

The proteasome subunit LMP2/ β 1i in the female genital system

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Abstract: Protein degradation by the ubiquitin-proteasome system is central to cell homeostasis and survival. Defects in this process are associated with diseases such as cancer and neurodegenerative disorders. The 26S proteasome consists of a 20S proteasome core and two 19S regulatory subunits. The 20S proteasome core is composed of 28 subunits that are arranged in four stacked rings, resulting in a barrel-shaped structure. The two end rings are each formed by seven α subunits, and the two central rings are each formed by seven β 1 subunits. Replacement of LMPY by LMP2/ β 1i increases the capacity of the immunoproteasome to cleave model peptides after hydrophobic and basic residues. LMP2/ β 1i mediates the cell survival pathway. Embryo implantation involves the invasion of placental extravillous trophoblast cells (EVTs) into the uterus. Normal human placentas or placentas from hydatidiform mole patients were collected and the expression of LMP2/ β 1i in different cell types including trophoblastic column (TC), cytotrophoblast cells (CTB) and syncytiotrophoblasts (STBs) was examined under different pathological states by pathological analysis. LMP2/ β 1i expression in TC of partial hydatidiform mole and complete hydatidiform mole placentas was higher than that in TC of normal human placentas. The overexpression of LMP2/ β 1i in trophoblast cells of hydatidiform moles may contribute to its highly invasive phenotype. LMP2/ β 1i-deficient mice reportedly exhibit uterine neoplasms, with a disease prevalence of 36% by 12 months of age. Further experiments with human and mouse uterine tissues clarified the biological significance of LMP2/ β 1i in malignant myometrium transformation and the cell cycle, which implicated LMP2/ β 1i as an anti-tumorigenic candidate. In this mini review, we covered recent insights into the molecular and cellular pathways involved in LMP2/ β 1i-mediated biological functions, with a particular focus on embryo implantation and uterine mesenchymal tumorigenesis.

Keywords: LMP2/ β 1i, Implantation, Trophoblast, Leiomyosarcoma, Leiomyoma

1. Introduction

The proteasome is an ATP-dependent, multisubunit,

multicatalytic protease responsible for the majority of nonlysosomal degradation in eukaryotic cells that recognizes and degrades ubiquitinated proteins, including

misfolded, damaged, and other regulatory proteins [1,2]. The ubiquitin-proteasome pathway (UPP) is involved in regulating the cell cycle, degrading some transcription factors, modifying some membrane proteins, assembling ribosomes, and antigen presentation of major histocompatibility complex (MHC) class I [1,3,4]. 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 [5]. The proteasome structure is a cylindrical complex containing a core of four stacked rings around a central pore, with each ring being composed of seven individual proteins. The inner two rings are made of seven β subunit that contain three to seven protease active sites [6,7]. Each proteolytic β subunits expressed with an NH₂-terminal threonine residue is critical for multiple peptidase activities, including chymotryptic-, tryptic-, and peptidylglutamyl-like activities [8–10]. Two of the β subunits with an NH₂-terminal threonine residue, low molecular mass polypeptide (LMP) 2/ β 1i and LMP7/ β 5i, which are induced by the pro-inflammatory cytokine interferon (IFN)- γ , are encoded within the class II region of the MHC, directly adjacent to the transporter associated with antigen presentation (TAP) 1 and TAP2 genes [11]. Several experiments have shown that incorporation of LMP2/ β 1i and LMP7/ β 5i into the 20S proteasome is responsible for antigen presentation, and inhibiting proteasome activity affected the capacity of cells to present antigenic peptides [12,13]. Furthermore, the 20S proteasome reconstructed by LMP2/ β 1i and LMP7/ β 5i, referred to as an immuno-proteasome, produced increased chymotryptic and tryptic protease activities and modulated cleavage-site preferences of the proteasome [14,15]. The proteasome is localized both in the nucleus and in cytoplasm of eukaryotic cells, while LMP2/ β 1i and LMP7/ β 5i individually appear to be more intense in the endoplasmic reticulum [16,17]. Thus, proteasomes in different cells normally differ in subunit composition and functional activities in a way that correlates with the cell's capacity for antigen presentation [1]. This review showed that LMP2/ β 1i communication is important for maintaining embryo implantation and transforming mesenchymal cell in the female genital system.

