has a histomorphology similar to that of spindle cell leiomyoma, cellular leiomyoma, epithelioid leiomyoma, intravenous leiomyomatosis, and leiomyoma with bizarre nuclei [4][5][6][7]. A CDL is a uterine leiomyoma with a very rare placental lobed tissue morphology[2] that can be misdiagnosed as a malignant mesenchymal tumor, i.e., uterine leiomyosarcoma due to its rarity and characteristic appearance on gross examination.

In one such case, contrast-enhanced computed tomography (CT) revealed a mass that was continuous with the

myometrium of the uterine corpus, suggesting that it may have arisen from there [8]. However, there was no invasion of tumor cells into the vein and smooth muscle layer. Therefore, uterine leiomyoma growing outside the uterus corpus and exhibiting a morphology like that of the placental leaf is called a CDL [8]. CDL, which extends into the myometrium and broad ligament as well as extrauterinally in a beaded manner, is accompanied by marked edematous changes and macroscopically resembles a placental lobe cotyledon [4][8][9].

Other clinical group reported the case of a 49-year-old woman with CDL diagnosed by molecular pathological examination using a postoperative paraffin-embedded tissue section [1]. She presented with a history of progressive constipation that lasted for 6 months and a palpable left lower abdominal mass for a month [1]. Contrast-enhanced CT imaging studies performed on her showed no enlarged pelvic or para-aortic lymph nodes. The blood test results for her showed that serological tumor markers, namely, cancer antigen (CA) 125, CA19-9, carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP) levels, were normal. Transvaginal ultrasonography revealed a massive mass comprising two subserous fibroids measuring 9.9 × 6.9 × 6.3 cm and 8.1 × 6.6 × 6.8 cm with peripheral and internal probing blood flow signals [1]. Rapid examination using intraoperative frozen sections revealed an angioleiomyoma with edema [1]. Truncal CT performed 6 months after surgery revealed no abnormal findings.

Recently, our clinical team recently experienced the clinical treatment for a case of CDL (Supplementary Figure 1). In November 2020, a 57-year-old woman visited a hospital because of abnormal vaginal bleeding, and the development of a chocolate cyst was suspected. Magnetic resonance imaging (MRI) revealed a mass in the patient's pelvis that could not be adjudged as malignant or benign. However, ovarian cancer was suspected because a solid component was identified on the MRI examination image. Therefore, the patient was referred to our hospital that has a gynecological team for thorough examination. Transvaginal ultrasonography revealed a solid mass measuring 115 mm × 57 mm with an indistinct margin in the right ovary. The area of origin of this mass suggested ovarian cancer or a retroperitoneal tumor. In March 2021, the patient underwent a simple hysterectomy and bilateral salpingectomy. A degenerative uterine leiomyoma measuring 110 mm × 80 mm was found growing within the broad ligament attached to the right round ligament. No gross abnormalities were observed in the bilateral fallopian tubes and ovaries. The surgical pathological examination of the resected tissue demonstrated a CDL. There was no evidence of malignancy in the endometrial tissue, cervical tissue, or bilateral oviduct tissue. The patient is currently being followed up on an outpatient basis. Similar to the detection of a suspicious malignant mass during MRI examination by our medical staff, other healthcare professionals must understand the characteristic appearance of a CDL.

A CDL is an extremely rare benign uterine mesenchymal tumor. Gross examination during surgical treatment is often misdiagnosed as malignant in appearance, which can lead to overtreatment. Serological tumor markers for gynecological tumors, such as CA125 and CA19-9 levels are elevated in many cases of gynecologic malignancies [10][11]. On the other hand, elevated levels of CEA *etc.*, markers for gastrointestinal malignancies, are not observed in many cases of gynecologic malignancies. On the contrary, blood test results for uterine leiomyomas show normal results for serological tumor marker levels. If medical staff are aware of and experienced in the appearance and oncological features of CDL, unnecessary surgical procedures, namely total abdominal hysterectomy (TAH) and bilateral salpingo-oophorectomy

(BSO), can be avoided in patients of reproductive age.

All CDLs reported to date were clinically diagnosed as benign gynecological tumors, and only one case recurred after initial segmental resection [12]. This case was observed in a 33-year-old woman who underwent an incomplete resection to preserve fertility, and recurrence was reported 5 years after the initial surgical intervention [12]. In such cases, it is natural to think that the remaining tumor cells, even if they are benign tumors, proliferate again after being stimulated by estrogen or the like. However, tumor recurrence or advanced tumor has not been reported in cases where complete resection (hysterectomy) for CDL was performed. In the case reported by other clinical group, no evidence of recurrence or advanced tumor was observed in the patient 18 months after hysterectomy [1]. Recently, other research facilities have reported the cases of epithelioid CDL variants and CDLs with intravenous leiomyomatosis [13][14]. Histologically, the CDL-derived neoplastic mesenchymal cells form the disorganized bundles, contrary to the organized pattern observed in common uterine leiomyomas [15].

