Potential biomarkers associated with malignancy in uterine mesenchymal tumors

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Uterine mesenchymal tumors, including uterine leiomyosarcoma (uLMS), are gynaecologic tumours difficult to treat clinically. To our knowledge, no standard clinical treatment for uLMS has been established in any clinical practice guideline. The primary reason for the lack of established treatment is the difficulty of conducting clinical trials because of the low incidence of uterine sarcoma. In many patients, uterine sarcoma develops within the body of the uterus. The development of sarcomas outside the body of the uterus (i.e., vagina, vulva, ovaries, etc.) is rare. A clinical study of uterine sarcoma revealed that the incidence of uterine sarcoma was 8% of malignancies that developed in the body of the uterus [1]. Uterine sarcoma is classified into carcinosarcoma, a mixed epithelial and mesenchymal tumour, and mesenchymal tumour (uLMS, endometrial stromal sarcoma, adenosarcoma, etc.). Approximately 50% of all uterine sarcomas are carcinosarcomas, and most of the other 50% are uLMSs, adenosarcomas and endometrial stromal sarcomas (ESS). Based on the results of clinical studies so far, uterine sarcomas are roughly classified into three. In clinical practice, cancer sarcoma (46%), leiomyosarcoma (36%) and ESS (13%) are found in descending order of frequency of uterine sarcoma [2, 3]. The peak age of onset is approximately 50 years for uLMS and ESS, whereas that for carcinosarcoma is ≥ 60 years [2–4]. The 50% survival for ESS is approximately 76 months, whereas that for carcinosarcoma or uLMS is approximately 27 and 30 months, respectively. Due to the low incidence of uterine sarcoma and the subtle differences in the same histology, the molecular pathological diagnosis of uterine sarcoma is often difficult in clinical practice. The clinical treatment strategy and prognosis of uterine sarcoma are determined based on the molecular pathological diagnosis of the resected uterine sarcoma tissue. Therefore, gynaecologists, radiologists and pathologists need to share molecular pathological information to determine the clinical, surgical and pathological diagnosis of uterine sarcoma.

Uterine carcinosarcoma is one of the mixed epithelial-mesenchymal tumours. The epithelial component of uterine carcinosarcoma is carcinoma, and the nonepithelial component is sarcoma. From the results of clinical research so far, single-cell origin, i.e., changes to cancer stem epithelial cells to mesenchymal cells, is considered the origin of uterine carcinosarcoma. In many patients, uterine carcinosarcoma forms a polyp-like ridge that protrudes into the uterine lumen, visible to the naked eye. Clinical studies have proposed three theories for tissue formation in uterine sarcoma, namely combination, collision and compositional tumour theory. Single-cell analysis reveals that most uterine carcinosarcoma is formed by single-cell-derived malignant tumour cells. Furthermore, it has been shown that in the process of uterine carcinosarcoma development, malignant tumour cells derived from single cells differentiate into tissues showing epithelial- and stromal-like morphologies [5]. Clinicopathological analysis reveals that uterine carcinosarcoma has more biological properties than sarcoma because of risk factors and multiple lymphoid metastases in uterine carcinosarcoma. Therefore, clinical treatment for uterine carcinosarcoma is performed according to clinical practice guidelines for high-grade uterine endometrial cancer. The 50% survival time (mOS) of patients with uterine carcinosarcoma is approximately 28 months. In other words, uterine carcinosarcoma is a poor prognosis disease. Thus, further clinical trials are needed to establish effective chemotherapy for uterine carcinosarcoma. If the uterine sarcoma component does not show a differentiation tendency, it is called homologous. The other hand, if it shows differentiation into mesenchymal tissue that does not naturally exist in the uterus, such as striated muscle, cartilage and bone, an uterine sarcoma is called heterologous. The incidence rate of uterine adenosarcoma is rare: < 1% of all malignant tumours of the uterus. Therefore, uterine adenosarcoma is a malignant mixed tumour comprising benign epithelial and sarcoma components (mostly low-grade ESS). In uterine adenosarcoma, the prognosis in sar-
comatous overgrowth, in which the sarcoma component accounts for >25% of the tumour, is poor. The age of uterine adenosarcoma onset is younger than that of carcinosarcoma onset.