2. Biological Significance of LMP2/ β 1i In Embryo Implantation

Implantation of the embryo into the uterine endometrial is a highly regulated event critical for the establishment of pregnancy. Successful embryo implantation depends upon the synchronized development of both the invasiveness of the embryo and receptivity of the endometrium [18]. This process is accompanied by extensive degradation and remodeling of the extracellular matrix (ECM). Numerous studies in mice, primates, and humans have shown that matrix metalloproteinases (MMPs), which are responsible

for degrading the ECM, are key regulators of blastocyst implantation [19-21]. Ubiquitin-related proteins were shown to be present in human, baboon, rhesus monkey, cow, sheep, and mouse pregnant uteri [22-26], and may be essential for endometrial modification and placental development during early pregnancy. However, no direct evidence has shown whether the UPP is involved in embryo implantation or has a regulatory effect on mRNA expression and the activities of MMP-2 and MMP-9.

LMP2/ β 1i and LMP7/ β 5i expression levels significantly increased with the elongation of pregnancy. LMP2/ β 1i and LMP7/ β 5i mRNAs were mainly expressed in the luminal and glandular epithelia on Day 12 of pregnancy. On Days 18 and 26 of pregnancy, strong signals of LMP2/ β 1i and LMP7/ β 5i mRNAs were detected in the placental villi, trophoblastic column, and arterial endothelial cells close to the implantation site, and moderate expressions were found in the trophoblastic shell and glandular epithelium (Fig. 1). LMP2/ β 1i and LMP7/ β 5i mRNAs were extensively distributed in the stroma on Day 26 of pregnancy. The expression patterns of LMP2/ β 1i and LMP7/ β 5i proteins were similar to those of their transcripts, whereas weak immunostaining of LMP2/ β 1i and LMP7/ β 5i proteins was detected in stroma at all stages of pregnancy. LMP2/ β 1i and LMP7/ β 5i may be involved in placental villi invasion, degradation of ECM, immune tolerance, glandular secretion, and angiogenesis.

The regulatory mechanism of LMP2/ β 1i on the expression and activities of MMP-2 and MMP-9 was examined using the human invasive extra villous trophoblast cell line, HTR8/Svneo. The expression of mRNA encoding the NF- κ B subunits p50 and p65 remained normal in LMP2/ β 1i-inhibited cells, whereas the p50 protein levels significantly decreased in cytosolic and nuclear extracts, and p65 protein levels were markedly reduced in the nuclear extract only [27]. LMP2/ β 1i contributes to I κ B α degradation and p50 generation, and the inhibition of LMP2/ β 1i suppresses the expression and activities of MMP-2 and MMP-9 by blocking the transfer of active NF- κ B1 p50/p65 heterodimers into the nucleus.

Embryo implantation involves the invasion of placental extravillous trophoblast cells (EVTs) into the uterus. Hyperactive EVT invasion occurs in hydatidiform moles and choriocarcinomas. Normal human placentas or placentas from hydatidiform mole patients were collected and the expression of LMP2/ β 1i in different cell types including trophoblastic column (TC), cytotrophoblast cells (CTB) and syncytiotrophoblasts (STB) was examined under different pathological states by immunohistochemical analysis. The expression of LMP2/ β 1i in TC of partial hydatidiform mole and complete hydatidiform mole placentas, was higher than that in TC of normal human placentas. The overexpression of the 20S proteasome β -subunit LMP2/ β 1i in trophoblast cells of hydatidiform moles may contribute to its highly invasive phenotype (Fig. 1).

