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GESDAV

LMP2/β1i as a potential biomarker of human uterine mesenchymal tumors when combined with the candidate molecules, cyclin E and calponin h1

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ABSTRACT

Uterine leiomyosarcoma (Ut-LMS) develops more frequently in the myometrium of the uterine body than in the uterine cervix. Although, the development of gynecological tumors is often correlated with the secretion of female hormones that of Ut-LMS does not, and its risk factor(s) remain unknown. Importantly, a diagnostic biomarker that can distinguish malignant tumor Ut-LMS from benign tumor leiomyoma (LMA) has yet to be established. Therefore, the risk factor(s) associated with Ut-LMS need to be examined in order to establish a diagnosis and clinical treatment method. Mice with a homozygous deficiency for the proteasome β-ring subunit, low-molecular mass polypeptide (LMP) 2/β1i spontaneously develop Ut-LMS, with a disease prevalence of ~40% by 14 months of age. In a recent study, we showed that LMP2/β1i expression was absent in human Ut-LMS, but present in other human uterine mesenchymal tumors including uterine LMA. Moreover, LMP2/β1i is also known to negatively regulate human Ut-LMS tumorigenesis. Additional experiments furthermore revealed the differential expression of cyclin E and calponin h1 in human uterine mesenchymal tumors. Therefore, LMP2/β1i is a potential diagnostic biomarker when combined with the candidate molecules, cyclin E and calponin h1 for human Ut-LMS, and may be a targeted molecule for a new therapeutic approach.

KEY WORDS: Calponin h1, cyclin E, low-molecular mass polypeptide 2/β1i, uterine leiomyoma, uterine leiomyosarcoma

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 that envelops the uterus. The term uterine tumor generally refers to an epithelial malignant tumor in the uterus, which is roughly classified as a tumor of the uterine cervix or uterine body. Because of the prevalence of medical check-ups, the rate of mortality from uterine cervix malignant tumors is decreasing, and is commonly detected at a very early stage. In contrast, the mortality rate for malignant tumors of the uterine body is increasing, and this disease is 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 tumors and adenocarcinomas. Uterine mesenchymal tumors, that is, smooth muscle tumors (SMTs) that develop in the myometrium, have

been traditionally divided into benign uterine usual leiomyoma (LMA), cellular LMA, and malignant uterine leiomyosarcoma (Ut-LMS) based on cytological atypia, mitotic activity, and other criteria. Ut-LMS is relatively rare, having an estimated annual incidence of 0.64/100,000 women [1]. Ut-LMS accounts for 2∼5% of tumors of the uterine body and develops more frequently in the muscle layer of the uterine body than in the uterine cervix. Surgical intervention is virtually the only means of treatment because human Ut-LMS is resistant to chemotherapy and radiotherapy [2-4]. The prognosis for human Ut-LMS is poor, and the five-year survival rate is approximately 35% [5]. However, the development of an efficient adjuvant therapy is expected to improve the prognosis for human Ut-LMS. Uterine LMA may occur in 70∼80% of women by the age of 50 years [6]. Difficulties have been reported in distinguishing uterine LMA from human Ut-LMS, and a diagnosis generally requires surgery and cytoscopy [7]. Diagnostic categories for uterine SMTs and morphological criteria are used to assign cases [8,9](Note 1). The non-standard subtypes of uterine SMTs such as the epithelioid and myxoid types are classified in a different manner using these features; therefore, a diagnostic method that can identify non-standard smooth muscle differentiation needs to be established [8,9].

High estrogen levels have been shown to significantly influence the development of tumors in the uterine body [10-12]. Although the molecular mechanisms underlying the transformation of uterine LMA and human Ut-LMS transform remain unknown tumors that have been initiated and grown in the myometrium gradually increase in size due to the influence of the female hormone, estrogen, and generate more tumors. However, no correlation has been reported between the development of human Ut-LMS and hormonal conditions, and no obvious risk factors have been identified. Although cases accompanied by hypocalcaemia or eosinophilia have been reported, neither clinical abnormality is an initial risk factor for human Ut-LMS.

