



A Novel Diagnostic Marker: Proteasome Lmp2- Differential Expression in Human Uterine Neoplasms

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ABSTRACT

Uterine leiomyosarcoma (LMS) develops more often in the muscle tissue layer of the uterine body than in the uterine cervix. The development of gynecologic tumors is often correlated with female hormone secretion; however, the development of uterine LMS is not substantially correlated with hormonal conditions, and the risk factors are not yet known. Importantly, a diagnostic-biomarker which distinguishes malignant LMS from benign tumor leiomyoma (LMA) is yet to be established. Accordingly, it is necessary to analyze risk factors associated with uterine LMS, to establish a treatment method. Proteasome LMP2-deficient mice spontaneously develop uterine LMS, with a disease prevalence of ~37% by 12 months of age. We found LMP2 expression to be absent in human LMS, but present in human LMA. Therefore, defective-LMP2 expression may be one of the risk factors for LMS. LMP2 is a potential diagnostic-biomarker for uterine LMS (Ut-LMS), and may be a targeted-molecule for a new therapeutic approach.

Keywords : LMP2, uterine leiomyosarcoma, uterine leiomyoma, biomarker

Introduction

The uterus is composed of three layers, the uterine endometrium which serves as a bed for the embryo; the myometrium of the wall which protects the embryo; and a serous membrane enveloping the uterus. In general, the term uterine tumor refers to an epithelial malignant tumor of the uterus, which is roughly classified as a tumor of the uterine cervix or the uterine body. Because of the prevalence of medical checkups, the rate of mortality from uterine cervix malignant tumor is decreasing, and usually detected at a very early stage. In contrast, the mortality rate for malignant tumor of the uterine body is increasing, and the disease is rarely detected at the initial stages. While most tumors of the uterine body are adenocarcinomas, the uterine cervix tumors are classified into squamous cancer and adenocarcinoma. Uterine mesenchymal tumors, which develop in the myometrium have been traditionally divided into benign LMA and malignant Ut-LMS based on cytological atypia, mitotic activity and other criteria. Ut-LMS is relatively rare, having an estimated annual incidence of 0.64 per 100,000 women.¹ Ut-LMS accounts for 2% to 5% of tumors of the uterine body and develops more often in the muscle layer of the uterine body than in the uterine cervix. As Ut-LMS is resistant to chemotherapy and radiotherapy, surgical intervention is virtually the only means of treatment.^{2,3} The prognosis for Ut-LMS is not good, and the five-year survival rate is approximately 35%. However, developing an efficient adjuvant therapy is expected to improve this. Uterine LMA may occur in as many as 70% ~ 80% of women by the age of 50 years.⁴ Distinguishing uterine LMA from Ut-LMS is very difficult, and a diagnosis generally requires surgery and cytосcopy. Diagnostic categories for uterine mesenchymal tumors and morphological criteria are used to assign cases. The non-standard subtypes of uterine mesenchymal tumors such as the epithelioid and myxoid types are classified in a different way using these features, so the establishment of a diagnostic method for the identification of non-standard smooth muscle differentiation is important.^{5,6}

High estrogen levels are considered to significantly influence

the development of tumors in the uterine body.⁷ The mechanisms by which uterine LMA and Ut-LMS develop are not yet known, though tumors that have developed in the myometrium for some reason gradually become larger due to the influence of the female hormone, estrogen, and generate tumors. However, no correlation between the development of Ut-LMS and hormonal conditions, and no obvious risk factors have been found. Although cases accompanied by hypocalcaemia or eosinophilia have been reported, neither clinical abnormality is an initial risk factor for Ut-LMS. The identification of a risk factor associated with the development of Ut-LMS would significantly contribute to the development of preventive and therapeutic treatments.