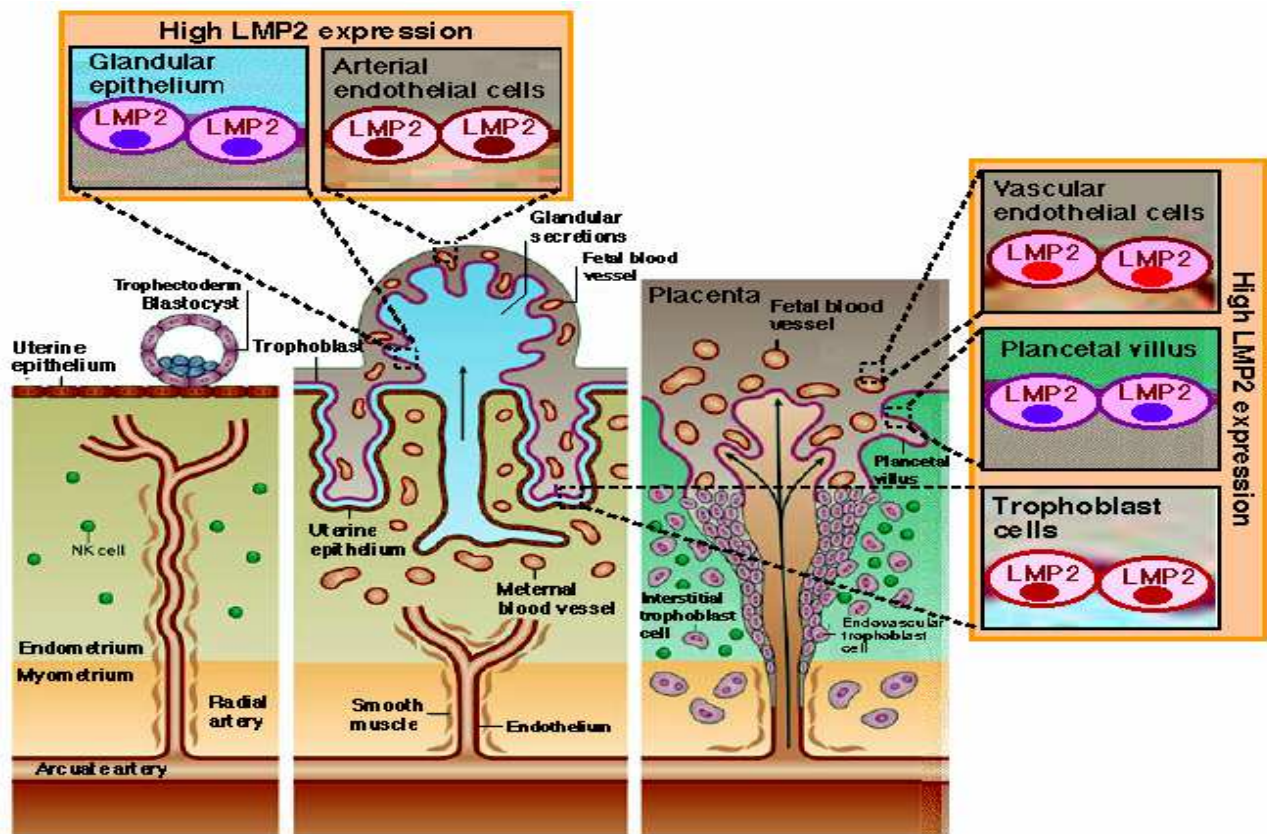


Figure 1. This picture shows implantation of the blastocyst, an early stage in embryo development, into the uterine epithelium. Cooperative interactions between trophoblast cells and maternal cells then form the placenta. In mammals, trophoblast cells lie adjacent to the surface epithelium of the uterus, but they do not invade it. Natural killer (NK) cells are also not present. Nutrients are transferred to the fetus from maternal blood vessels close to the uterine epithelium and in glandular secretions. This arrangement is known as an epitheliochorial placenta. The endometrium does not transform into the decidua, which is the name given to an endometrium that has differentiated under the influence of progesterone. In human placentation, trophoblast cells invade blood vessels as in rhesus macaques, but they replace the vascular endothelium in the myometrium to a greater degree. Invasion extends beyond the endometrium into the myometrium, whereas it is restricted to the endometrium in rhesus macaques. In addition, trophoblast cells invade the decidua, replacing the medial smooth muscle with fibrinoid material. Accompanying these changes is the presence of numerous NK cells. Strong signals of LMP2/ β 1i mRNAs were detected in the placental villi, trophoblastic column, and arterial endothelial cells close to the implantation site, and the moderate expression was found in the trophoblastic shell and glandular epithelium. LMP2/ β 1i expression in trophoblast cells of hydatidiform moles may contribute to its highly invasive phenotype.