For the molecular pathological diagnosis of uLMS, the surgical pathological diagnostic criteria proposed by Hendrickson and Kempson’s clinical group are widely used [6]. As a surgical pathological diagnosis for uLMS, nuclear division (index), cell atypia and coagulative tumour necrosis are comprehensively evaluated. For uLMS, simple abdominal hysterectomy and bilateral adnexectomy are performed as the basic initial treatment for the excised possible cases. In uLMS, the incidence of lymphatic metastases is very low. Therefore, there is no clear evidence that extended surgery or surgical treatment of lymph node dissection improves the prognosis of patients with uLMS. Postoperative chemotherapy may be given to prevent recurrence of uLMS, but current clinical trials have not shown the efficacy of postoperative chemotherapy for uLMS. Radiation therapy for uLMS does not suppress recurrence [7].

Previously, endometrial stromal sarcoma was classified as low-grade endometrial stromal sarcoma (LG-ESS) and high-grade endometrial stromal sarcoma (HG-ESS). However, since HG-ESS not always similar to the endometrial stroma, according to the latest World Health Organisation classification, HG-ESS is classified as undifferentiated endometrial sarcoma (UES) [8]. LG-ESS of the prognosis is good, and a 50% survival period (mOS) is reportedly 76 months. Alternatively, the prognosis of UES is poor. As a treatment method for ESS, surgical treatment by simple abdominal hysterectomy and bilateral adnexectomy similar to the treatment method for uLMS is performed. However, since pelvic and para-aortic lymph node metastases were found at a rate of 9%–33% in LG-ESS and 15%–18% in UES, lymph node dissection is required for some patients with LG-ESS or UES [9, 10]. Since there are many cases in which estrogen or progesterone or both receptors are expressed in LG-ESS, hormone therapy is effective as a treatment method for LG-ESS [11]. Therefore, long-term survival of patients is recognised by hormone therapy for recurrent LG-ESS [11]. In the results of clinical trials conducted so far, the efficacy of radiation therapy and chemotherapy for ESS, regardless of malignancy, has not been confirmed.

The proteasome is a proteolytic enzyme complex consisting of multiple subunits that degrades ubiquitinated proteins in eukaryotic cells and plays a central role in proteolytic degradation. Stimulation of interferon-gamma (IFN-\(\gamma\)) induces the expression of beta subunits and constitutes the immunoproteasome, which regulates gene expression and cell proliferation by controlling the degradation of intracellular proteins. The expression of the major histocompatibility complex-linked low molecular mass polypeptide 2/\(\beta_{I}\) (LMP2/\(\beta_{I}\)) subunit, which is increased by treatment with IFN-\(\gamma\), amplifies specific endopeptidase activities of the immunoproteasome. Reports demonstrated that uLMS spontaneously developed after six months of age in Lmp2/\(\beta_{I}\)-deficient (mouse) female mice [12–14]. Studies have shown that the prevalence of uLMS in Lmp2/\(\beta_{I}\)-deficient mice is approximately 37% at 12 months of age [12–14]. Hematogenous metastases were also found in the Lmp2/\(\beta_{I}\)-deficient female mouse [12–14]. The incidence of other malignancies (i.e., hepatocellular carcinoma, etc.) in Lmp2/\(\beta_{I}\)-deficient mice has been reported to be 1% or less [12–14]. Therefore, in clinical research by a collaboration of medical institutions, the expression status of LMP2/\(\beta_{I}\) (Human) was examined in 74 cases with normal myometrium, uterine leiomyoma, uLMS and other uterine mesenchymal tumour tissues obtained from the pathological file by immunohistochemical (IHC) staining using an anti-human LMP2/\(\beta_{I}\) monoclonal antibody [15, 16]. As a result, the expression level of LMP2/\(\beta_{I}\) was significantly explicitly reduced in the uLMS tissues compared with those in the uterine leiomyoma and normal myometrium tissues. Therefore, it may be easy to distinguish between uLMS and uterine leiomyoma depending on the expression status of LMP2/\(\beta_{I}\), even in cases where differential diagnosis is difficult by the surgical, pathological diagnosis method currently performed in clinical practice [15].

Based on the markedly reduced expression level of LMP2/\(\beta_{I}\), candidate factors as biomarkers specifically expressed in uLMS have been sought using genome-wide experimental methods with human-extracted tissues. As a result, CAVEOLIN 1, CYCLIN B, CYCLIN E, Ki-67/MIB1 and LMP2/\(\beta_{I}\) were identified as biomarker candidate factors expressed explicitly in uLMS. The differential diagnostic method with IHC staining using a combination of several monoclonal antibodies to LMP2/\(\beta_{I}\) and other candidate cellular factors such as CAVEOLIN 1, CYCLIN B, CYCLIN E, Ki-67/MIB1 and 5’-nucleotidase domain containing two has been investigated for uterine mesenchymal tumours, including uLMS (Table 1) [17, 18]. Over-expression of cyclin E correlates with tumorigenesis, it is involved in various malignant tumours, including breast, colon, bladder, skin and lung cancer [19]. Recent reports have demonstrated that cyclin E-deficient cells actively proliferate in conditions of continuous cell cycling but are unable to re-enter the cell cycle from the G1 phase to the S phase and are resistant to chemical-induced oncogenic transformation [20–22]. Cyclin E, a regulator of the cell cycle and Ki-67/MIB1, which is used as a diagnostic biomarker in proliferating cancer or malignant tumour cells, affect the behaviour of human breast cancer cells and uLMS [22–24]. Clinical studies suggested that patients with uLMS with high expression levels of cyclin E and Ki-67/MIB1 have a poor prognosis. In other words, the expression status of cyclin E and Ki-67/MIB1 is considered to correlate with the uLMS malignancy.

