Scientific Research

Candidate Molecules and ki-67/MIB1 as Novel Diagnostic Biomarker for Human Uterine Mesenchymal Tumors

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ABSTRACT

Human 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 uterine 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 clinical treatment method. Protea some β-ring subunit LMP2/β1i-deficient mice spontaneously develop uterine LMS, with a disease prevalence of ~40% by 14 months of age. We found LMP2/β1i expression to be absent in human uterine LMS, but present in human LMA. Therefore, defective-LMP2/β1i expression may be one of the risk factors for human uterine LMS. LMP2/β1i is a potential diagnostic-biomarker under the combination of candidate molecules, for instance cyclin B1, cyclin E and calponin h1 and ki-67/MIB1 counts for uterine mesenchymal tumors, especially human uterine LMS, and may be a targeted-molecule for a new therapeutic approach.

Keywords: LMP2/β1i; Uterine Leiomyosarcoma; Uterine Leiomyoma; Biomarker

1. Introduction

The uterus, the organ in which the embryo grows, 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 screening, uterine cervix cancer is decreasing in incidence, and usually detected at a very early stage. In contrast, cancer of the uterine body is increasing in incidence, and rarely detected at the initial stages. While most tumors of the uterine body are adenocarcinomas (derived from the subintimal gland), tumors of the uterine cervix are classified into squamous cancer and adenocarcinoma. Smooth muscle tumors (SMTs) which develop in the

myometrium have been traditionally divided into benign uterine leiomyoma (LMA) and malignant uterine leiomyosarcoma (LMS) based on cytological atypia, mitotic activity and other criteria. Uterine LMS is relatively rare, having an estimated annual incidence of 0.64 per 100,000 women [1]. Uterine 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 uterine LMS is resistant to chemotherapy and radiotherapy, surgical intervention is virtually the only means of clinical treatment [2-4]. The prognosis for uterine LMS is not good, and the five-year survival rate is approximately 35% [5]. However, developing an efficient adjuvant therapy is expected to improve survival rate. Uterine LMA may occur in as many as 70% ~ 80% of women by the age of 50 years [6]. Distinguishing uterine LMA from uterine LMS is very difficult, and a diagnosis generally requires surgery and cytoscopy [7].

Diagnostic categories for uterine SMTs and morphological criteria are used to assign cases [8,9] (**Attention 1**). The non-standard subtypes of uterine SMTs 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 [8,9].

High estrogen levels are considered to significantly influence the development of tumors in the uterine body [10-12]. The mechanisms by which uterine LMA and uterine 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 and generate tumors. However, no correlation between the development of uterine LMS and hormonal conditions, and no obvious risk factors has been found. Although cases accompanied by hypocalcaemia or eosinophilia have been reported, neither clinical abnormality is an initial risk factor for uterine LMS. The identification of a risk factor associated with the development of uterine LMS would significantly contribute to the development of preventive and therapeutic treatments.

2. Spontaneous Development of Uterine Leiomyosarcoma in LMP2/β1i-Deficient Mice

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 [13,14]. The proteasomal degradation is essential for many cellular processes, including the cell cycle, the gene expression and immunological function [15]. Interferon (IFN)-γ induces the expression of large numbers of responsive genes, proteasome subunits, *i.e.*, low-molecular mass polypeptide (LMP)2/β1i, LMP7/β5i, and LMP10/β2i [16] (**Figure 1**).

The individual expression of LMP2/β1i, LMP7/β5i and LMP10/β2i subunits in various cell types or tissues is believed to contribute to the initiation and development of disorders. A recent study revealed a unique role for LMP7/β5i in controlling pathogenic immune responses and provided a therapeutic rationale for targeting LMP7/β5i in autoimmune disorders, especially rheumatoid arthritis [17].

Homozygous mice deficient in LMP2/β1i show tissue- and substrate-dependent abnormalities in the biological functions of the proteasome [18]. Here we identify LMP2/β1i, as obligatory for tumor surveillance and demonstrate a tissue-specific role for LMP2/β1i in protection from spontaneous uterus neoplasms. In short, uterine LMS reportedly occurred in female LMP2/β1i-deficient mice at age 6 months or older, and the incidence at 14 months of age was about 40% [19,20] (**Figure 2**).
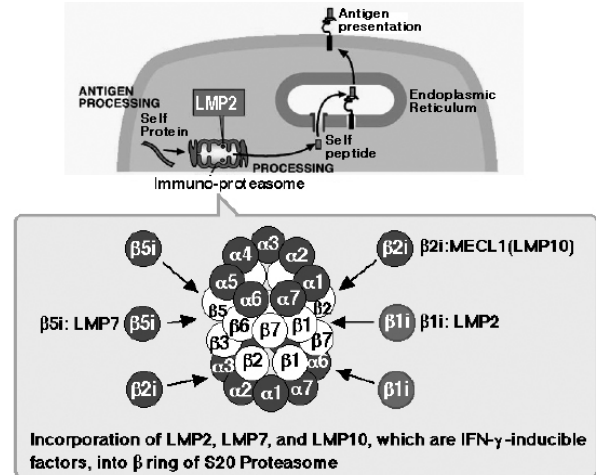