In many cases of uterine mesenchymal tumors, differentiating between uterine leiomyoma and uterine leiomyosarcoma is difficult. Uterine leiomyoma is the most frequently occurring uterine sarcoma and accounts for 1%–2% of all uterine malignant tumors [8]. Most uterine leiomyosarcomas occur in women aged >40 years. Unlike uterine benign smooth muscle tumors, uterine leiomyosarcomas are frequently observed in postmenopausal women [16]. In uterine mesenchymal tumors that develop from the uterine smooth muscle layer, the rapid growth of the uterine mesenchymal tumor does not immediately suggest the development of uterine leiomyosarcoma. However, in postmenopausal women who are not undergoing hormone replacement therapy, malignancy should be suspected by medical staff when uterine mesenchymal tumors are growing or recurring. However, in the case reported by Ye H et al., neither an epithelioid pattern nor an intravascular neoplastic component was observed [1]. Therefore, recurrence of tumor after surgical treatment will presumably not occur in this patient.

In conclusion, a CDL is a rare variant of uterine leiomyoma[15][17]. Its gross appearance and ultrasonographic features may indicate malignancy. Furthermore, it demonstrates >10 increases in mitotic activity/10 high-power fields, tumor cell necrosis, and no evidence of cellular atypia. Thus, histologically, CDL is a benign tumor. To date, recurrence and metastasis have not been reported in a majority of the cases. Therefore, in clinical practice, prognosis is considered favorable. Clinicians and pathologists must understand the oncologic features of CDL to prevent misdiagnosis of malignancy and consequent overtreatment.

Molecular pathological features of STUMP

A recent clinical study reported the analysis of immunohistochemical examination findings of smooth muscle tumors of uncertain malignant potential (STUMPs), which are considered difficult to pathologically diagnose based on prognosis [18]. Uterine smooth muscle tumors are the most common uterine mesenchymal tumors that have the properties of uterine smooth muscle cells. Uterine mesenchymal tumors are classified into three major types according to their malignant potential: benign leiomyoma (also called as uterine fibroid), malignant uterine leiomyosarcoma, and STUMP, whose

degree of malignancy cannot be clarified [19]. Surgery is the only treatment for uterine leiomyosarcoma, and the prognosis is poor. Therefore, determining the malignancy of uterine mesenchymal tumors is important. In clinical practice, this is carried out by histopathological analysis based on the observation of indicators such as nuclear atypia, mitotic count, and coagulative necrosis. Uterine smooth muscle cells are characterized by a proliferation of spindle-shaped cells consisting of obtuse, elongated nuclei at both ends and eosinophilic cytoplasm, which are oriented perpendicular to each other and multiply in bundles.

Recently, our clinical team recently experienced the clinical treatment for a case of CDL (Supplementary Figure 2). In October 2020, a 47-year-old woman visited a hospital because of abnormal vaginal bleeding, and the development of a endometrial stromal sarcoma or uterine leiomyosarcoma was suspected. In many cases, uterine leiomyomas and uterine leiomyosarcomas exhibit the same morphological characteristics as those of uterine smooth muscle cells [20] (Figure 1A, C, D, F). Therefore, the diagnosis of mesenchymal tumors depends on the morphological features of the uterine smooth muscle cells [21]. However, uterine leiomyomas and uterine leiomyosarcomas have similar morphological characteristics; thus, differentiating between them is a key challenge. During surgical pathological examination, including malignancy determination, the cell morphology of STUMP and that of uterine leiomyosarcoma are very similar, which makes differential diagnosis difficult (Figure 1B, C, E, F). A recent clinical research report revealed that the Ki-67/MIB1-positivity rates in uterine leiomyosarcoma and uterine leiomyoma tissues were approximately 40.78%–45.68% and approximately 02.46%–04.61%, respectively [21] (Figure 2A). On the contrary, the Ki-67/MIB1-positivity rate in STUMP tissues was approximately 38.54%–44.75% (Figure 2A). However, the relationship between Ki-67/MIB1-positivity rate and prognosis has not been clarified. In other words, among uterine mesenchymal tumors with more mitotic numbers than in uterine leiomyomas but fewer ones than in uterine leiomyosarcomas, those without clear tumor-induced coagulative necrosis are identified as STUMPs [22].

