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Review Article

Differential expression of LMP2/β1i: As a potential biomarker of human uterine mesenchymal tumors*

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Abstract

Uterine leiomyosarcoma (Ut-LMS) develops more often in myometrium 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 Ut-LMS is not substantially correlated with hormonal conditions, and the risk factor(s) are not yet known. Importantly, a diagnostic-biomarker, which distinguishes malignant tumor Ut-LMS from benign tumor leiomyoma (LMA), is yet to be established. Accordingly, it is necessary to examine risk factor(s) associated with Ut-LMS, to establish a clinical treatment method. The mice with a homozygous deficiency for proteasome subunit, low-molecular mass polypeptide (LMP) 2/β1i spontaneously develop Ut-LMS, with a disease prevalence of ~40% by 14 months of age. In the recent study, we found LMP2/β1i expression to be absent in human Ut-LMS, but present in human uterine LMA. Moreover, LMP2/β1i is reported to negatively regulate human Ut-LMS tumorigenesis. Therefore, LMP2/β1i is a potential diagnostic-biomarker for human Ut-LMS, and may be a targeted-molecule for a new therapeutic approach.

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 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 (derived from the subintimal gland), tumors of the uterine cervix are classified into squamous cell carcinoma and adenocarcinoma. Uterine mesenchymal tumors, i.e. smooth muscle tumors (SMTs), which develop in the myometrium, have been traditionally divided into benign uterine 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 human Ut-LMS is resistant to chemotherapy and radiotherapy, surgical intervention is virtually the only means of treatment [2, 3, 4]. The prognosis for human Ut-LMS is not good, and the five-year survival rate is approximately 35% [5]. However, developing an efficient adjuvant therapy is expected to improve the prognosis for human Ut-LMS. Uterine LMA may occur in as many as 70%~80% of women by the age of 50 years [6]. Distinguishing uterine LMA from human Ut-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] (Note 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, 11, 12]. The molecular mechanisms by which uterine LMA and human 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 human Ut-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 human Ut-LMS.

The proteasomal degradation pathway is essential for many cellular processes, including the cell cycle and the regulation of gene expression. In structure, the proteasome is a cylindrical complex containing a core of four stacked rings around a central pore, each ring composed of seven individual proteins. The inner two rings are made of seven *β subunits* that contain three to seven protease active sites. Alternative β forms denoted LMP2/β1i can be expressed in the myometrium in response to exposure to pro-inflammatory signals such as cytokines, in particular, IFN-γ. Ut-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%. The determination of 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, i.e. LMP2/β1i, would significantly contribute to the development of preventive and therapeutic treatments.

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 the cytoplasm [13, 14]. The ubiquitin proteasome degradation pathway is essential for many cellular processes, including the cell cycle, the regulation of gene expression and immunological function [15]. Interferon (IFN)-γ induces the expression of large numbers of responsive genes, proteasome subunits, i.e., 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 reports demonstrate LMP2/β1i as obligatory for tumor surveillance and a tissue- specific role for LMP2/β1i in protection from 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 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 1). The disease prevalence in mice is similar to that of human Ut-LMS, which occurs after menopause [19]. Pathological studies of LMP2/β1i- deficient uterine tumors have revealed characteristic abnormalities of human Ut-LMS [19]. The tumors lacked lymphoid infiltrates, a sign of immune recognition, and consisted of uniform elongated myometrium cells arranged into bundles (Figure 1). The nuclei of the tumor cells varied in size and shape, furthermore, mitosis was frequent. The tumor consisted of uniform elongated myometrium cells arranged into bundles. In contrast, the myometrium cells of C57BL/6 mice were normal in appearance [19, 20]. Whereas relatively few MIB-1(Ki-67) positive cells, the proliferating cells, were observed in the basal cell layer of the normal myometrium, most of the basal cells in LMP2/β1i-deficient mice vividly

expressed MIB-1 (Ki-67) [19] (Figure 1). This immunological staining indicates abnormal proliferation of the LMP2/β1i-lacking cells in the basal layer. LMP2/β1i-deficient mice that have developed Ut-LMS undergo considerable weight loss, and then die by 14 months of age. They also potentially exhibit skeletal muscle metastasis from the Ut-LMS [21]. Therefore it is likely that LMP2/β1i-deficient mice with Ut-LMS die as a result of tumor growth and metastasis. In general, it is not easy to distinguish uterine LMA from human Ut-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., an Ut-LMS [19, 20].