LMP2/ β 1i, candidate molecule as diagnostic biomarker

Cytoplasmic proteins are mostly degraded by a protease complex, which has many substrates consisting of twenty-eight 20 to 30-kDa subunits, referred to as the 20S proteasome.^{8,9} The proteasomal degradation is essential for many cellular processes, including the cell cycle, the regulation of gene expression and immunological function.¹⁰ Interferon- γ (IFN- γ) induces the expression of large numbers of responsive genes, proteasome subunits, i.e., low-molecular mass polypeptide (LMP) 2, LMP7, and LMP10.¹¹ A molecular approach to studying the correlation of IFN- γ with tumor cell growth has drawn attention.

Homozygous mice deficient in LMP2 show tissue- and substrate-dependent abnormalities in the biological functions of the proteasome.¹² Ut-LMS reportedly occurred in female LMP2^{-/-} mice at age 6 months or older, and the incidence at 14 months of age was about 40%.¹³ Histological studies of LMP2-lacking uterine tumors have revealed characteristic abnormalities of Ut-LMS.¹³ The tumors consisted of uniform elongated myometrium cells arranged into bundles. The nuclei of the tumor cells varied in size and shape, furthermore, mitosis was frequent. In contrast, the myometrium cells of C57BL/6 mice were normal in appearance. Whereas relatively few ki-67-positive cells, the proliferating cells of solid

tumors, were observed in the basal cell layer of the normal myometrium, most of the basal cells vividly expressed ki-67 in LMP2^{-/-} mice. 13 LMP2^{-/-} mice that have developed Ut-LMS undergo considerable weight loss, and then die by 14 months of age. The LMP2^{-/-} mice also exhibit skeletal muscle metastasis from Ut-LMS. Therefore it is likely that LMP2^{-/-} mice with Ut-LMS die as a result of the tumor mass and metastasis.

The non-standard subtypes of uterine mesenchymal tumors such as the epithelioid and myxoid types are classified in a different way using these features, so the establishment of a diagnostic method for the identification of non-standard smooth muscle differentiation is important.^{5,6} Pathological studies were performed to demonstrate the validity and reliability of LMP2 as a diagnostic biomarker under the combination of other candidate molecules, for instance cyclin E and calponin h1, which reportedly function as anti-tumorigenic factor in human Ut-LMS. Pathological studies revealed a serious loss in the ability to induce LMP2 and calponin h1 expressions in human Ut-LMS tissues in comparison with uterine LMA or normal myometrium located in the same section, and markedly cyclin E expression in only human Ut-LMS tissues.^{14,15,16,17} Histological findings were consistent with metastatic LMS for the skeletal muscle and rectum lesions.^{14,15} In western blotting and RT-PCR experiments, LMP2 was expressed in normal myometrium, but not in human Ut-LMS, both strongly supportive of the pathological findings.^{14,15,17} Although we have previously demonstrated that the abnormal expression of the ovarian steroid receptors, p53 and ki-67 and mutations of p53 were frequently associated with Ut-LMS, defective LMP2 expression appears to be more characteristic of Ut-LMS than these factors.^{15,16}

Final Consideration

In the case of gynecological cancers, a female hormonal im-

balance is often a risk factor for developing tumors.⁷ As in the case of uterine LMA, however, a correlation between the development of Ut-LMS, the female hormone, and hormone receptors has yet to be elucidated. A recent report showed the expression of *Lmp2* mRNA and protein in luminal and glandular epithelia, placenta villi, trophoblastic shells, and arterial endothelial cells.^{18,19,20} These results implicate LMP2 in the invasion of placental villi, degradation of the extracellular matrix, immune tolerance, glandular secretion, and angiogenesis, but no more information for tumorigenesis. Further experiments are also required to elucidate the molecular mechanism of human Ut-LMS tumorigenesis involved biological significance of LMP2. We are investigating the reliability and characteristics of LMP2 as a diagnostic indicator with several clinical research facilities. Histologic characteristics of uterine mesenchymal tumors including mitotically active leiomyoma, bizarre leiomyoma, lipoleiomyoma, uterine mesenchymal tumors of uncertain malignant potential (STUMP), leiomyomatoid angiomatous neuroendocrine tumor (LANT) are summarized.^{21,22} Clarification of the correlation between these factors and the development of human Ut-LMS and the identification of specific risk factors may lead to the development of new clinical treatments for the disease.

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