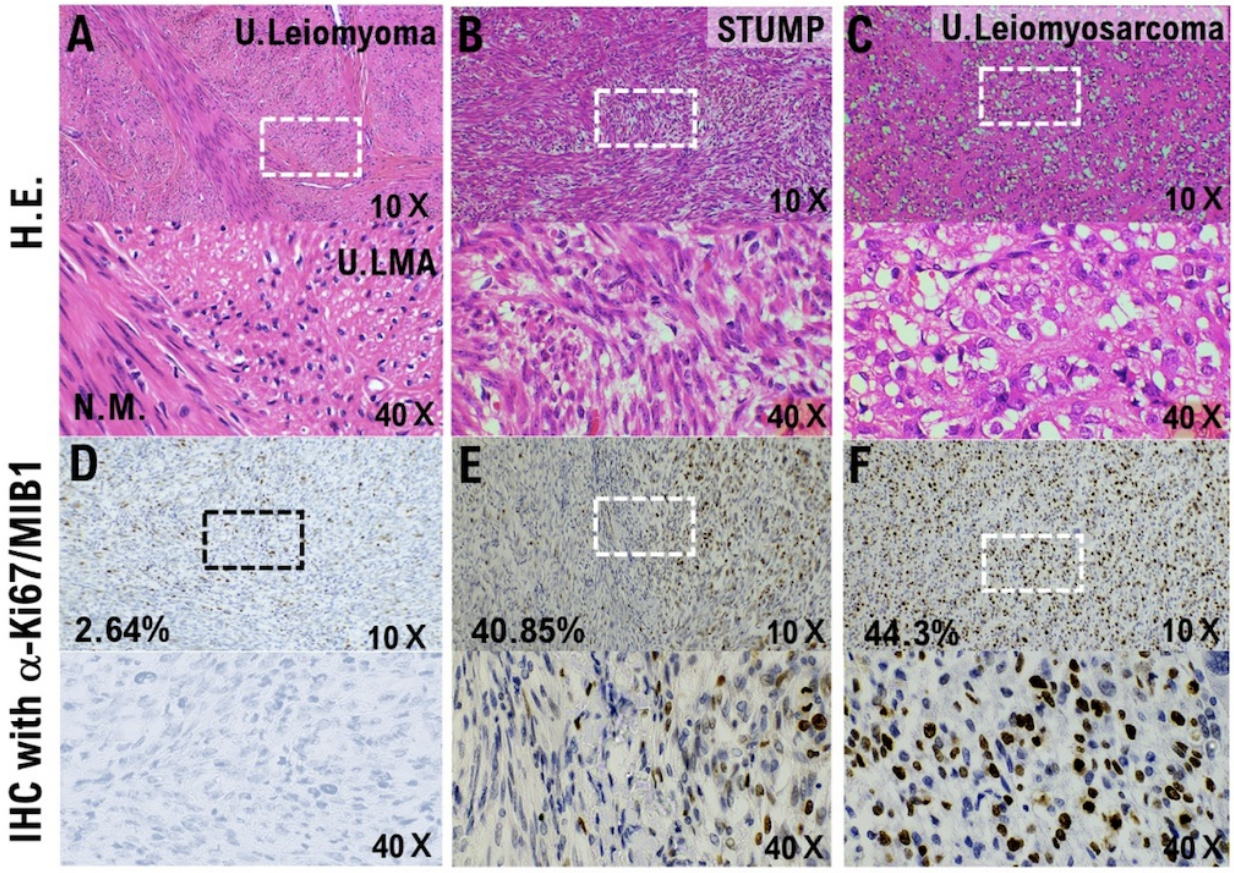
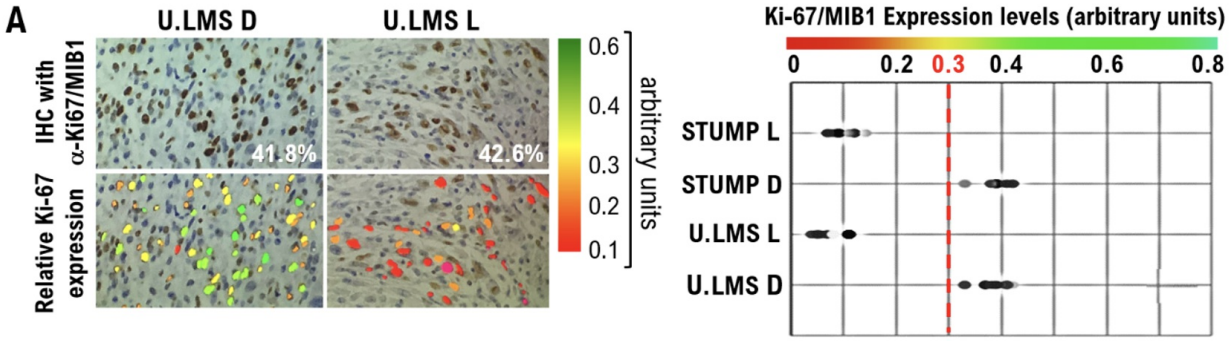