Figure 1. The immuno-proteasomal degradation pathway is essential for antigen presentation by MHC class I.

ure 2). The curve indicating the incidence in mice is similar to that indicating the incidence of human uterine LMS, which occurs after menopause. Histological studies of LMP2/β1i-lacking uterine tumors have revealed characteristic abnormalities of uterine LMS, and the tumors lacked lymphoid infiltrates, a sign of immune recognition, and consisted of uniform elongated myo- metrium cells arranged into bundles [19] (**Figure 2**).

The nuclei of the tumor cells varied in size and shape, furthermore, mitosis was frequent. In contrast, the myometrium cells of its parental mice, C57BL/6 mice were normal in appearance. Whereas relatively few ki-67/ MIB1-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/MIB1 in LMP2/β1i-deficient mice [19] (**Figure 2**). Immunohistochemistry (IHC) study indicates abnormal proliferation of the LMP2/β1i-lacking cells in the basal layer. LMP2/β1i-deficient mice that have developed uterine LMS undergo considerable weight loss, and then die by 14 months of age [19,20]. The LMP2/β1i-deficient mice also exhibit skeletal muscle metastasis from uterine LMS. Therefore it is likely that LMP2/β1i-deficient mice with uterine LMS die as a result of the tumor mass and metastasis [19,20]. In general, it is not easy to distinguish uterine LMA from uterine LMS, however, in mice, because of such characteristic pathological findings, significant weight loss, and skeletal muscle metastasis, a tumor that develops in the uterus of an LMP2/β1i-deficient mouse can be considered malignant, *i.e.*, uterine LMS [19,20].

3. Defective LMP2/β1i Expression in Human Leiomyosarcoma

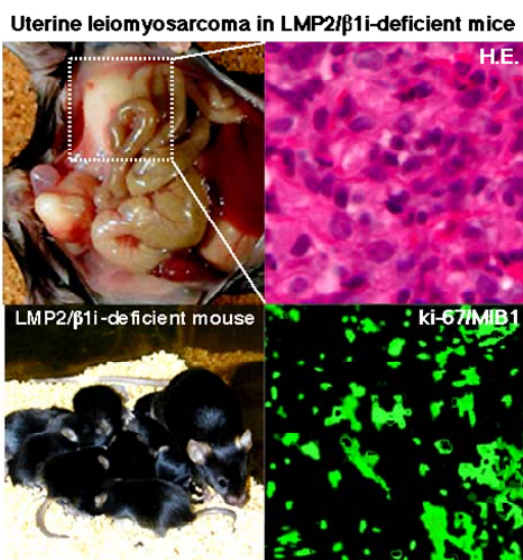IHC studies were performed to demonstrate the validity and reliability of LMP2/β1i as a diagnostic biomarker

Figure 2. Homozygous mice deficient in LMP2/β1i, an IFN-γinducible factor, show tissue- and substrate-dependent abnormalities in the biological functions of the proteasome. uterine LMS reportedly occurred in female LMP2/β1i deficient mice at age 6 months or older, and the incidence at 14 months of age was about 40%.

under the combination of other candidate molecules, for instance cyclin B1, cyclin E and calponin h1, which reportedly function as anti-tumorigenic factor in human uterine LMS [21-25]. IHC experiments revealed a serious loss in the ability to induce LMP2/β1i and calponin h1 expression in human uterine LMS tissue in comparison with uterine LMA or normal myometrium located in the same section [21-25]. Of the 54 cases we examined with uterine LMS, 46 were negative for LMP2/β1i expression, 4 were focally positive, and 2 were partially positive [21,22,24]. Two uterine LMS cases were stained for LMP2/β1i. LMP2/β1i levels were also evaluated in skeletal muscle and rectum metastases from individual uterine LMS patients. Pathological examination of surgical samples showed the presence of a mass measuring 3 cm in its largest diameter in the lumbar quadrate muscle without a fibrous capsule. All lymph nodes were negative for uterine LMS metastases, and IHC analyses showed positivity for ki-67/MIB1 and negativity for LMP2/β1i. Histological findings were consistent with metastatic LMS for the skeletal muscle and rectum lesions. In western blotting and RT-PCR experiments, LMP2/β1i was expressed in normal myometrium, but not in human uterine LMS, both strongly supportive of the IHC findings [21,22]. Although we has previously demonstrated that the abnormal expression of the ovarian steroid receptors, TP53 and ki-67/MIB1 and mutations of TP53 were frequently associated with uterine LMS, defective LMP2/β1i expression appears to be more characteristic of uterine LMS than these factors [21-25].

