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Identification of Novel Biomarker for Human Uterine Leiomyosarcoma

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Abstract

Sarcomas are neoplastic malignancies that typically arise in tissues of mesenchymal origin. The identification of novel molecular mechanisms leading to sarcoma formation and the establishment of new therapies has been hampered by several critical factors. Human uterine leiomyosarcoma (Ut-LMS) develops more frequently in the muscle tissue layer of the uterine body than in the uterine cervix. Although the development of gynecologic tumors is often correlated with the secretion of female hormones; that of human Ut-LMS does not and its risk factors remain unknown. Importantly, a diagnostic biomarker that can distinguish malignant Ut-LMS from benign tumor uterine leiomyoma (LMA) has yet to be established. Therefore the risk factor(s) associated with human Ut-LMS to establish a diagnosis and novel therapeutic method. Proteasome *b-ring* subunit LMP2/b1i-deficient mice spontaneously develop Ut-LMS, with a disease prevalence of ~40% by 14 months of age. We shown that LMP2/b1i expression was absent in human Ut-LMS, but present in other human uterine mesenchymal tumors including uterine LMA. Therefore, defective-LMP2/b1i expression may be one of the risk factors for human Ut-LMS. LMP2/b1i is a potential diagnostic biomarker for human Ut-LMS, and may be a targeted-molecule for a new therapeutic approach.

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Background

Sarcomas are a rare form of malignant tumor with less than 15,000 new cases diagnosed each year in United States. Though rare, sarcomas are highly debilitating malignancies as they are often associated with significant morbidity and mortality. Sarcomas are biologically very heterogeneous as evidenced by the fact that these malignant tumors arise from a plethora of different tissues and cell types. They are classically defined by their tissue of origin and additionally stratified by their histopathology or patient's age at diagnosis. While most tumors of the uterine body are adenocarcinomas, tumors of uterine cervix are classified into squamous tumors and adenocarcinomas. Uterine mesenchymal tumors that develop in the myometrium have been traditionally divided into benign uterine usual LMA, cellular 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% ~ 5% of tumors of the uterine body and develops more frequently in the muscle layer of the uterine body than in the uterine cervix. As Surgical intervention is virtually the only means of treatment, because Ut-LMS is resistant to chemotherapy and radiotherapy.[2,3] The prognosis for Ut-LMS is poor, and the five-year survival rate is approximately 35%. 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.[4] Difficulties have been reported in distinguishing Ut-LMS from other uterine mesenchymal tumors including uterine LMA, and a diagnosis generally requires surgery and cytoscopy. 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 manner using these features; therefore a diagnostic method that can identify non-standard smooth muscle differentiation needs to be established.[5,6]

High estrogen levels have been shown to significantly influence the development of tumors in the uterine body.[7] Although the molecular mechanisms underlying the transformation of uterine LMA and Ut-LMS develop remain unknown. The tumors that have been initiated and grown in the myometrium 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 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 identification of a risk factor associated with the development of human Ut-LMS would significantly contribute to the development of preventive and therapeutic treatments.

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, regulation of gene expression, and immunological function.[10] Interferon (IFN)-g treatment induces the expression of large numbers of responsive genes, the *b-ring* subunits of proteasome, i.e., low-molecular mass polypeptide (LMP)2/b1i, LMP7/b5i, and LMP10/multicatalytic endopeptidase complex-like (MECL)-1/b2i.[11] A molecular approach to studying the correlation of IFN-g with tumor cell growth has drawn attention. Homozygous mice deficient in LMP2/b1i show tissue- and substrate-dependent abnormalities in the biological functions of the proteasome.[12] Ut-LMS was reported in female LMP2/b1i-deficient mice at 6 months or older, with its incidence at 14 months being approximately 40%.[13] Histopathological studies of LMP2/b1i-lacking uterine tumors have revealed the characteristic abnormalities of Ut-LMS.[13] These tumors consist of uniform elongated myometrium cells arranged into bundles. The nuclei of tumor cells vary in size and shape, and mitosis is frequent. In contrast, the myometrium cells of its parental mice, C57BL/6 mice were shown to be normal in appearance. Whereas relatively few ki-67-positive cells, the proliferating cells of solid tumors, have been reported in the basal cell layer of a normal myometrium, the expression of ki-67/MIB1 was marked in most basal cells in LMP2/b1i-deficient mice. [13] Marked body weight loss has been reported in LMP2/b1i-deficient mice that develop Ut-LMS, and these mice die by 14 months of age. The LMP2/b1i-deficient mice also exhibit skeletal muscle