Figure 1. Cell morphology of uterine leiomyoma, STUMP, and uterine leiomyosarcoma. (A, D) Uterine leiomyoma (spindle cell leiomyoma). Low-power view (10×) shows a well-circumscribed tumor nodule in the myometrium comprising broad fascicles of spindle cells. High-power view (40×) shows spindle cells having bland cytological features, elongated nuclei, and fine nuclear chromatin. **(B, E)** Epithelioid smooth muscle tumor of uncertain malignant potential. Low-power view (10×) shows a tumor with multinodular growth at its periphery that might recur. The tumor has an irregular border with the surrounding myometrium. High-power view (40×) shows tumor recurrence in the peritoneum as multiple nodules. **(C, F)** Uterine leiomyosarcoma (spindle cell leiomyosarcoma). Low-power view (10×) shows a cellular tumor with fascicular growth and enlarged hyperchromatic nuclei. High-power view (40×) shows epithelioid leiomyosarcoma with round tumor cells having eosinophilic granular cytoplasm and irregularly shaped nuclei. **(A, B, C)** The photographs show tissue sections stained by Hematoxylin and Eosin (H.E.). **(D, E, F)** The photographs show the tissue sections stained by IHC stained with anti-Ki-67/MIB1 monoclonal antibody. N.M.; normal myometrium, STUMP; smooth muscle tumors of uncertain malignant potential, U.LMA; uterine leiomyoma.

A



B

Ki-67/MIB1 positive cells/total cells (%)		
U.Liomyoma	STUMP	U.Leiomyosarcoma
02.48% – 04.61%	38.54% – 44.75%	40.78% – 45.68%

	Relative Ki-67/MIB1 expression (arbitrary units)	
	≤ 0.3	> 0.3
STUMP	Survival over 5 years after surgery	Death within 5 years after surgery
U.LMS	Survival over 5 years after surgery	Death within 5 years after surgery

Figure 2. Important of Expression level of Ki-67/MIB on uterine mesenchymal malignancy. (A) The photographs show the results of IHC using anti-human Ki-67 monoclonal antibody (clone; MIB1) on the sections of uterine leiomyosarcomas. Ki-67/MIB1 positivity rate (%) in uterine leiomyosatcomas are indicated in the upper photographs. The expression levels of Ki-67/MIB1 were quantified by an image analysis device; Mantra 2™ Quantitative Pathology Workstation (Akoya Biosciences, Inc. Marlborough, MA, USA). **(B)** The table shows Ki-67/MIB1 positivity rate (%) in uterine mesenchymal tumors; uterine leiomyoma, STUMP, uterine leiomyosarcoma. These values are approximately 40.78%–45.68%, 02.46%–04.61%, and 38.54%–44.75% in uterine leiomyosarcoma, uterine leiomyoma, and STUMP tissues, respectively. Relative Ki-67/MIB1 expression (arbitrary units) in uterine mesenchymal tumors. As a result, it was revealed that in patients with uterine leiomyosarcoma or STUMP, those with a Ki-67/MIB1 expression level of 0.3 (arbitrary units) or higher died within 5 years after surgery, and those with a Ki-67/MIB1 expression level of less than 0.3 (arbitrary units) lived for more than 5 years after surgery. N.M.; normal myometrium, STUMP; smooth muscle tumors of uncertain malignant potential, U.LMA; uterine leiomyoma. STUPM L or U.LMS L; Survival over 5 years after surgery, STUMP D or U.LMS D; Death within 5 years after surgery.