The proteasome is a cylindrical complex containing a core of four stacked rings around a central pore, with each ring being composed of seven individual proteins [13,14]. The inner two rings are made of seven β ring subunits that contain three to seven protease active sites [13,14]. The ubiquitin-proteasomal degradation pathway is essential for many cellular processes, including the cell cycle, regulation of gene expression, and onset of human disease [15-17]. Alternative β ring forms denoted as low-molecular mass polypeptide (LMP) 2/β1i can be expressed in the myometrium in response to exposure to pro-inflammatory signals such as cytokines, in particular, interferon (IFN)-γ [16]. Ut-LMS was reported in female LMP2/β1i-deficient mice at 6 months or older, with its incidence at 14 months of age being approximately 40% [18-20]. Determining the malignant potential of smooth muscle neoplasm also represents a significant diagnostic conundrum with important therapeutic ramifications. However, the genetic changes underlying the neoplastic transformation of uterine smooth muscle cells have not been fully characterized. Moreover, diagnostic biomarkers that are able to distinguish between human Ut-LMS and LMA have yet to be established. The identification of a risk factor and/or biological candidate(s) associated with the development of human Ut-LMS, that is, LMP2/β1i, may significantly contribute to the development of preventive and therapeutic treatments [19,20].

Development of Ut-LMS 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, and it is located in the nucleus and cytoplasm [13,14]. The ubiquitin-proteasome degradation pathway is essential for many cellular processes, including the cell cycle, regulation of gene expression, and immunological function [15]. IFN-γ induces the expression of a large number of responsive genes, the proteasome subunits, that is, LMP2/β1i, LMP7/β5i, and LMP10/β2i [16]. 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].

Recent studies demonstrated that LMP2/β1i was obligatory for tumor surveillance and played a tissue-specific role in protecting against spontaneous uterus neoplasms [18,19]. Homozygous mice deficient in LMP2/β1i show tissue-and substrate-dependent abnormalities in the biological functions of the proteasome [18,20]. Ut-LMS was reported in female LMP2/β1i-deficient mice at 6 months or older, with its the incidence at 14 months being approximately 40% [19,20][Figure 1].

The prevalence of Ut-LMS in mice is similar to that of human Ut-LMS, which develops after the menopause [19]. Pathological studies of LMP2/β1i-deficient uterine tumors have revealed the characteristic abnormalities of human Ut-LMS [19]. These tumors lack lymphoid infiltrates, a sign of immune recognition, and consist of uniform elongated myometrium cells arranged into bundles [Figure 1]. The nuclei of tumor cells vary in size and shape, and mitosis is frequent. The tumor consists of uniform elongated myometrium cells arranged into bundles. In contrast, the myometrium cells of its parental mice, C57BL/6 mice were shown to be normal in appearance [19,20]. Whereas relatively few Ki-67/MIB1 and cyclin E-positive cells, which are proliferating cells, have been reported in the basal cell layer of a normal myometrium, the expression of MIB1/Ki-67 and cyclin E was marked in most of the basal cells in LMP2/β1i-deficient mice [19] [Figure 1]. This immunochemical staining revealed the abnormal proliferation of LMP2/β1i-lacking cells in the basal layer. Marked body-weight loss has been reported in LMP2/β1i-deficient mice that develop Ut-LMS and these mice die by 14 months of age. They also potentially exhibit skeletal muscle metastasis from Ut-LMS [21]. Therefore, these research findings suggest that LMP2/β1i-deficient mice with Ut-LMS die as a result of tumor growth and metastasis. It is generally not easy to distinguish uterine LMA from human Ut-LMS; however, because of such characteristic pathological findings