metastasis from Ut-LMS. Therefore, these research findings suggest that LMP2/b1i-deficient 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 manner using these features; therefore, a diagnostic method that can the identify non-standard smooth muscle differentiation needs to be established.[5,6] Pathological studies have been performed to demonstrate the validity and reliability of LMP2/b1i as a diagnostic biomarker when combined with other candidate molecules, such as cyclin E and calponin h1, which reportedly function as anti-oncogenic factors in human Ut-LMS. Pathological examinations revealed that the ability to induce the expression of LMP2/b1i and calponin h1 was markedly lower in human Ut-LMS tissues than in uterine LMA or a normal myometrium located in the same section, and markedly expression of cyclin E in human Ut-LMS tissues only.[14-17] Histological findings for the skeletal muscle and rectum lesions were consistent with metastatic Ut-LMS.[14,15] Western blotting and RT-PCR experiments revealed that LMP2/b1i was expressed in a normal myometrium, but not in human Ut-LMS, and both findings strongly supported the pathological results.[14,15,17,18] Although we has previously demonstrated that abnormal expression of the ovarian steroid receptors, Tp53, ki-67 and mutations in Tp53 were frequently associated with Ut-LMS, defective LMP2/b1i expression appears to be more characteristic of human Ut-LMS than these factors.[15,16]

A female hormonal imbalance is often a risk factor for development of tumors in the case of gynecological cancers.[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. Recent study reported the expression of *Lmp2/b1i* mRNA and protein in luminal and glandular epitheliua, placenta villi, trophoblastic shells, and arterial endothelial cells.[19-21] These findings implicate LMP2/b1i in the invasion of placental villi, degradation of the extracellular matrix, immune tolerance, glandular secretion, and angiogenesis, but no more information for sarcomagenesis. Further studies should help to elucidate the molecular mechanism of human Ut-LMS tumorigenesis involved biological significance of LMP2/b1i; we are currently investigating the reliability and characteristics of LMP2/b1i as a diagnostic indicator with several clinical research facilities. Histopathological characteristics of human uterine mesenchymal tumors including mitotically active leiomyoma, bizarre leiomyoma, lipoleiomyoma, undifferentiated endometrial sarcoma, epithelioid variant leiomyosarcoma, myxoid variant leiomyosarcoma, smooth muscle tumors of uncertain malignant potential (STUMP), leiomyomatoid angiomatous neuroendocrin tumor (LANT) have been summarized already [22,23,24,25] (Table 1, Table 2). Clarifying correlations between these factors and the development of human Ut-LMS and the identifying specific risk factors may lead to the development of new clinical treatments for the disease.

	Age	n	LMP2 expression			
			-	-/+1	focal+2	+++3
Normal	32~83	59				57
Leiomyoma (Ordinaly leiomyoma) (Cellular leiomyoma) (Tumor of uncertain malignantpotential)	33~83	53 (32) (9) (12)				53
Bizarre Leiomyoma	44,49,55	3				3
Leiomyosarcoma	32~83	59	51	2	4	2

-/+1: partially positive (5% to 10% of cells stained), focal+2: focal-positive (focal or sporadic staining with less than 5% of cells stained), +++3: diffuse-positive (homogeneous distribution with more than 90% of cells stained), -: negative (no stained cells).