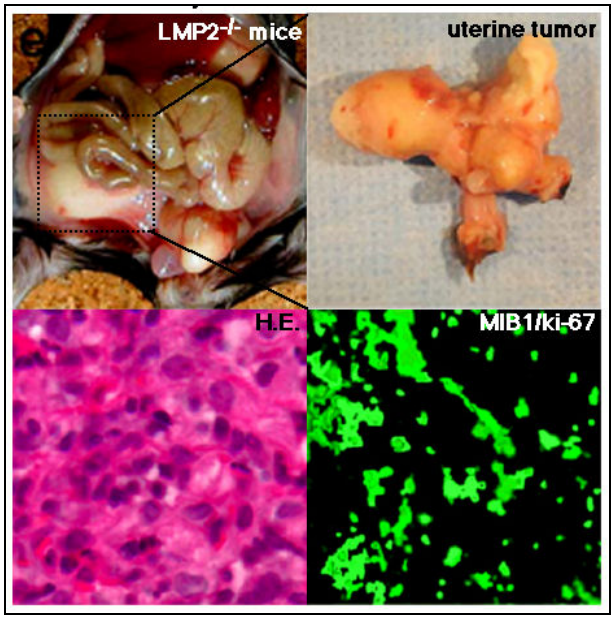

Figure 1. Uterine leiomyosarcoma in LMP2-deficient mice. Homozygous mice deficient in LMP2/β1i, an interferon (IFN)-γ-inducible factor, show tissue- and substrate-dependent abnormalities in the biological functions of the proteasome [19,20]. Ut-LMS reportedly occurred in female LMP2/β1i -null mice at age 6 months or older, and the incidence at 14 months of age was about 40% [19,20].

LOSS OF 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 way using these features, so the establishment of a diagnostic method for the identification of non-standard smooth muscle differentiation is important [7, 8, 9]. Immunohistochemistry (IHC) studies were performed to demonstrate the validity and reliability of LMP2/β1i 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 (Table 1, Table 2). IHC studies revealed a serious loss in the ability to induce LMP2/β1i and calponin h1 expressions in human Ut-LMS tissue in comparison with LMA or normal myometrium located in the same section [22, 23]. Of the 54 cases we examined with human Ut-LMS, 46 were negative for LMP2/β1i expression, 4 were focally positive, and 2 were partially positive [23]. Two human Ut-LMS cases were stained for LMP2/β1i. LMP2/β1i levels were also evaluated in skeletal muscle and rectum metastases from individual human Ut-LMS patients [23]. 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 human Ut-LMS metastases, and IHC analyses showed positivity for MIB1/ki-67 and negativity for LMP2/β1i [23]. 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 Ut-LMS, both strongly supportive of the IHC findings [22, 23]. To increase tumor incidence and better assess the role of systemic expression of TP53 in responses to initiation of uterine LMS tumorigenesis, LMP2/β1i-deficient mice were bred with TP53-deficient mice to create *Lmp2-/-Tp53-/-* double knockout mice. Uterine LMS incidence and death rates were similar in *Lmp2-/-Tp53-/-* mice and closely matched those for control *Lmp2-/-Tp53+/+* mice. The correlation between defective TP53 function and uterine LMS tumorigenesis is unclear. Although we previously demonstrated that the abnormal expression of ovarian steroid receptors, TP53 and ki-67 and mutations of 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).

In the case of gynecological cancers, such as breast cancer, a female hormonal imbalance is often a risk factor for developing tumors [10, 11, 12]. As in the case of uterine LMA, however, a correlation between the development of human Ut-LMS, the female hormone, and hormone receptors has been unclear. A recent report showed the expression of *Lmp2/β1i* mRNA and protein in luminal and glandular epithelia, placenta villi, trophoblastic cells, and arterial endothelial cells [25, 26, 27]. These results implicate LMP2/β1i in the invasion of placental villi, degradation of the extracellular matrix, immune tolerance, glandular secretion, and angiogenesis [25, 26, 27]. Further study should help to elucidate the regulatory role of LMP2/β1i in the implantation of embryos [25, 26, 27]. The LMP2/β1i-deficient mouse was the first animal

model of spontaneous Ut-LMS to be established [19, 22, 23]. In the recent studies, LMP2/β1i is reported to negatively regulate human Ut-LMS independently of its role in the proteasome [28, 29, 30, 31]. Moreover, several lines of evidence indicate that calcium binding protein, calponin h1 clearly affects LMP2-induced cellular morphological chances [30, 31]. Further experiments are also required to elucidate the molecular mechanism of human Ut-LMS tumorigenesis involved biological significance of LMP2/β1i. To demonstrate whether LMP2/β1i is a potential biomarker for distinguishing human Ut-LMS from uterine LMA under the combination with other candidate molecules, especially cyclin E and calponin h1 which are identified as potential diagnostic candidates [28, 29, 30, 31]. We are investigating the reliability and characteristics of LMP2/β1i as a diagnostic indicator with several clinical research facilities [28]. The clinical research is yet to be concluded, and large-scale clinical studies need to be performed with additional clinical research facilities.

Table 1. Expression of ER, PR, p53, Ki-67, p53, LMP2, and Calponin h1 in human uterine leiomyosarcoma.

Patient No.	Age in yrs	Immunohistochemical staining									Somatic mutations					Follow Up (months)
		TMN stage	MF	CCN	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)

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 disease; A, alive; MF, mitotic figure/10 high power fields; CCN, coagulative cell necrosis.

Table 2. Classification of uterine mesenchymal tumours.