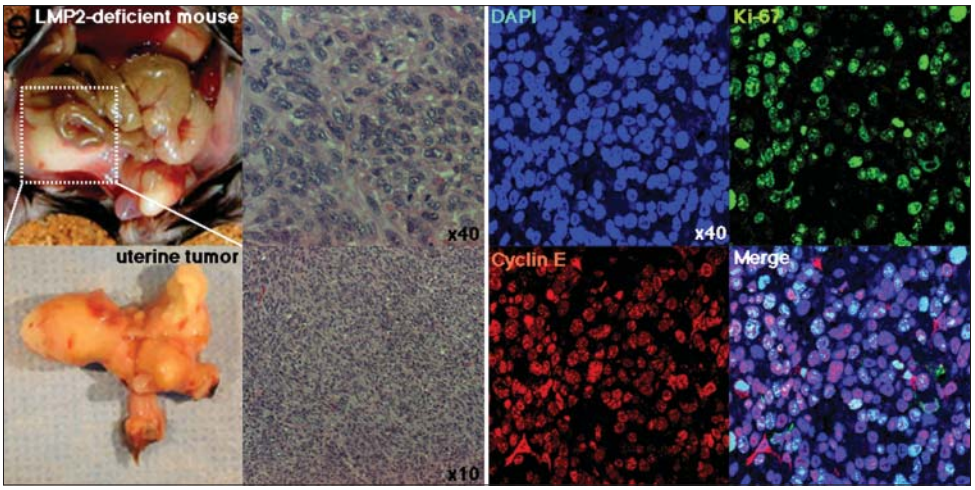

Figure 1: Homozygous mice deficient in low-molecular mass polypeptide (LMP) 2/β1i, an interferon (IFN)-γ-inducible factor, show tissue-and substrate-dependent abnormalities in the biological functions of the proteasome [19,20]. Uterine leiomyosarcoma (Ut-LMS) has been reported occurred in female LMP2/β1i-deficient mice at 6 months or older, with its incidence at 14 months of age being approximately 40% [19,20]. Pathological studies of LMP2/β1i-deficient uterine tumors have revealed the characteristic abnormalities of human Ut-LMS [19]. The expression of Ki-67/MIB1 and cyclin E was shown to be marked in the basal cells in LMP2/β1i-deficient mice vividly expressed [19,20]

in mice, including significant body-weight loss and skeletal muscle metastasis, a tumor that develops in the uterus of a LMP2/β1i-deficient mouse can be considered malignant, that is, Ut-LMS [19-21].

Defective LMP2/β1i expression in human Ut-LMS

The non-standard subtypes of uterine SMTs such as the epithelioid and myxoid types are classified in a different manner using these features; therefore, a diagnostic method that can identify non-standard smooth muscle differentiation needs to be established [7-9]. Immunohistochemistry (IHC) studies have been performed to demonstrate the validity and reliability of LMP2/β1i as a diagnostic biomarker when combined with other candidate molecules, such as cyclin E and calponin h1, which reportedly function as anti-tumorigenic factors in human Ut-LMS [Table 1]. IHC studies revealed a serious loss in the ability to induce the expression of LMP2/β1i and calponin h1 in human Ut-LMS tissues in comparison with uterine LMA or a normal myometrium located in the same section, and the expression of cyclin E was higher in human Ut-LMS tissues only [22,23]. The 54 cases of human Ut-LMS were examined in our previous studies, 46 cases were negative for the expression of LMP2/1i, 4 cases were focally positive, and 2 cases were partially positive [23]. Two human Ut-LMS cases were stained for LMP2/β1i. The expression levels of LMP2/β1i were also evaluated in skeletal muscle and rectum metastases from individual human Ut-LMS patients [23]. Pathological studies of surgical samples revealed the presence of a mass measuring 3 cm at its largest diameter in the lumbar quadrate muscle without a fibrous capsule. All lymph nodes were negative for human Ut-LMS metastases, and IHC analyses were positive for Ki-67/MIB1 and negative for LMP2/β1i [23]. Histological findings for the skeletal muscle and rectum lesions were consistent with metastatic LMS. Western blotting and RT-PCR experiments revealed that LMP2/β1i was expressed in a normal myometrium, but not in human Ut-LMS, and both findings strongly supported the IHC results [22,23][Figure 2].