Table 2. LMP2 expression levels in human myometrium, uterine leiomyoma (Ordinaly leiomyoma, Cellular leiomyoma, Tumor of uncertain malignant potential), bizarre leiomyoma, uterine leiomyosarcoma. IHC experiments individually performed at several medical facilities revealed a serious loss in the ability to induce LMP2/b1i expression in human uterine leiomyosarcoma tissues compared to that in uterine leiomyoma or bizarre leiomyoma located in the same tissue section. Normal total: 59 cases, uterine leiomyoma total: 53 cases, Bizarre leiomyoma total: 3 cases, uterine leiomyosarcoma total: 59 cases.

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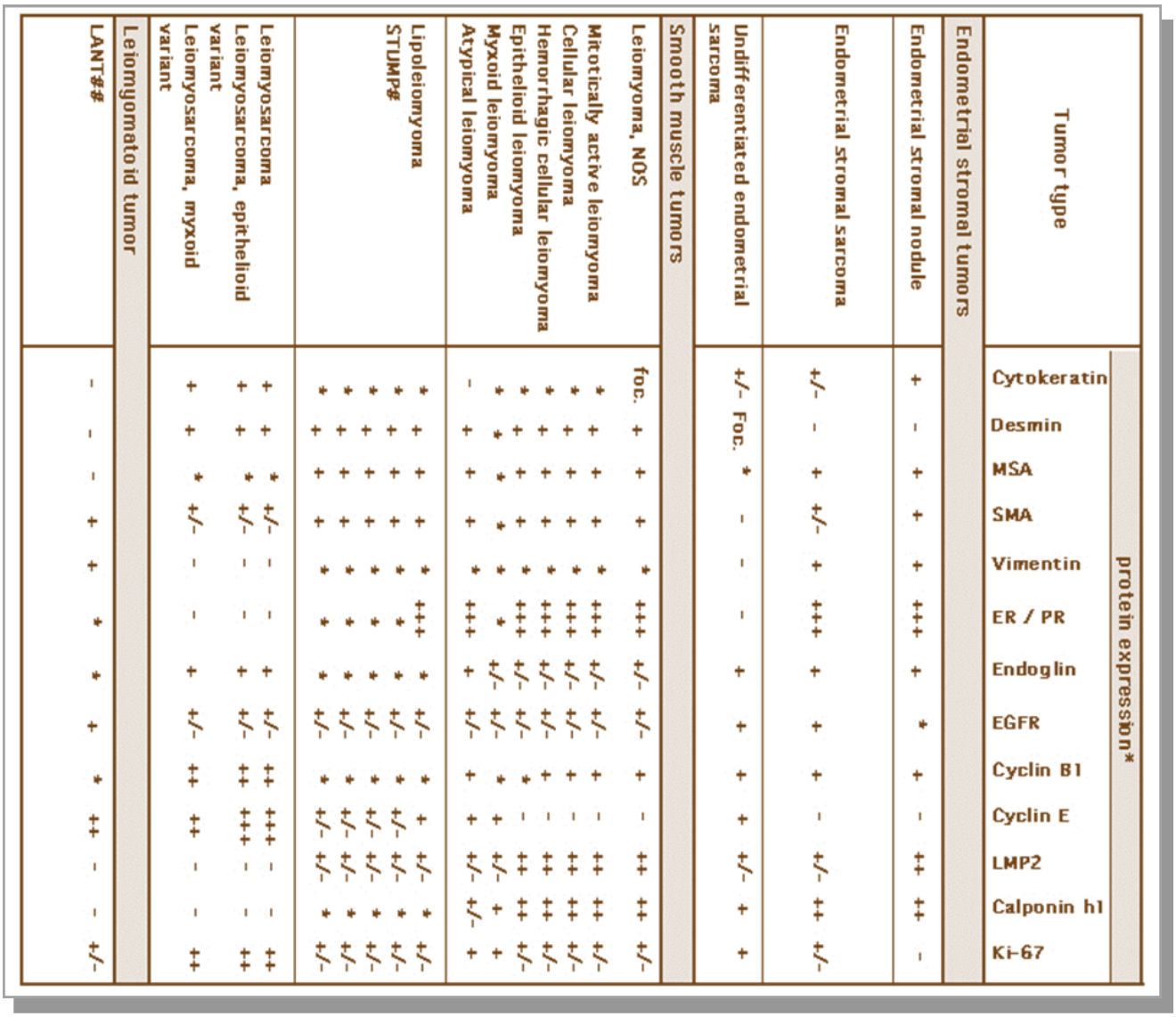