In a recent clinical study, of the 54 cases of uterine leiomyosarcoma, 43 cases were included in the cohort that died within 5 years after surgery and 11 cases were in the cohort that survived for more than 5 years after surgery. Furthermore, of the 12 cases of STUMP, 5 cases were included in the cohort that died within 5 years after surgery, and 7 cases were included in the cohort that survived for more than 5 years after surgery. In this clinical study, the expression level of Ki-67/MIB1 was quantified by image analysis device; Mantra 2™ Quantitative Pathology Workstation (Akoya Biosciences, Inc. Marlborough, MA, USA) (Supplementary Materials and Methods). As a result, it was revealed that in patients with uterine leiomyosarcoma or STUMP, those with a Ki-67/MIB1 expression level of 0.3 (arbitrary units) or higher died within 5 years after surgery (Figure 2 A). In addition, those with a Ki-67/MIB1 expression level of less than 0.3 (arbitrary units) lived for more than 5 years after surgery (Figure 2 A). Rather than the Ki-67/MIB1 positive rate, the expression level of Ki-67/MIB1 is considered to be related to the malignancy of uterine mesenchymal tumors (Figure 2 B). Therefore, the expression level of Ki-67/MIB1 is considered to be useful as candidate factors for prognostic marker for patients who develop uterine mesenchymal tumors. STUMPs typically occur in reproductive-aged or postmenopausal women with a

mean age of approximately 43 years, which is a decade less than that of patients with uterine leiomyosarcoma [23]. Uterine leiomyomas are sensitive to female hormones, and female hormones induce the proliferation of uterine leiomyoma cells. However, the growth of uterine leiomyosarcoma cells is female hormone independent. It is not clear how this 10-year difference affects the development of uterine leiomyosarcoma, a malignant tumor.

An increased mitotic rate in uterine leiomyoma-like uterine mesenchymal tumors is highly suggestive of a uterine leiomyosarcoma. However, uterine mesenchymal tumors with poor nuclear atypia and no tumor-induced coagulation necrosis are diagnosed as uterine leiomyomas with increased nuclear mitotic activity, and these tumors are benign [21]. In many of these cases, the mitotic rate is around 5-9/10 high-power field (HPF), although mitotic rates as high as 10-20/10 HPF also occurs. A uterine mesenchymal tumor with a mitotic rate of ≥20/10 HPF in the absence of nuclear atypia and tumor coagulation necrosis is diagnosed as STUMP. Uterine leiomyoma with nuclear atypia is referred to as uterine leiomyoma with bizarre nuclei that until recently was considered a benign tumor. However, in new clinical studies, foci of atypical cells with nuclear atypia have been found, and recurrence has been observed in uterine mesenchymal tumors with low mitotic numbers [24]. According to the latest World Health Organization classification, uterine leiomyoma with bizarre nuclei is categorized as STUMP [19][24].

A meta-analysis of the results of 11 clinical studies involving the follow-up of patients with STUMP revealed a 10% postoperative recurrence rate (15/150 cases) [25]. However, uterine STUMP is an exceedingly rare uterine mesenchymal tumor among gynecologic tumors. Therefore, there is no standardized pathologic classification or definitive preoperative contrast-enhanced computed tomography (CT), magnetic resonance imaging or hematological examination for STUMP.

Recently, a Taiwanese clinical research group examined the medical history, etiology, risk factors, and prognosis of six patients with STUMP to establish a standardized pathologic classification [18]. Immunohistochemistry examination using appropriate monoclonal antibodies revealed marked expression of the cyclin-dependent kinase inhibitor 2A (CDKN2A), tumor protein p53 (TP53), and tumor antigen Ki-67/MIB1 in all six patients [1]. The expression rates of estrogen receptor (ER) and progesterone receptor (PgR) in this series were 50.0% (3/6) and 33.3% (2/6), respectively. Furthermore, no correlation was found between the expression of these immunohistochemical biomarkers and mitotic count or tumor recurrence, thus leading to the conclusion that the expression status of current immunohistochemical biomarkers is ineffective in determining the malignant potential in patients with STUMP who wish to conceive [1]. The identification of STUMP pathogenic variants by genome sequencing and gene expression profiling using next-generation molecular techniques may facilitate malignant potential prediction, surgical pathological diagnosis, clinical treatment, and prognostic assessment.

Previous clinical studies have demonstrated immunohistochemical positivity for CDKN2A and TP53 in STUMP cases with postoperative recurrence [26][27]. However, in recent years, cancer genome testing in clinical practice has also revealed pathogenic variants in cell cycle regulators, such as CDKN2A, TP53, CDKN1A, and CDKN1B, in gynecologic tumors, including uterine carcinosarcoma, and endometrial stromal sarcoma [28]. Therefore, the immunohistochemical findings related to CDKN2A, TP53, CDKN1A, CDKN1B, ER, and PgR have limited application in the differentiation between uterine leiomyomas and uterine leiomyosarcomas [29].