3. Biological Role of LMP2/ β 1i in Uterine Mesenchymal Tumorigenesis

Proteasomal degradation is essential for many cellular processes, including the cell cycle, regulation of gene expression, and immunological function. The abnormal expression of proteasome subunits has been correlated with tumor initiation and progression [27-29]. The individual expression of LMP2/ β 1i, LMP7/ β 5i, and LMP10 (MECL-1)/ β 2i subunits is believed to contribute to the initiation and development of disorders. A recent study revealed a unique role for LMP7/ β 5i in controlling pathogenic immune responses and provided a therapeutic rationale for targeting LMP7/ β 5i in autoimmune disorders, especially rheumatoid arthritis [30]. Selective inhibition of LMP7/ β 5i blocked the production of interleukin-23 (IL-23) by activated monocytes and interferon- γ and IL-2 by T cells. In mouse models of rheumatoid arthritis, a LMP7/ β 5i

inhibitory treatment reversed the signs of disease and resulted in reductions in cellular infiltration, cytokine production, and autoantibody levels. Recent reports have demonstrated that LMP2/ β 1i is obligatory for tumor surveillance and the tissue-specific role of LMP2/ β 1i in protection from spontaneous uterus neoplasms [31,32]. The presentation of antigenic peptides by MHC class I molecules is important for tumor rejection by CTLs. Such antigenic peptides are generated as a result of the degradation of intracellular proteins by the proteasome pathway, a process that is influenced by the LMP2/ β 1i subunit of the proteasome complex. Therefore, LMP2/ β 1i-deficient mice exhibit a defect in proteasome function. LMP2/ β 1i-deficient mice have now been shown to develop uterine neoplasms, with a disease prevalence of 36% by 12 months of age. This finding indicates that proteasome function is essential for MHC class I-mediated tumor rejection by CTLs. Homozygous mice deficient in LMP2/ β 1i exhibit tissue- and substrate-dependent abnormalities in the biological

functions of the proteasome [31, 32]. Ut-LMS reportedly occurred in female LMP2/ β 1i-deficient mice at the age of 6 months or older and the incidence at 14 months of age was about 40% [32, 33]. Disease prevalence in mice is similar to that of human Ut-LMS, which occurs after menopause. Histological studies of LMP2/ β 1i-deficient uterine tumors revealed the characteristic abnormalities of human Ut-LMS [32]. These tumors lacked lymphoid infiltrates, a sign of immune recognition, and consisted of uniformly elongated smooth muscle cells arranged into bundles. The nuclei of tumor cells varied in size and shape; furthermore, mitosis was frequently observed. The tumors lacked lymphoid infiltrates, a sign of immune recognition, and consisted of uniformly elongated myometrium cells arranged into bundles. The nuclei of tumor cells varied in size and shape. Furthermore, mitosis was frequently observed. In contrast, the myometrium cells of C57BL/6 mice were normal in appearance [32]. Whereas relatively few ki-67-positive cells, which are proliferating cells, were observed in the basal cell layer of the normal myometrium, most of the basal cells in LMP2/ β 1i-deficient mice strongly expressed ki-67 [32]. This immunological staining indicates the abnormal proliferation of LMP2/ β 1i-lacking cells in the basal layer. LMP2/ β 1i-deficient mice that have developed Ut-LMS exhibit marked weight loss, and then die by 14 months of age. They may also have skeletal muscle metastasis from the Ut-LMS [34]. Therefore, LMP2/ β 1i-deficient mice with Ut-LMS may die as a result of tumor growth and metastasis. In general, it is not easy to distinguish uterine LMA from Ut-LMS in humans; however, in mice, because of the characteristic pathological findings in mice, including significant weight loss and possible skeletal muscle metastasis, a tumor that develops in the uterus of an