Further experiments demonstrated the differential expression of cycline E and calponin h1 in human uterine mesenchymal tumors [22-24]. To increase the incidence of tumors and better assess the role of the systemic expression of TP53 in response to the initiation of uterine LMS tumorigenesis, LMP2/β1i-deficient mice were bred with TP53-deficient mice to create LMP2−/− Tp53−/−double knockout mice. The incidence of Ut-LMS and death rates were similar between Lmp2−/−Tp53−/−mice and closely matched those for control LMP2−/−Tp53+/+ mice. The correlation between defective TP53 function and Ut-LMS tumorigenesis remains unclear. Although we previously demonstrated that abnormal expression of the ovarian steroid receptors, TP53 and MIB1/Ki-67, and mutations in TP53 were frequently associated with human Ut-LMS, defective LMP2/β1i expression appears to be more characteristic of Ut-LMS than any of these factors [22-24][Table 1].

A female hormonal imbalance is often a risk factor for the development of tumors in gynecological cancers, such as breast cancer [10-12]. However, a correlation between the development of human Ut-LMS, the female hormone, and hormone receptors has been unclear in uterine LMA. A recent study reported the expression of LMP2/β1i mRNA and protein in luminal and glandular epithelia, placenta villi, trophoblastic shells, and arterial endothelial cells [25-27]. These findings implicate LMP2/β1i in the invasion of placental villi, degradation of the extracellular matrix, immune tolerance, glandular secretion, and angiogenesis [25-27]. Further studies should help to elucidate the regulatory role of LMP2/β1i in the implantation of embryos [25-27]. The LMP2/β1i-deficient mouse was the first animal model of spontaneous Ut-LMS to be established [19,22,23]. LMP2/β1i was recently shown reported to negatively regulate human Ut-LMS independently of its role in the proteasome [28-31]. Moreover, several lines of evidence indicate that the calcium

Table 1: Expression of cyclin E, ER, PR, Ki-67, p53, LMP2, and calponin h1 in human uterine leiomyosarcoma

Patient number	Age in years	Immunohistochemical staining								Somatic mutations					Follow-up (months)
		TMN stage	MF	Cy.E	ER	PR	Ki-67	p53	LMP2	Cal	P53	JAK1	JAK2	STAT1	LMP2 pro	
1	37	T4N1M0	97	++	−	−	3000	+++	−	−	SM	ND	ND	ND	ND	D (1)
2	58	T3N0M0	24	++	−	−	3500	+	±	−	SM	ND	ND	ND	SM	D (23)
3	45	T2N0M0	32	++	±	±	2150	+++	−	−	SM	M	ND	SM	SM	D (24)
4	65	T1N0M0	30	++	±	±	1700	+++	−	−	SM	M	ND	ND	ND	D (20)
5	52	T1N0M0	107	+	−	+	2600	++	+	−	ND	M	ND	ND	ND	D (13)
6	49	T1N0M0	46	++	−	−	4300	+	−	−	ND	ND	ND	ND	ND	D (24)
7	55	T1N0M0	75	++	−	−	4000	+++	−	−	ND	ND	ND	SM	SM	D (18)
8	43	T3N0M0	57	++	+	−	2000	−	±	±	ND	ND	ND	ND	ND	D (10)
9	67	T1N0M0	13	++	−	±	1430	−	−	−	ND	M	ND	ND	ND	A (34)
10	67	T1N0M0	37	++	−	−	2100	−	−	−	ND	ND	ND	SM	SM	A (15)
11	51	T1N0M0	93	++	−	−	4500	−	−	−	ND	ND	ND	SM	ND	A (94)
12	48	T1N0M0	14	++	−	−	900	+++	+	+	ND	ND	ND	ND	ND	A (58)
13	51	T1N0M0	22	++	±	+	450	+	−	−	ND	M	ND	ND	SM	A (34)
14	67	T1N0M0	64	+	−	+	1450	++	−	−	ND	ND	ND	ND	ND	A (15)
15	52	T1N0M0	65	++	−	−	1780	++	−	−	ND	M	ND	SM	ND	D (23)
16	42	T3N0M0	73	++	−	−	2130	++	−	−	ND	ND	ND	ND	SM	A (21)
17	80	T1N0M0	98	++	−	−	1980	+++	−	−	ND	M	ND	ND	ND	D (19)
18	56	T1N0M0	78	++	−	−	1860	++	−	−	ND	ND	ND	ND	ND	A (11)
19	58	T1N0M0	40	++	−	−	1750	++	−	−	ND	ND	ND	ND	ND	A (10)
20	65	T2N0M0	67	++	−	−	780	+++	−	−	ND	M	ND	ND	SM	A (12)