Owing to its high incidence, many patients present with the typical features of uterine leiomyoma. Therefore, it can be easily identified by surgical pathological examination. However, determining the malignancy of uterine mesenchymal tumors that exhibit atypical features is difficult. In cases of uterine leiomyoma, which has a high morbidity rate in actual clinical practice, a diagnostic exclusion method for uterine leiomyosarcoma (a malignant tumor) has not yet been established [30]. Therefore, an in-depth investigation of the relationship between cell morphology and prognosis of various uterine mesenchymal tumors, including uterine leiomyoma, is key to understanding the oncological characteristics of uterine mesenchymal tumors. In clinical practice, STUMP should be conclusively diagnosed. Furthermore, the detailed pathological findings and clinical information about uterine mesenchymal tumors must be documented to establish a more appropriate pathological concept of STUMP.

Conclusion

Considering a standardized classification for uterine mesenchymal tumors has not yet been established, the surgical pathological diagnosis of uterine mesenchymal tumors is often difficult. By using a next-generation sequencer to identify key biomarkers, i.e., pathogenic variants involved in the progression and tumorigenesis of various uterine mesenchymal tumors, the prediction of survival among patients with STUMP may be possible. In the future, the establishment of personalized treatment in clinical practice for uterine mesenchymal tumors, including STUMPs, is expected.

Statements and Declarations

Author Contributions

S.N. and T.H. performed most of the clinical work and coordinated the project. T.H. conducted the diagnostic pathological studies. T.H. conceptualized the study and wrote the manuscript. T.H., N.Y. K.A. and I.K. carefully reviewed this manuscript and commented on the aspects of medical science. I.K. shared information on clinical medicine and oversaw the entirety of the study. All authors have read and agreed to the published version of the manuscript.

Funding

This clinical research was performed with research funding from the following: Japan Society for Promoting Science for TH (Grant No. 19K09840), START-program Japan Science and Technology Agency for TH (Grant No. STSC20001), and the National Hospital Organization Multicenter clinical study for TH (Grant No. 2019-Cancer in general-02), and The Japan Agency for Medical Research and Development (AMED) (Grant No. 22ym0126802j0001), Tokyo, Japan.

Institutional Review Board Statement

This study was reviewed and approved by the Central Ethics Review Board of the National Hospital Organization

Headquarters in Japan (Tokyo, Japan) on November 08, 2019 and Shinshu University (Nagano, Japan) on August 17, 2019, with approval codes NHO H31-02 and M192. The completion numbers for the authors are AP0000151756, AP0000151757, AP0000151769, and AP000351128. As this research was considered clinical research, consent to participate was required. After briefing regarding the clinical study and approval of the research contents, the participants signed an informed consent form.

Materials and Methods

1. Tissue Collection.

A total of 101 patients between 32 and 83 years of age and diagnosed as having smooth muscle tumors of the uterus were selected from pathological files. Serial sections were cut from at least 2 tissue blocks from each patient for hematoxylin and eosin staining and immunostaining.

2. Immunohistochemistry (IHC)

IHC staining for Caveolin 1, Cyclin B, Cyclin E1, large multifunctional peptidase 2/β1i (LMP2/β1i), Ki-67, desmin, and myogenin was performed using serial human uterine mesenchymal tumor sections obtained from patients with uterine mesenchymal tumor

3. Ethical approval and consent to participate

This study was reviewed and approved by the Central Ethics Review Board of the National Hospital Organization Headquarters in Japan (Tokyo, Japan) and Shinshu University (Nagano, Japan). Ethical approval was obtained on August 17, 2019 and the code was NHO H31-02.

Details of Materials and Methods are indicated in Supplementary Materials.

Supplementary Materials

Supplementary Materials: 1.Materials and Methods, 2.Patient #1 findings on contrast-enhanced MRI imaging, 3.Patient #2 findings on contrast-enhanced MRI imaging.

Informed Consent Statement

The applicable for studies involving humans. We have obtained Informed Consent Statements from people participating in clinical studies.

Data Availability Statement

The study did not report any data.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

We appreciate Dr. Susumu Tonegawa (M.I.T., Cambridge, MA, USA.) and Dr. Zhang W (Roche Tissue Diagnostics, Tucson, AZ, USA.) for critical research assistance. We thank all medical staff for clinical research at Kyoto University School of Medicine and the National Hospital Organization Kyoto Medical Center.