LMP2/ β 1i-null mouse can be considered malignant, i.e., a Ut-LMS [32,33]. Research experiments with LMP7/ β 5i knock out mice have demonstrated that a LMP7/ β 5i deficiency also affects the cleavage site preference of the 20S proteasome, and the reduced MHC class I antigen presentation of LMP7/ β 5i knock out mice is most likely due to an impairment in peptide generation [35]. Although the 20S proteasome from LMP7/ β 5i knock out mice showed altered proteolytic activities and cleavage site preferences, no report has shown that LMP7/ β 5i knock out mice exhibit uterine neoplasms [35].

Furthermore, immunohistochemistry (IHC) revealed a serious loss in the ability to induce LMP2/ β 1i expression in human Ut-LMS tissue relative to that in LMA or a normal myometrium located in the same section [36,37] (Fig. 2) ^(NOTE 1). Of the 54 cases we examined with human Ut-LMS, 46 were negative for LMP2/ β 1i expression, 4 were focally positive, and 2 were partially positive [37]. Two Ut-LMS cases stained for LMP2/ β 1i. LMP2/ β 1i levels were also evaluated in skeletal muscle and rectum metastases from individual Ut-LMS patients [37,38]. A pathological examination of surgical samples revealed the presence of a mass measuring 3 cm in its largest diameter in the lumbar quadratus muscle without a fibrous capsule. All lymph nodes were negative for human Ut-LMS metastases, and immunohistological studies showed positivity for ki-67 and negativity for LMP2/ β 1i [37]. Histological findings were consistent with metastatic Ut-LMS for the skeletal muscle and rectum lesions [37]. In western blotting and RT-PCR experiments, LMP2/ β 1i was expressed in the normal myometrium and LMA, but not in human Ut-LMS, both of which are strongly supportive of the IHC findings [36,37,39].

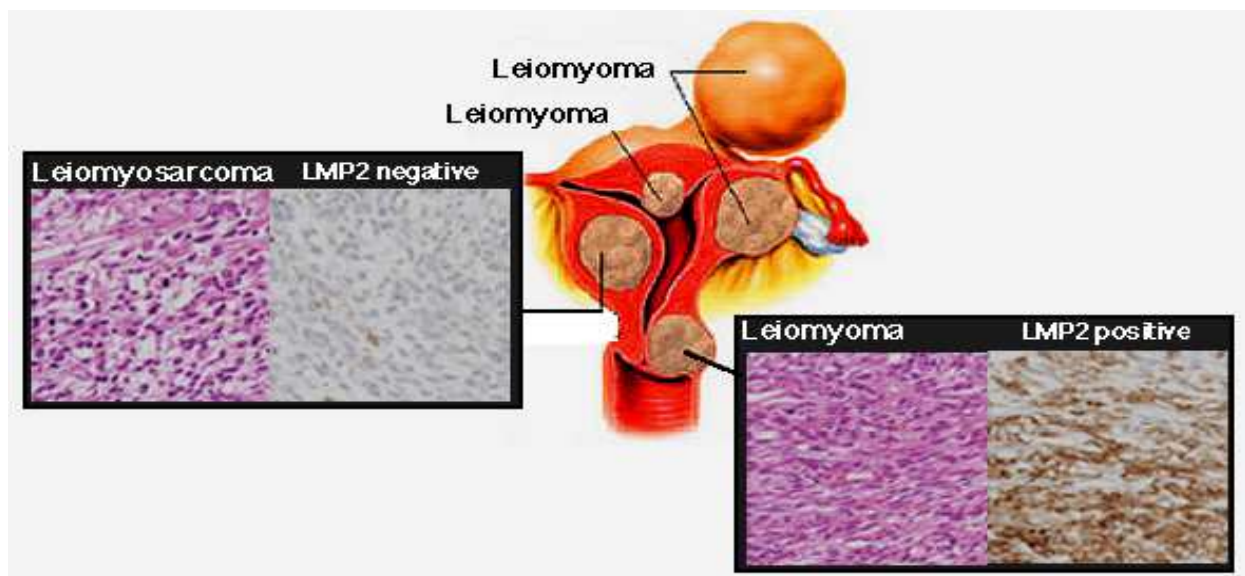


Figure 2. The differential expression levels of LMP2/ β 1i in uterine mesenchymal tumors, leiomyoma and leiomyosarcoma. Immunohistochemistry (IHC) revealed a serious loss in the ability to induce LMP2/ β 1i expression in human uterine leiomyosarcoma tissue relative to that in leiomyoma or normal myometrium.