Mediator complex subunit 12 (MED12) is a transcriptional mediator of RNA polymerase II transcription. Previous research has shown that mutations in MED12 are found in both primary and metastatic uLMSS, including uLMS.
Protein blotting examinations revealed the expression of MED12 protein in all uterine leiomyomas and uLMS, irrespective of MED12 exon 2 mutation status or histological grade [29]. These findings suggest that MED12 has transforming roles in a broad range of smooth muscle neoplasia in addition to uterine mesenchymal tumours. As a health and welfare activity of national and international governments, uterine cancer screening (including uterine leiomyoma with high incidence regardless of race) is recommended for women aged ≥20 years. Micro-RNAs are short nucleic acids that regulate gene expression and protein activity in the body. About 2500 types of micro-RNAs are present in the blood. In recent years, it has been clarified that the type and amount of micro-RNAs contained in the blood fluctuate depending on the onset of cancer or malignant tumour. Using the biological properties of micro-RNAs, diagnostic techniques for cancer and malignant tumours have been investigated. Also, for preoperative uterine mesenchymal tumour screening, the usefulness of serum micro-RNA as liquid biopsy samples with high diagnostic performance has been investigated. However, because uterine mesenchymal tumours are a mixture of tumours of various morphologies and pathologies, many problems must be resolved before the results of the clinical studies can be applied to clinical practice.

Currently approved treatments for other malignancies and cancers have not been shown to be effective against uterine mesenchymal tumours, including uLMS. In international clinical research, current clinical evidence has been weighing to establish various preoperative and postoperative surgical pathological diagnostic or clinical treatment methods or both for uLMS at an early stage. Based on data obtained from clinical studies, the administration of adjuvant chemotherapy seems to show no improvement in progression-free survival or overall survival of patients with uLMS at the early stage. Due to the low prevalence of uterine mesenchymal malignancies compared to other malignant tumours, large prospective, randomised, multi-institutional studies are required to assess better the value of different adjuvant strategies for clinical treatment against uLMS. Innovative targeted therapies must be tested by a large cohort to improve outcomes in patients with uterine mesenchymal malignancies such as uLMS, which remain inadequately effective. Pathogenic variants, including impaired expression of LMP2/βi or MED12 oncogenic mutations or both, may be one of the risk factors for developing human uLMS, as are Lmp2/βi-deficient mice. Thus, combining defective LMP2/βi expression or MED12 oncogenic mutations or both with other functional candidates may be useful as a novel diagnostic biomarker to distinguish human uLMS from other mesenchymal tumours. To our knowledge, no effective therapy has been established for unresectable human uLMS; therefore, gene therapy with LMP2/βi expression vectors might be a new treatment for human uLMS that exhibits a defect in LMP2/βi expression.

ULMS is a malignant mesenchymal tumour with a poor prognosis because of repeated recurrence and distant metastasis. Preoperative diagnosis of uLMS is difficult in clinical practice. Therefore, it is important to establish a simple diagnostic method using diagnostic biomarkers to distinguish uLMS from other uterine mesenchymal tumours. Currently, clinical studies are being conducted to verify the specificity and superiority of candidate cellular factors specific to uLMS as differential diagnostic biomarkers that can predict prognosis. Healthcare workers and patients hope that a simple diagnostic method would be established based on the findings of future clinical studies.
Author contributions

TH wrote the manuscript. ST and NY provided research materials and scientific information. NY and IK carefully reviewed the manuscript and commented on aspects of clinical medicine, shared information on clinical medicine.

Ethics 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). The authors attended a 2020 educational lecture on medical ethics supervised by the Japanese government. The completion numbers of the authors are AP0000151756 AP0000151757 AP0000151769 AP000351128. This research is not clinical study, therefore consent to participate is not required.

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Conflict of interest

The authors declare no conflict of interest.

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