4. LMP2/β1i Differential Expressions in Human Uterine Mesenchymal Tumors

In the case of gynecological cancers, such as breast cancer, a female hormonal imbalance is often a risk factor for developing tumors [10-12]. As in the case of uterine LMA, however, a correlation between the development of uterine LMS, the female hormone, and hormone receptors has yet to be elucidated. A recent report showed the expression of *Lmp*2 mRNA and protein in luminal and glandular epitheliua, placenta villi, trophoblastic shells, and arterial endothelial cells [26-27]. These results implicate LMP2/β1i in the invasion of placental villi, degradation of the extracellular matrix, immune tolerance, glandular secretion, and angiogenesis [26,27]. The present study should help to elucidate the regulatory role of LMP2/β1i in the implantation of embryos [26-29]. Unfortunately, it is unclear whether defective LMP2/β1i expression is involved in the onset of uterine LMS. Risk factors for its development however, have not been identified because of the absence of a suitable animal model. The LMP2/β1i-deficient mouse was the first animal model of spontaneous uterine LMS to be established [19,20]. Defective LMP2/β1i expression may be one of the causes of uterine LMS. To demonstrate whether LMP2/β1i is a potential biomarker for distinguishing uterine LMS from uterine LMA, we are investigating the reliability and characteristics of LMP2/β1i as a diagnostic indicator with several clinical research facilities. To demonstrate whether LMP2/β1i is a potential biomarker for distinguishing human uterine LMS from uterine LMA under the combination with other candidate molecules, especially cyclin B1, cyclin E calponin h1, which are identified as potential diagnostic candidates, and ki-67/MIB1 counts [21,22,24,25,30-37], we are investigating the reliability and characteristics of LMP2/β1i as a diagnostic indicator with several clinical research facilities [22-25,30-32]. The clinical research is yet to be concluded, and large-scale clinical studies need to be performed with additional clinical research facilities. Histologic and IHC characteristics of uterine mesenchymal tumors including mitotically active leiomyoma, bizarre leiomyoma, lipoleiomyoma, uterine smooth muscle tumors of uncertain malignant potential (STUMP), leiomyomatoid angiomatous neuroendocrin tumor (LANT) are summarized [32-37]. Clarification of the correlation between these factors and the development of human uterine LMS and the identification of specific risk factors may lead to the development of new treatments for the disease. Uterine LMS is refractory to chemotherapy and has a poor prognosis. The molecular biological and cytological information obtained from LMP2/β1i-deficient mice will contribute remarkably to the development of preventive methods, a potential diagnostic-biomarker, and new therapeutic approaches against human uterine

LMS.

5. Final Considerations

Human uterine LMS is refractory to chemotherapy and has a poor prognosis. Defective LMP2/β1i expression is likely to be one of the risk factors in the development of human uterine LMS as it is in the LMP2/β1i-deficient mouse. LMP2/β1i might function as an anti-tumorgenic factor in human uterine LMS. The molecular biological and cytological information obtained from LMP2/β1i-deficient mice will contribute remarkably to the development of preventive methods, a potential diagnostic biomarker, and new therapeutic approaches against human mesenchymal tumors, especially human uterine LMS.

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Attention 1

The typical gross appearance is a large (>10 cm), poorly circumscribed mass with a soft, fleshy consistency and a variegated cut surface that is grey-yellow to pink, with foci of hemorrhage and necrosis [8,9]. The histologic classification of uterine sarcomas is based upon homology to normal cell types and include uterine LMS (analogous to myometrium), stromal sarcoma (analogous to endometrial stroma), and other heterologous cell types (*i.e.*, osteosarcoma, liposarcoma). Microscopically, most human uterine LMS are overtly malignant, with hypercellularity, coagulative tumor cell necrosis, abundant mitoses [>10 to 20 mitotic figures (mf) per 10 high power fields (hpf)], atypical mitoses, cytologic atypia, and infiltrative borders. Mitotic rate is the most important determinant of malignancy, but is modified by the presence of necrosis and cytologic atypia. The diagnosis of human uterine LMS may be made in the presence of tumor necrosis and any mitoses. In the absence of tumor necrosis, the diagnosis can be made with moderate to severe cytologic atypia and a mitotic index greater than 10mf/10hpf. Without tumor necrosis and significant atypia, a high mitotic index is compatible with a benign clinical course; however, data is limited [8,9].