4. Final Consideration

In conclusion, the 20S proteasome b ring subunit LMP2/β1i was highly overexpressed in trophoblast cells of hydatidiform moles, and LMP2/β1i expression in aggressive EVT cells directly regulated cell invasion. Human Ut-LMS is refractory to chemotherapy and has a poor prognosis. Defective LMP2/β1i expression may be one of the risk factors for the development of human Ut-LMS similar to LMP2/β1i-deficient mouse. LMP2/β1i may function as an anti-tumorigenic 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. The biological communication of LMP2/β1i with other factors is important to maintain embryo implantation and the transformation of mesenchymal cells in the female genital system.

(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 [40,41]. The histologic classification of uterine sarcomas is based upon homology to normal cell types and includes human Ut-LMS (analogous to myometrium), stromal sarcoma (analogous to endometrial stroma), and other heterologous cell types (i.e., osteosarcoma and 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. The mitotic rate is the most important determinant of malignancy, but is modified by the presence of necrosis and cytologic atypia. A diagnosis of human Ut-LMS may be made in the presence of tumor necrosis and any mitosis. A diagnosis can be made in the absence of tumor necrosis with moderate to severe cytologic atypia and a mitotic index greater than 10mf/10hpf. Without tumour necrosis and significant atypia, a high mitotic index is compatible with a benign clinical course; however, data is limited [40, 41].

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References

- [1] Coux O, Tanaka K, Goldberg AL. (1996) Structure and functions of the 20S and 26S proteasomes. *Annu Rev Biochem* 65:801–847.
- [2] Voges D, Zwickl P, Baumeister W. (1999) The 26S proteasome: a molecular machine designed for controlled proteolysis. *Annu Rev Biochem* 1999; 68:1015–1068.
- [3] Ciechanover A. (1998) The ubiquitin-proteasome pathway: on protein death and cell life. *EMBO J* 17: 7151–7160.
- [4] Craiu A, Gaczynska M, Akopian T, Gramm CF, Fenteany G, Goldberg AL, Rock KL. (1997) Lactacystin and clasto-lactacystin b-lactone modify multiple proteasome subunits and inhibit intracellular protein degradation and major histocompatibility complex class I antigen presentation. *J Biol Chem* 272: 13437–13445.
- [5] Rivett AJ, Bose S, Brooks P, Broadfoot KI. (2001) Regulation of proteasome complexes by interferon-g and phosphorylation. *Biochemie* 2001; 83: 363–366.
- [6] Lowe J, Stock D, Jap B, Zwickl P, Baumeister W, Huber R. (1995) Crystal structure of the 20S proteasome from the archaon *T. acidophilum* at 3.4 Å resolution. *Science* 268:533–539.
- [7] Groll M, Ditzel L, Lowe J, Stock D, Bochtler M, Bartunik HD, Huber R. (1997) Structure of 20S proteasome from yeast at 2.4 Å resolution. *Nature* 386:463–471.
- [8] Baumeister W, Walz J, Zuhl F, Seemuller E. (1998) The proteasome: paradigm of a self- compartmentalizing protease. *Cell* 92:367–380.
- [9] Schmidtke G, Kraft R, Kostka S, Henklein P, Frommel C, Lowe J, Huber R, Kloetzel PM, Schmidt M. (1996) Analysis of mammalian 20S proteasome biogenesis: the maturation of b subunits is an ordered two-step mechanism involving autocatalysis. *EMBO J* 15:6887–6898.
- [10] Kuchelkorn U, Frentzel S, Kraft R, Kostka S, Groettrup M, Kloetzel PM. Incorporation of major histocompatibility complex-encoded subunits LMP2 and LMP7 changes the quality of the 20S proteasome polypeptide processing products independent of interferon-g. *Eur J Immunol* 25:2605–2611.
- [11] Glynne R, Powis SH, Beck S, Kelly A, Kerr LA, Trowsdale J. (1991) A proteasome-related gene between the two ABC transporter loci in the class II region of the human MHC. *Nature* 353:357–360.
- [12] Fehling HJ, Swat W, Laplace C, Kuhn R, Rajewsky K, Muller U, von Boehmer H. (1994) MHC class I expression in mice lacking proteasome subunit LMP-7. *Science* 265:1234–1237.
- [13] Rock KL, Gramm C, Rothstein L, Clark K, Stein R, Dick L, Hwang D, and Goldberg A. (1994) Inhibition of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. *Cell* 78:761–771.
- [14] Gaczynska M, Goldberg AL, Tanaka K, Hendil KB, Rock KL. (1996) Proteasome subunits X and Y alter peptidase activities in opposite ways to the interferon-g-induced