Tumor type	Atypia	Mitotic activity	Necrosis	Protein expression*													Clinical comments
				Cyt	Des	MSA	SMA	Vim	ER/PR	End	EGFC	yB	CyE	LMP2	Cal	Ki67	
Endometrial stromal tumors.																	
Endometrial stromal nodule	minimal	infrequent	-/inconspicuous	+	-	+	+	+	+++	+	*	+	-	++	++	-	Absence of myometrial infiltration
Endometrial stromal sarcoma	-	infrequent	-/inconspicuous	-/+	-	+	+	+	+++	+	+	+	-	-/+	++	-/+	
Undifferentiated endometrial sarcoma	marked	Frequent (atypical MF)	+	-/+	foc	*	*	-	-	+	+	+	+	-/+	+	+	Lack specific differentiation
Smooth muscle tumors																	
Leiomyoma, NOS	-	<5 MF/10HPF	-	foc	+	+	+	*	+++	-/+	-/+	+	-	++	++	-/+	Well-circumscribed
Mitotically active leiomyoma	-	>5 MF/10HPF	-	*	+	+	+	*	+++	-/+	-/+	+	-	++	++	-/+	Pseudocapsul
Cellular leiomyoma	-	infrequent	-	*	+	+	+	*	+++	-/+	-/+	+	-	++	++	-/+	Increased cellularity
Hemorrhagic cellular leiomyoma	-	infrequent	-	*	+	+	+	*	+++	-/+	-/+	+	-	++	++	-/+	Hormone induced changes
Epithelioid leiomyoma	-	<5 MF/10HPF	-	*	+	+	+	*	+++	-/+	-/+	*	-	++	++	-/+	Epithelial-like cells
Myxoid leiomyoma	-	<5 MF/10HPF	-	*	*	*	*	*	*	-/+	-/+	*	+	-/+	+	+	Myxoid material
Atypical leiomyoma	moderate	<10 MF/10HPF	-	-	+	+	+	*	+++	+	-/+	+	+	-/+	-/+	+	Separates tumor cells
	-	infrequent	-	*	+	+	+	*	+++	*	-/+	*	+	-/+	*	-/+	
	-	infrequent	-	*	+	+	+	*	*	*	-/+	*	-/+	-/+	*	-/+	
Lipoleiomyoma	-/+	>10 MF/10HPF	+/uncertain	*	+	+	+	*	*	*	-/+	*	-/+	-/+	*	-/+	Scattered adipocytes
STUMP#	Marked	borderline	-	*	+	+	+	*	*	*	-/+	*	-/+	-/+	*	-/+	
	-	infrequent	+/difficult classify	*	+	+	+	*	*	*	-/+	*	-/+	-/+	*	-/+	
Leiomyosarcoma	moderate	>10 MF/10HPF	+	+	+	*	-/+	-	-	+	-/+	++	+++	-	-	++	Infiltrative
Leiomyosarcoma epitheliod variant	moderate	>5 MF/10HPF	+	+	+	*	-/+	-	-	+	-/+	++	+++	-	-	++	Infiltrative, >50% epithelioid cells
Leiomyosarcoma myxoid variant	moderate	Any MF	+	+	+	*	-/+	-	-	+	-/+	++	++	-	-	++	Infiltrative, myxoid extracellular matrix
Leiomyomatoid tumor																	
LANT#	absent	frequent	+	+	-	-	+	+	*	*	+	+	++	-	-	-/+	NOTE1

*insufficient data or not applicable.
Cyt., cytokeratin; Des., Desmin; MSA, muscle specific actin; SMA, smooth muscle actin; Vim., vimentin; ER/PR, estrogen receptor/progesterone receptor; End., Endoglin, CD105/TGFb receptor (stem cell marker); EGF, EGFR, epidermal growth factor receptor; CyB, cyclin B1; CyE, cyclin E, LMP2, low-molecular mass polypeptide; Cal., calponin h1; CD56, neural cell adhesion molecule (N-CAM); WT-1, Wilms tumor 1; NOS, not otherwise specified; MF, magnification factor; HPF, high power field; Foc., focal; STUMP, smooth muscle tumors of uncertain malignant potential. Protein expression*, estimated-protein expressions by immunoblot analysis, immunohistochemistry (IHC) and/or RT-PCR (quantitative-PCR), +/-, partial expression; +, expression; ++, medium expression; +++, high expression; -, no evidence of expression; ER/PR [24], LMP2 [22, 23], cyclin E [24, 32], calponin h1 [29, 30, 31], Ki-67 [24, 33]. STUMP# [33, 34]. Cyclin E, LMP2, calponin h1 are potential bio-marker for human uterine mesenchymal tumours. LANT#, leiomyomatoid angiomatous neuroendocrin tumor (LANT) is described as a dimorphic neurosecretory tumor with a leiomyomatous vascular component [35, 36]. NOTE1, Low-grade neuroendocrine tumor possibly related to null cell adenoma.

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 in Table 2. 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.

CONCLUSION

Human Ut-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 Ut-LMS as it is in the LMP2/β1i-deficient mouse. LMP2/β1i might function as a tumor suppressor in human Ut-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 Ut-LMS.

Note 1: The typical gross appearance is a large (>10cm), 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 human Ut-LMS (analogous to myometrium), stromal sarcoma (analogous to endometrial stroma), and other heterologous cell types (i.e., osteosarcoma, liposarcoma). Microscopically, most human Ut-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 Ut-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].

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