Proteasome Subunit LMP2/β1i As a Biomarker for Human Uterine Mesenchymal Tumors

Abstract

The proteasomal degradation pathway is essential for many cellular processes, including the cell cycle, regulation of gene expression, and responses to oxidative stress. The proteasome, most exclusively used in mammals, is the cytosolic 20S proteasome, which has a molecular mass of approximately 2,000 kilodaltons (kDa) and contains one 20S protein subunit and two 19S regulatory cap subunits. The 20S particle is assembled from two half-proteasomes, each of which consists of a seven-membered pro-β-ring attached to a seven-membered α-ring. Component of β-ring, low-molecular mass polypeptide (LMP)2β1i-deficient mice spontaneously develop uterine leiomyosarcoma (UT-LMS), with a disease prevalence of 40% by 14 months of age. Recent studies on human and mouse uterine tissues revealed the defective expression of LMP2β1i in human UT-LMS, and this was attributed to the interferon (IFN)-γ pathway and the specific effects of Janus kinase (JAK)-1 somatic mutation on LMP2β1i transcriptional activation. Furthermore, an analysis of a human UT-LMS cell line clarified the biological significance of LMP2β1i in malignant myometrium transformation and the cell cycle; thus, implicating LMP2β1i as an anti-tumorigenic candidate. The development of gynecological tumors is often correlated with the secretion of female hormones; however, the development of UT-LMS has not been correlated with hormonal conditions, and the risk factors remain unknown. Therefore, the defective expression of LMP2β1i may be a risk factor for human UT-LMS. LMP2β1i is a potential diagnostic biomarker for UT-LMS, and may be a targeted molecule for a new clinical therapeutic approach.

Introduction

Proteins play crucial roles in all biological processes, and, the finely tuned equilibrium between their synthesis and degradation regulates cellular homeostasis. Controlling the quality of proteome informational content is essential for cell survival and function. After their initial synthesis, membrane and secretory proteins are modified, folded, and assembled in the endoplasmic reticulum, whereas other proteins are synthesized and processed in the cytosol. Cells have different protein quality control systems, the molecular chaperones, which assist protein folding and stabilization, and the ubiquitin-proteasome system (UPS) and lysosomes, which degrade proteins. UPS and lysosomes are generally assumed to be regulated independently and serve distinct functions. Proteasomes, which are located in the nucleus and cytoplasm, have a hollow barrel-shaped multiprotein structure of 2.5 MDa and are comprised of a core particle (20S proteasome) flanked on the both sides by a regulatory particle (19S proteasome) [1,2]. The functions of the 19S proteasome include the recognition of ubiquitinated proteins, denaturing of these proteins, de-ubiquitination, which allows ubiquitin molecules to be recycled, and the delivery of target proteins to the 20S proteasome [3].

The 20S proteasome consists of four rings of seven member proteins, each of which are joined stuck one on the other. The two peripheral rings are identical and called α-rings (with subunits α1 to α7). The two central rings are also identical and called β-rings (with subunits β1 to β7). The proteasome possesses three enzymatic activities; trypsin-like (cleavage of post-basic residues) activity, a chymotrypsin-like (cleavage of post-hydrophobic residues) activity, and a post-glutamyl (cleavage of caspase-like or post-acidic residues) activity.

The proteasome is a multiprotein complex that regulates the stability of hundreds of cellular proteins and is thus, implicated in virtually all cellular functions, including cell proliferation, apoptosis, angiogenesis, and motility, which are of particular importance for tumorigenesis.

Abbreviations


Alternative β-forms of low molecular weight protein (LMP2)/β1i are expressed in the myometrium following exposure to pro-inflammatory signals such as cytokines, especially IFN-γ. Uterine leiomyosarcoma (Ut-LMS) was shown to occur in female LMP2/β1i-null mice at 6 months or older, and its incidence at 14 months was shown to occur in female LMP2/β1i-null mice at 6 months or older, and its incidence at 14 months was approximately 40% [5,6]. Human uterine mesenchymal tumors, i.e., smooth muscle tumors (SMTs), which develop in the myometrium, have traditionally been divided into benign leiomyoma (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 [7]. Surgical intervention is virtually the only means of treatment because Ut-LMS is resistant to chemotherapy and radiotherapy [8-10]. The prognosis of patients with 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 of patients with Ut-LMS. Uterine LMA may occur in as 70%-80% of women by the age of 50 years [11]. However, difficulties are associated with distinguishing uterine LMA from Ut-LMS, and a diagnosis generally requires surgery and cytology [12]. Diagnostic categories for uterine SMTs and morphological criteria are used to assign cases [13,14]. The non-standard subtypes of uterine SMTs such as the epithelioid and myxoid types are classified differently using these features; therefore establishing a diagnostic method to identify non-standard smooth muscle differentiation is important [13,14].