- subunits LMP2 and LMP7. *J Biol Chem* 271:17275–17280.
- [15] Ustrell V, Pratt G, Rechsteiner M. (1995) Effects of interferon- γ and major histocompatibility complex encoded subunits on peptidase activities of human multicatalytic proteases. *Proc Natl Acad Sci U S A* 92: 584–588.
- [16] Reits EA, Benham AM, Plougaste B, Neeffjes J, Trowsdale J. (1997) Dynamics of proteasome distribution in living cells. *EMBO J* 16:6087–6094.
- [17] Brooks P, Murray RZ, Mason GGF, Hendil KB, Rivett AJ. (2000) Association of immunoproteasome with the endoplasmic reticulum. *Biochem J* 352:611–615.
- [18] Finn CA. (1996) Implantation, menstruation and inflammation. *Biol Rev Camb Philos Soc* 61:313–328.
- [19] Waterhouse P, Denhardt DT, Khokha R. (1993) Temporal expression of tissue inhibitors of metalloproteinases in mouse reproductive tissues during gestation. *Mol Reprod Dev* 35:219–226.
- [20] Blankenship TN, King BF. (1994) Identification of 72-kilodalton type IV collagenase at sites of trophoblastic invasion of macaque spiral arteries. *Placenta* 15:177–187.
- [21] Hurskainen T, Hoyhtya M, Tuuttila A, Oikarinen A, Autio-Harmanen H. (1996) mRNA expressions of TIMP-1, -2, and -3 and 92-KD type I collagenase in early human placenta and decidual membrane as studied by *in situ* hybridization. *J Histochem Cytochem* 44:1379–1388.
- [22] Bebington C, Bell SC, Doherty FJ, Fazleabas AT, Fleming SD. (1999) Localization of ubiquitin and ubiquitin cross-reactive protein in human and baboon endometrium and decidua during the menstrual cycle and early pregnancy. *Biol Reprod* 60:920–928.
- [23] Bebington C, Doherty FJ, Fleming SD. (2000) Ubiquitin and ubiquitin-protein conjugates are present in human cytotrophoblast throughout gestation. *Early Pregnancy* 4:240–252.
- [24] Johnson GA, Austin KJ, Van Kirk EA, Hansen TR. (1998) Pregnancy and interferon- τ induce conjugation of bovine ubiquitin cross-reactive protein to cytosolic uterine proteins. *Biol Reprod* 58: 898–904.
- [25] Johnson GA, Spencer TE, Hansen TR, Austin KJ, Burghardt RC, Bazer FW. (1999) Expression of the interferon τ inducible ubiquitin crossreactive protein in the ovine uterus. *Biol Reprod* 61:312–318.
- [26] Austin KJ, Bany BM, Belden EL, Rempel LA, Cross JC, Hansen TR. (2003) Interferon-stimulated gene-15 (ISG15) expression is up-regulated in the mouse uterus in response to the implanting conceptus. *Endocrinology* 144:3107–3113.
- [27] Hayashi T, Kodama S, Faustman DL. (2000) LMP2 expression and proteasome activity in NOD mice. *Nature Med.* 6:1065–6.
- [28] Higashitsuji H, Liu Y, Mayer RJ, Fujita J. (2005) The oncoprotein gankyrin negatively regulates both p53 and RB by enhancing proteasomal degradation. *Cell Cycle* 4: 1335–1337.