Cy.E: Cyclin E, ER: Estrogen receptor, PR: Progesterone receptor, Cal: Calponin h1, Ki-67: Positive cell number/10 high power fields, SM: Somatic mutation, ND: Not detected, D: Died of the disease, A: Alive, MF: Mitotic figure/10 high power fields, CCN: Coagulative cell necrosis was observed in all samples tested

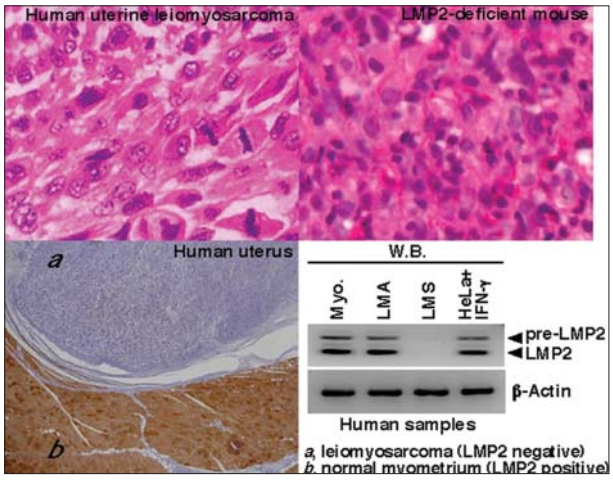

Figure 2: Defective low-molecular mass polypeptide (LMP) 2/β1i expression in human uterine leiomyosarcoma. H and E stained tissue sections of uterine leiomyosarcoma (Ut-LMS) of LMP2/β1i-deficient mouse and patient. Immunohistochemically stained tissue section of human Ut-LMS with an anti-human LMP2/β1i monoclonal antibody. Extracts of 50 μg were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. LMP2 and β-actin levels were examined by western blot analysis with appropriate antibodies. Myo: Human myometrium, LMA: Human leiomyoma, LMS: Human leiomyosarcoma, HeLa + IFN-γ; HeLa cells treated with IFN-γ

binding protein, calponin h1 clearly affects LMP2/β1i-induced cellular morphological changes [30,31]. Further experiments are also required to elucidate the molecular mechanism of human Ut-LMS tumorigenesis and biological significance of LMP2/β1i. To demonstrate whether LMP2/β1i is a potential biomarker that can distinguish human Ut-LMS from uterine LMA when combined with other candidate molecules, especially cyclin E and calponin h1, which have been identified as potential diagnostic candidates [28-31], we are currently investigating the reliability and characteristics of LMP2/β1i as a diagnostic indicator in several clinical research facilities [28]. This clinical research has not yet been concluded, and large-scale clinical studies need to be performed in additional clinical research facilities. The histological and IHC characteristics of uterine mesenchymal tumors including mitotically active LMA, bizarre LMA, lipoleiomyoma, uterine SMTs of uncertain malignant potential, and leiomyomatoid angiomatous neuroendocrin tumor have been summarized already [32-37]. Clarifying correlations between these factors and the development of human Ut-LMS and the identifying of specific risk factors may lead to the development of new clinical treatments for the disease.

FINAL CONSIDERATIONS

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

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