High estrogen levels are considered to significantly influence the development of tumors in the uterine body [15-17]. The molecular mechanisms by which uterine LMA and Ut-LMS develop have not yet been elucidated, however, tumors that develop in the myometrium gradually increase in size due to the influence of the female hormone and, estrogen, and generate tumors. No correlation between the development of Ut-LMS and hormonal conditions, and no obvious risk factors have been identified. Although cases accompanied by hypocalcemia or eosinophilia have been reported, neither clinical abnormality is an initial risk factor for Ut-LMS. Determining the malignant potential of smooth muscle neoplasms 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 can distinguish between Ut-LMS and uterine mesenchymal tumors including LMA have yet to be established. The protein homology of the proteasome β-subunit, LMP2/β1i in humans and mice is 88.1%, and further scores, based on the homogeneity of mouse LMP2/β1i between other species, were shown to reverse the signs of disease and resulted in reductions by T cells. In mouse models of RA, LMP7/β5i-inhibitory treatments were shown to reverse the signs of disease and resulted in reductions in cellular infiltration, cytokine production, and autoantibody levels. Recent studies have suggested that LMP2/β1i is necessary for tumor cell proliferation and may play a tissue-specific role in protection against spontaneous uterus neoplasms [5,24]. 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/β1i subunit of the proteasome complex (Figure 1a). Homozygous mice deficient in LMP2/β1i exhibit tissue- and substrate-dependent abnormalities in the biological functions of the proteasome [5,24]. Ut-LMS has been reported in female LMP2/β1i-null mice at 6 months or older, and its incidence at 14 months was approximately 40% [5,6] (Figure 1b). The disease prevalence in mice is similar to that of human Ut-LMS, which occurs after menopause. Pathological studies on LMP2/β1i-null uterine tumors revealed the characteristic abnormalities of human Ut-LMS [5]. These tumors lacked lymphoid infiltrates, a sign of immune recognition, and consisted of uniformly elongated smooth muscle cells arranged into bundles. The nuclei of these tumor cells varied in size and shape; furthermore, mitosis was frequent. In contrast, the myometrium cells of C57Bl/6 mice were normal in appearance [5]. Whereas relatively few ki-67-positive cells, which are proliferating cells, were observed in the basal cell layer of the normal myometrium, most basal cells in LMP2/β1i-null mice vividly expressed ki-67 [5] (Figure 1b). This immunological staining revealed the abnormal proliferation of LMP2/β1i-lacking cells in the basal layer. LMP2/β1i-null mice with Ut-LMS lose a marked amount of weight and die by 14 months. They may also exhibit skeletal muscle metastasis from Ut-LMS [25]. Therefore, LMP2/β1i-null mice with Ut-LMS are more likely to die as a result of tumor growth and metastasis. It is generally not easy to distinguish uterine LMA from Ut-LMS in humans, however, in mice, because of the characteristic pathological findings, including significant weight loss, and possible skeletal muscle metastasis, a tumor that develops in the uterus of an LMP2/β1i-null mouse can be considered as malignant, i.e., Ut-LMS [5,6] (Figure 1b).

To examine whether the biological significance of LMP2/β1i to uterine sarcomagenesis observed in mice applies to other species, the mouse LMP2/β1i protein sequence was compared with all protein sequences in euteleostomi using BLAST protein. Pairwise Alignment Scores, based on the homogeneity of mouse LMP2/β1i between other species, were 66.5–95% (Figure 2). The protein homology of LMP2/β1i in humans and mice was found to be 88.1% (Table 1 (included as supplementary data)). Furthermore, immunohistochemistry (IHC) revealed that the ability of human Ut-LMS tissue to induce LMP2/β1i expression was markedly weaker than that of LMA or the normal...
Figure 1: a. Mediation of the proteasomal degradation pathway to antigen presentation by MHC class I. The immuno-proteasomal degradation pathway is essential for antigen presentation by MHC class I. The defective expression of LMP2 results in tissue-and substrate-dependent abnormalities in immuno-proteasomal functions. Therefore, an impaired proteasome may promote the initial development of disease including tumorigenesis. b. 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 [5,27]. Ul-LMS reportedly occurred in female LMP2/β1i-null mice at 6 months or older, and its incidence at 14 months was approximately 40% [5,27].