Tumor type	protein expression*												
Endometrial stromal tumors													
Endometrial stromal nodule	+	-	+	+	+	+++	+	*	+	-	++	++	-
Endometrial stromal sarcoma	+/-	-	+	+/-	+	+++	+	+	+	-	+/-	++	+/-
Undifferentiated endometrial sarcoma	+/- Foc.	*	-	-	-	-	+	+	+	+	+/-	+	+
Smooth muscle tumors													
Leiomyoma, NOS	foc.	+	+	+	*	+++	+/-	+/-	+	-	++	++	+/-
Mitotically active leiomyoma	*	+	+	+	*	+++	+/-	+/-	+	-	++	++	+/-
Cellular leiomyoma	*	+	+	+	*	+++	+/-	+/-	+	-	++	++	+/-
Hemorrhagic cellular leiomyoma	*	+	+	+	*	+++	+/-	+/-	+	-	++	++	+/-
Epithelioid leiomyoma	*	+	+	+	*	+++	+/-	+/-	+	-	++	++	+/-
Myxoid leiomyoma	*	*	*	*	*	*	+/-	+/-	+	-	+/-	+	+/-
Atypical leiomyoma	-	+	+	*	*	+++	+	+/-	+	+	+/-	+	+/-
Lipoleiomyoma STUMP#	*	*	+	*	*	+++	*	+/-	+	+	+/-	+	+/-
	*	*	*	*	*	*	*	+/-	+	+	+/-	*	+/-
	*	*	*	*	*	*	*	+/-	+	+	+/-	*	+/-
	*	*	*	*	*	*	*	+/-	+	+	+/-	*	+/-
	*	*	*	*	*	*	*	+/-	+	+	+/-	*	+/-
	*	*	*	*	*	*	*	+/-	+	+	+/-	*	+/-
	*	*	*	*	*	*	*	+/-	+	+	+/-	*	+/-
Leiomyosarcoma Leiomyosarcoma, epithelioid variant Leiomyosarcoma, myxoid variant	+	+	*	+/-	-	-	+	+/-	++	-	-	-	++
	+	+	*	+/-	-	-	+	+/-	++	+++	-	-	++
	+	+	*	+/-	-	-	+	+/-	++	-	-	-	++
Leiomyomatoid tumor													
LANT##	-	-	+	+	+	*	*	+	*	++	-	-	+/-

Table 1. Differential protein expression in Uteriene tumours

SMA, smooth muscle actin; MSA, muscle specific actin; ER/PR, estrogen receptor/ progesterone receptor; Endoglin, CD105/TGFb receptor (stem cell marker); EGFR, epidermal growth factor receptor; LMP2, low-molecular mass polypeptide; 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(ref.16), LMP2(ref.14,15), cyclin E(ref.16,28), calponin h1(ref.17,26,27), Ki-67(ref.16,29). STUMP#(ref.29,30). Cyclin E, LMP2, calponin h1 are potential bio-marker for human uterine mesenchymal tumours. LANT##, leiomyomatoid angiomatous neuroendocrin tumor (LANT) is desvribed as a dimorphic neurosecretory tumor with a leiomyomatous vascular component (ref.22,23).

Competing interests

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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