References

1. [a, b, c, d, e, f, g, h, i] *Ye H, Qi X, Tian Y, Yang K, Zuo X, Peng Z. Case report: cotyledonoid dissecting leiomyoma in a 49-year-old woman. Transl Cancer Res 2022;11(11):4189-4193. doi: 10.21037/tcr-22-1521*
2. [a, b, c] *Uterine leiomyoma. Female Genital Tumours WHO Classification of Tumours, 5th ed., Vol.4. WHO Classification of Tumours Editorial Board. WORLD HEALTH ORGANIZATION. 2020; pp272-276.*
3. [^] *WHO classification of mesenchymal tumours of the lower genital tract. Female Genital Tumours WHO Classification of Tumours, 5th ed., Vol.4. WHO Classification of Tumours Editorial Board. WORLD HEALTH ORGANIZATION. 2020; pp13.*
4. [a, b] *Zaloudek CJ, Hendrickson MR, Soslow RA. Mesenchymal tumors of the uterus. In Kurman R (ed): Blaustein's Pathology of the Female Genital Tract, 6th ed. Springer, New York, 2010, pp453-527.*
5. [^] *Intravenous leiomyotosis. Female Genital Tumours WHO Classification of Tumours, 5th ed., Vol.4. WHO Classification of Tumours Editorial Board. WORLD HEALTH ORGANIZATION. 2020; pp277-278.*
6. [^] *Tamura S, Hayashi T, Ichimura T, Yaegashi N, Abiko K, Konishi I. Characteristic of Uterine Rhabdomyosarcoma by Algorithm of Potential Biomarkers for Uterine Mesenchymal Tumor. Curr Oncol. 2022, 29(4): 2350-2363. doi: 10.3390/curroncol29040190.*
7. [^] *Tamura S, Hayashi T, Tokunaga H, Yaegashi N, Abiko K, Konishi I. Oncological Properties of Intravenous Leiomyomatosis: Involvement of Mesenchymal Tumor Stem-Like Cells. Curr Issues Mol Biol. 2021, 43(2): 1188-1202. doi: 10.3390/cimb43020084.*
8. [a, b, c, d] *Kuman RJ, Carcangiu MI, Herrington CS, et al. (eds): WHO Classification of Tumours of Female Reproductive Organs, 4th ed, IARC Press, Lyon, 2014.*
9. [^] *Ip PP, Tse KY, Tam KF. Uterine smooth muscle tumors other than the ordinary leiomyomas and leiomyosarcomas: a review of selected variants with emphasis on recent advances and unusual morphology that may cause concern for malignancy. Adv Anat Pathol. 2010 Mar;17(2):91-112. doi: 10.1097/PAP.0b013e3181cfb901.*
10. [^] *Dolscheid-Pommerich RC, Keyver-Paik M, Hecking T, Kuhn W, Hartmann G, Stoffel-Wagner B, Holdenrieder S. Clinical performance of LOCI™-based tumor marker assays for tumor markers CA15-3, CA125, CEA, CA19-9 and AFP in gynecological cancers. Tumour Biol. 2017 Oct;39(10):1010428317730246. doi: 10.1177/1010428317730246.*
11. [^] *Babacan A, Kizilaslan C, Gun I, Muhcu M, Mungen E, Atay V. CA125 and other tumor markers in uterine leiomyomas*

and their association with lesion characteristics. Int J Clin Exp Med. 2014 Apr 15;7(4):1078-83. eCollection 2014.