Figure 2: LMP2/β1i genes and proteins conserved in Euteleostomi.
myometrium located in the same section [26,27]. Of the 54 cases examined with human Ut-LMS, 46 were negative for the expression of LMP2/β1i, 4 were focally positive, and 2 were partially positive [27]. Two Ut-LMS cases stained positive for LMP2/β1i. LMP2/β1i levels were also evaluated in the skeletal muscle and rectum metastases from individual Ut-LMS patients [27]. A pathological examination of surgical samples revealed the presence of a mass measuring 3 cm in diameter in the lumbar quadrate muscle without a fibrous capsule. All lymph nodes were negative for human Ut-LMS metastases, and IHC studies were positive for ki-67 and negative for LMP2/β1i [27]. Histological findings were consistent with metastatic Ut-LMS for the skeletal muscle and rectum lesions [27]. Western blotting and RT-PCR experiments revealed that LMP2/β1i was expressed in the normal myometrium, but not in human Ut-LMS, and both these results were strongly supportive of the IHC findings [26,27] (Figure 2b). To increase the tumor incidence and better assess the role of the systemic expression of TP53 in response to the initiation of Ut-LMS tumorigenesis, LMP2/β1i-null mice were bred with TP53-null mice to create Lmp2−/−Tp53−/− double knockout mice. The incidence of Ut-LMS 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 Ut-LMS tumorigenesis remains unclear. Although we previously demonstrated that the abnormal expression of ovarian steroid receptors, TP53, ki-67, and mutations of TP53 were frequently associated with human Ut-LMS, defective LMP2/β1i expression appears to be more characteristic of human Ut-LMS than any of these factors [26-29].

The biological significance of LMP2/β1i in human Ut-LMS tumorigenesis

The IFN-γ pathway is important in the control of tumor growth and invasion and has been implicated in several cancers. Recent studies on human and mouse uterine tissues revealed the defective expression of LMP2/β1i in human Ut-LMS, and this was attributed
suggest that single-unit LMP2/β1i-mediated cellular factors other experiments on cell morphology following modified-gene expression be involved in the biological function of calponin h1. Biological that LMP2/β1i-induced cellular morphological phenotypes may biological experiments were performed to examine the biological cellular processes, such as the cell cycle and gene expression. Cellular were reportedly associated with cellular factor(s) that regulate human Ut-LMS remains incomplete [29-31]. LMP2/β1i molecules null mice do not exhibit Ut-LMS. The biological characterization of sarcomagenesis, and morphological changes in uterine LMS cells inoculated with the SKN-LMP2/β1i (Flatrevertant cell type) clones. The efficiency of colony formation and size of the colonies in soft agar were greatly reduced in LMP2/β1i (Flatrevertant cell type) clones. The growth rates, indicated as doubling time, were generally lower in the typical SKN-LMP2/β1i (Flatrevertant cell type) clones and SKN-LMP2/β1iK33A (Flatrevertant cell type) clones than in the control SKN-CEM9 (Transform cell type) clones. FACS analysis demonstrated that LMP2/β1i expression might have induced G1 arrest in the SKN-LMP2/β1i (Flatrevertant cell type) clones. The efficiency of colony formation and size of the colonies in soft agar were greatly reduced in the Flatrevertant cell type clones. Tumor growth was clearly observed in BALB/c nu/nu mice inoculated with SKN-CEM9 (Transform cell type) clones; however, a reduction in tumor growth was noted in mice inoculated with the SKN-LMP2/β1i (Flatrevertant cell type) clones. A recent study suggested that the suppression of cell proliferation, sarcomagenesis, and morphological changes in uterine LMS cells observed under stable LMP2/β1i expression may be attributed to the biological function of single-molecule LMP2/β1i only, without the involvement of its immunoproteasome function. An analysis of human Ut-LMS cells clarified the biological significance of LMP2/β1i in malignant myometrium transformation including the cellular morphological phenotype and cell cycle, thereby implicating LMP2/β1i as an anti-tumorigenic candidate [26,27,29].