- [29] Wang J, Maldonado MA. (2006) The Ubiquitin-Proteasome System and Its Role in Inflammatory and Autoimmune Diseases. *Cell Mol Immunol* 3: 255–261.
- [30] Muchamuel T, Basler M, Aujay MA, Suzuki E, Kalim KW, Lauer C, Sylvain C, Ring ER, Shields J, Jiang J, Shwonek P, Parlati F, Demo SD, Bennett MK, Kirk CJ, Groettrup M. (2009) A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis. *Nature Med* 15: 781–788.
- [31] Van Kaer L, Ashton-Rickardt PG, Eichelberger M, Gaczynska M, Nagashima K, Rock KL, Goldberg AL, Doherty PC, Tonegawa S. (1994) Altered peptidase and viral-specific T cell response in LMP2 mutant mice. *Immunity* 1: 533–541.
- [32] Hayashi T, Faustman DL. (2002) Development of spontaneous uterine tumors in low molecular mass polyubiquitin-2 knockout mice. *Cancer Res* 62: 24–27.
- [33] <http://www.informatics.jax.org/javawi2/servlet/WIFetch?page=alleleDetail&id=MGI:2152729#refs>
- [34] Hayashi T, Horiuchi A, Sano K, Hiraoka N, Kanai Y, Shiozawa T, Tonegawa S, Konishi I. (2011) Molecular approach on uterine leiomyosarcoma: LMP2-deficient mice as an animal model of spontaneous uterine leiomyosarcoma. *Sarcoma*. 476498. Epub 2011 Mar 8.
- [35] Stohwasser R, Kuckelkorn U, Kraft R, Kostka S, Kloetzel PM. (1996) 20 S proteasome from LMP7 knock out mice reveals altered proteolytic activities and cleavage site preferences. *FEBS Letters* 383: 109–113.
- [36] Hayashi T, Kobayashi Y, Kohsaka S, Sano K. (2006) The mutation in the ATP-binding region of JAK1, identified in human uterine leiomyosarcomas, results in defective interferon- γ inducibility of TAP1 and LMP2. *Oncogene* 25: 4016–4026.
- [37] Hayashi T, Horiuchi A, Sano K, Hiraoka N, Kasai M, Ichimura T, Nagase S, Ishiko O, Kanai Y, Yaegashi N, Aburatani H, Shiozawa T, Konishi I. (2011) Potential role of LMP2 as tumor-suppressor defines new targets for uterine leiomyosarcoma therapy. *Sci Rep* 1: 180|DOI:10.1038/srep00180
- [38] Hayashi T, Horiuchi A, Sano K, Hiraoka N, Kasai M, Ichimura T, Nagase S, Ishiko O, Kanai Y, Yaegashi N, Aburatani H, Shiozawa T, Konishi I. (2012) Potential role of LMP2 as an anti-oncogenic factor in human uterine leiomyosarcoma: morphological significance of calponin h1. *FEBS Letter* 586: 1824–31.
- [39] Hayashi T, Horiuchi A, Sano K, Hiraoka N, Ichimura T, Sudo T, Ishiko O, Yaegashi N, Aburatani H, Konishi I. Potential diagnostic biomarkers for human uterine mesenchymal tumors: Especially LMP2/ β 1i and Cyclin B1-differential expression. *Oncology Letter* 2013. in-press
- [40] Kurma RJ. (2001) *Pathology of the Female Genital Tract*, 4th ed. New York, Springer-Verlag 2001; 4: 499.
- [41] Diagnostic Criteria for LMS, Adapted from 2003 WHO Guidelines: World Health Organization Classification of Tumours: Pathology and Genetics, Pathology and Genetics of Tumours of the Breast and Female Genital Organs. 2003; IARC Press, France.