12. [a, b]*Roth LM, Kirker JA, Insull M, Whittaker J. Recurrent cotyledonoid dissecting leiomyoma of the uterus. Int J Gynecol Pathol. 2013 Mar;32(2):215-20. doi: 10.1097/PGP.0b013e318257dff4.*
13. [^]*Soleymani MH, Ismail L, Desai SA, Reginald PW. Epithelioid cotyledonoid dissecting leiomyoma: a case report and review of the literature. Arch Gynecol Obstet. 2011;283:771-4. doi: 10.1007/s00404-010-1716-6*
14. [^]*Jordan LB, Al-Nafussi A, Beattie G. Cotyledonoid hydropic intravenous leiomyomatosis: a new variant leiomyoma. Histopathology. 2002 Mar;40(3):245-52. doi: 10.1046/j.1365-2559.2002.01359.x.*
15. [a, b]*Weissferdt A, Maheshwari MB, Downey GP, Rollason TP, Ganesan R. Cotyledonoid dissecting leiomyoma of the uterus: a case report. Diag Pathol. 2007;2:18. doi: 10.1186/1746-1596-2-18.*
16. [^]*Gemma Toledo, Esther Oliva Smooth muscle tumors of the uterus: a practical approach. Arch Pathol Lab Med. 2008 Apr;132(4):595-605. doi: 10.5858/2008-132-595-SMTOTU.*
17. [^]*Jamal I, Gupta RK, Sinha RK, Bhadani PP. Cotyledonoid dissecting leiomyoma: an uncommon form of a common disease. Obstet Gynecol Sci. 2019 Sep;62(5):362-366. doi: 10.5468/ogs.2019.62.5.362.*
18. [a, b]*Lin YM, Hong SY, Teng SW, Chang CK, Lai TJ. Retrospective Analysis on Characteristics of Uterine Smooth Muscle Tumors of Uncertain Malignant Potential—13 Years' Experience. Clin. Exp. Obstet. Gynecol. 2022, 49(10), 234 DOI: 10.31083/j.ceog4910234*
19. [a, b]*WHO classification of mesenchymal tumours of the lower genital tract. Female Genital Tumours WHO Classification of Tumours, 5th ed., Vol.4. WHO Classification of Tumours Editorial Board. WORLD HEALTH ORGANIZATION. 2020; pp13.*
20. [^]*Gupta M, Laury AL, Nucci MR, Quade BJ. Predictors of adverse outcome in uterine smooth muscle tumours of uncertain malignant potential (STUMP): a clinicopathological analysis of 22 cases with a proposal for the inclusion of additional histological parameters. Histopathology 2018 73(2):284-298. doi: 10.1111/his.13515.*
21. [a, b, c]*Smooth muscle tumour of uncertain malignant potential of the uterine corpus. Female Genital Tumours WHO Classification of Tumours, 5th ed., Vol.4. WHO Classification of Tumours Editorial Board. WORLD HEALTH ORGANIZATION. 2020; pp279-280.*
22. [^]*Nishikawa S, Hayashi T, Amano Y, Yaegashi N, Abiko K, Konishi I. Characteristic of Concurrent Uterine Lipoleiomyoma and Hemangioma by Algorithm of Candidate Biomarkers for Uterine Mesenchymal Tumor. Diagnostics (Basel). 2022 Oct 12;12(10):2468. doi: 10.3390/diagnostics12102468.*
23. [^]*Guntupalli SR, Ramirez PT, Anderson ML, Milam MR, Bodurka DC, Malpica A. Uterine smooth muscle tumor of uncertain malignant potential: a retrospective analysis. Gynecol Oncol. 2009 Jun;113(3):324-6. doi: 10.1016/j.ygyno.2009.02.020.*
24. [a, b]*Watanabe K, Hayashi T, Katsumata M, Sano K, Abiko K, Konishi I. Development of Uterine Leiomyosarcoma During Follow-up After Caesarean Section in a Woman With Uterine Leiomyoma. Anticancer Res. 2021 Jun;41(6):3001-3010.*
25. [^]*Ip PP, Cheung AN. Pathology of uterine leiomyosarcomas and smooth muscle tumours of uncertain malignant potential. Best Pract Res Clin Obstet Gynaecol. 2011 25(6): 691-704. doi: 10.1016/j.bpobgyn.2011.07.003.*
26. [^]*Ip PP, Cheung AN, Clement PB. Uterine smooth muscle tumors of uncertain malignant potential (STUMP): a*

clinicopathologic analysis of 16 cases. Am J Surg Pathol. 2009 33(7): 992-1005. doi: 10.1097/PAS.0b013e3181a02d1c.

27. ^*Atkins KA, Arronte N, Darus CJ, Rice LW. The Use of p16 in enhancing the histologic classification of uterine smooth muscle tumors.Am J Surg Pathol. 2008 32(1): 98-102. doi: 10.1097/PAS.0b013e3181574d1e.*
28. ^*Hensley ML, Chavan SS, Solit DB, Murali R, Soslow R, Chiang S, Jungbluth AA, Bandlamudi C, Srinivasan P, Tap WD, Rosenbaum E, Taylor BS, Donoghue MTA, Hyman DM. Genomic Landscape of Uterine Sarcomas Defined Through Prospective Clinical Sequencing. Clin Cancer Res. 2020 Jul 15;26(14):3881-3888. doi: 10.1158/1078-0432.CCR-19-3959.*
29. ^*Mills AM, Ly A, Balzer BL, Hendrickson MR, Kempson RL, McKenney JK, Longacre TA. Cell cycle regulatory markers in uterine atypical leiomyoma and leiomyosarcoma: immunohistochemical study of 68 cases with clinical follow-up. Am J Surg Pathol. 2013 37(5): 634-42. doi: 10.1097/PAS.0b013e318287779c.*
30. ^*Hayashi T, Yaegashi N, Tonegawa S, Konishi I. Potential biomarkers associated with malignancy in uterine mesenchymal tumors. European Journal of Gynaecological Oncology. 2021, Vol. 42 Issue (5): 824-828 DOI: 10.31083/j.ejgo4205125*