In addition, recent studies reported an association between malignant transformation of the myometrium and the reduced expression of calponin h1, a calcium-binding protein, that is specifically expressed in smooth muscle and binds calmodulin, actin, and tropomyosin [29-31]. Calponin h1 reportedly inhibits the ATPase activity of myosin and may play a role in smooth muscle contraction [32,33]. Although calponin h1 may function as a tumor suppressor in Ut-LMS, unlike mice lacking LMP2/β1i, calponin h1-null mice do not exhibit Ut-LMS. The biological characterization of human Ut-LMS remains incomplete [29-31]. LMP2/β1i molecules were reportedly associated with cellular factor(s) that regulate cellular processes, such as the cell cycle and gene expression. Cellular biological experiments were performed to examine the biological connection between LMP2/β1i and calponin h1. The results obtained that LMP2/β1i-induced cellular morphological phenotypes may be involved in the biological function of calponin h1. Biological experiments on cell morphology following modified-gene expression suggest that single-unit LMP2/β1i-mediated cellular factors other than calponin h1, prevent cell proliferation and sarcomagenicity in Ut-LMS cells [26,27,29]. Elucidating the mechanism by which LMP2/β1i-induced biological events, including calponin h1 expression, are regulated may provide a valuable insight into the transformation of cellular phenotypes, control of cell proliferative activity, and the pathogenesis of human uterine mesenchymal tumors at the molecular level. In conclusion, we demonstrated that LMP2/β1i may be a tumor suppressor in human Ut-LMS. This role of LMP2/β1i may lead to new therapeutic targets in human Ut-LMS.

Conclusion

A female hormonal imbalance is often a risk factor for the development of tumors in gynecological cancers, such as breast cancer [15-17]. However, the relationship between the development of human Ut-LMS, a female hormone, and hormone receptors has yet to be clarified in uterine LMA. A recent study showed the expression of LMP2/β1i mRNA and protein in luminal and glandular epithelia, placenta villi, trophoblastic shells, and arterial endothelial cells [34-36]. These results implicate LMP2/β1i in the invasion of placental villi, degradation of the extracellular matrix, immune tolerance, glandular secretion, and angiogenesis [35]. Further studies may help to elucidate the regulatory role of LMP2/β1i in the implantation of embryos [28,34,35,37]. The LMP2/β1i-null mouse was the first animal model of spontaneous Ut-LMS to be established. The defective expression of LMP2/β1i may be one of the causes of human Ut-LMS. The growth of a cell line with normal JAK1 kinase activity was shown to be strongly inhibited by an IFN-γ treatment, whereas the growth of JAK1-deficient cell lines was unaffected [38]. The cellular effects of IFN-γ include the inhibition of cellular proliferation and influences on apoptosis. Exogenous LMP2/β1i expression resulted in cell growth inhibition in LMP2/β1i-transfected human Ut-LMS cells, which highly express LMP2/β1i [26,27]. In contrast the growth of LMP2/β1i-transfected human Ut-LMS cells was unaffected by the IFN-γ treatment [26,27]. Taken together, the effect of IFN-γ on cell growth inhibition may be attributed to the inducibility of LMP2/β1i. To demonstrate whether LMP2/β1i is a potential biomarker that can distinguish human Ut-LMS from uterine LMA, we are currently investigating the reliability and characteristics of LMP2/β1i as a diagnostic indicator in several clinical research facilities. This clinical research has yet to be concluded, and large-scale clinical studies need to be performed. The histological and IHC characteristics of uterine mesenchymal tumors including cellular LMA, bizarre LMA, mitotically active LMA, and smooth muscle tumor of uncertain malignant potential (STUMP) are now being examined at our clinical facilities. A clarification of the relationship between these factors and the development of human Ut-LMS, and the identification of specific risk factors may lead to the development of new treatments for this disease. Human Ut-LMS is refractory to chemotherapy and has a poor prognosis. The molecular biological and cytological information obtained from research studies with LMP2/β1i-null mice will markedly contribute to the development of preventive methods, a potential diagnostic biomarker, and new therapeutic approaches against human Ut-LMS.

References

ISSN: 2325-2340


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Acknowledgements

We sincerely thank Professor Luc Van Kaer (Vanderbilt University Medical Center). This study was supported in part by grants from the Ministry of Education, Culture, Science and Technology, and The Foundation of Osaka Cancer Research, The Ichiro Kanehara Foundation for the Promotion of Medical Science and Medical Care, The foundation for the Promotion of Cancer Research, The Kanzawa Medical Research Foundation, The Shinshu Medical Foundation, and The Takeda Foundation for Medical Science.