Somatic mutations in IFN-γ-Signal molecules in human uterine leiomyosarcoma

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Abstract

Uterus Leiomyosarcoma (uLMS), unfortunately, is a disease with a poor prognosis. In the report of the Ministry of Health, Labor and Welfare, the 50% survival time (mOS) summarized from Stage I to Stage IV was 31 months. Preoperative diagnosis of uLMS is very difficult. Although it is diagnosed as “uterine fibroids”, it is not uncommon in cases where the uterine leiomyosarcoma is definitely diagnosed again after surgical hysterectomy or removal of myoma. Human uLMS is neoplastic malignancy that typically arises in tissues of mesenchymal origin. The identification of novel molecular mechanism leading to human uLMS formation and the establishment of new clinical therapies has been hampered by several critical points. Mice with a homozygous deficiency for Proteasome beta subunit (Psmb)9/β1i spontaneously develop uLMS-like neoplasm. The use of research findings with mouse model has been successful in increasing our knowledge and understanding of how alterations, in relevant oncogenic, tumor suppressive, and signal pathways directly impact sarcomagenesis. The experiments with human clinical materials revealed a defective expression of PSMB9/β1i in human uLMS that was traced to the IFN-γ pathway and the specific effect of somatic mutations of Janus kinase (JAK) 1 molecule and/or promoter region on the locus coding PSMB9/β1i gene. Understanding the biological characters of human uLMS may lead to identification of new diagnostic candidates or therapeutic targets against human uLMS.

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stromal sarcoma. Targeted therapy has been developed rapidly with endometrial cancer, carcinosarcoma, and endometrial metastasis to the lungs and liver. However, the metastasis to retroperitoneal lymph node is as low as 6 to 11% compared to the case of leiomyosarcoma. In other words, (1) cell type, (2) fission (index), and (3) coagulation necrosis are evaluated for histological diagnosis of leiomyosarcoma. In other words, (1) cell type, (2) fission (index), and (3) coagulation necrosis are evaluated for histological diagnosis, it is important to confirm diagnosis and decisions and prognostic predictions are largely responsible for histological subtype of uterine sarcoma originating in the smooth muscles of the myometrium. It accounts for only 1% of cases of uterine sarcoma. However, only 25% of patients suffering from leiomyosarcoma are 28 months and 31 months respectively. It is noteworthy that, when adjusting for stage and mitotic count, human utLMS has a significantly worse prognosis than carcinosarcoma; developing an efficient adjuvant therapy is expected to improve the prognosis of the disease [7]. A trend towards prolonged disease-free survival is seen in patients with matrix metalloproteinase (MMP)-2-negative neoplasms [8]. Although typical presentations with hypercalcemia or eosinophilia have been reported, this clinical abnormality is not an initial risk factor for human uLMS. To the best of our knowledge, little is known regarding the biology of human uLMS; therefore, the risk factors that promote the initial development of human uLMS and regulate their growth in vivo remain poorly understood.

The mice with a targeted disruption of PSMB9/β1i, which is IFN-γ-inducible proteasome β-subunit, exhibited a defect in tissue- and substrate-dependent physiological function of immune-proteasome, and female PSMB9/β1i-deficient mice showed to develop uLMS-like neoplasms, with a disease prevalence of 37% by 14 months of age [9,10]. Histopathological examinations of PSMB9/β1i-deficient uterine neoplasms revealed common characteristic abnormalities of human uLMS. Defective expression of PSMB9/β1i is likely to be one of the risk factors for the development of human uLMS, as it is in PSMB9/β1i-deficient mice [10]. Recent report shows that stable expression of PSMB9/β1i contributes to cell proliferation, which directly correlates to the progressive deterioration with increasing stage and the neoplasm aggressive grade. While it has been established that IFN-γ markedly enhances PSMB9/β1i production through JAK-STAT signaling, the NF-κB pathway also reportedly induces PSMB9/β1i gene expression in an independent manner; inhibition of NF-κB pathway resulted in a decrease in PSMB9/β1i expression in human carcinoma cell lines and human lymphocytes (Figure 1). However, the essential signaling pathway for PSMB9/β1i expression in myometrium is not yet clearly understood. We performed experiments with IFN-γ-deficient mice and TNF-α-deficient mice to elucidate the molecular mechanism of PSMB9/β1i gene expression in myometrium. Although PSMB9/β1i expression was detected in several tissues (heart, ventriculus, esophagus, liver) obtained from IFN-γ- and TNF-α-deficient mice at a similar basal expression level as age-matched wild type mice, the myometrium of IFN-γ-deficient mice had non-identical PSMB9/β1i expression in comparison with TNF-α-deficient mice and wild-type mice. Immunohistochemistry (IHC) experiments revealed that IFN-γ pathway was especially required for PSMB9/β1i expression in myometrium, and other molecular experiments showed that IFN-γ-deficient mice had markedly decreased levels of PSMB9/β1i expression. Examination of mice lacking RelA or NF-κBp65 could not be performed due to embryonic lethality. IFN-γ pathway was required to allow IRF-1 binding to the PSMB9/β1i regulatory region of the genome in human uterine organs. Taken together, these findings demonstrated that the IFN-γ pathway likely played a key role in PSMB9/β1i expression in myometrium. We demonstrate that there are serious mutational defects in the factors on the IFN-γ pathway, which is the key signal cascade for PSMB9/β1i expression and promoter region of PSMB9/β1i gene, in human uLMS. The somatic mutational defects in the IFN-γ pathway may induce the initial development of uLMS. Recent advances in our understanding of the bi-

ological characters of uLMS have concentrated on the impaired IFN-γ pathway. Somatic mutations in key regulatory genes alter the behavior of cells and can potentially lead to the unregulated growth seen in malignant neoplasm. Therefore, continued improvement of our knowledge of the molecular biology of uLMS may ultimately lead to novel therapies and improved outcome.

Expressions of PSMB9/B1i were not markedly induced by IFN-γ treatment in human uLMS cell lines, although cervical epithelial adenocarcinoma cell lines and normal human uterus smooth muscle cells underwent strong induction of PSMB9/B1i following IFN-γ treatment [11]. Furthermore, the IHC experiments revealed a serious loss in the ability to induce expression of PSMB9/B1i in human uLMS tissues in comparison with normal myometrium tissues located in same tissue sections and 4 various mesenchymal neoplasm types. Of 58 uLMS, 50 cases were negative for PSMB9/B1i, 4 cases were focally positive, 2 cases were weakly positive, and 2 cases were positive. IHC analyses showed positivity for ki-67/MIB1 and differential expression of estrogen receptor (ER), progesterone receptor (PR), tumor protein 53 (TP53), and calponin h1. Histopathological differentiation of uterine smooth muscle benign or malignant tumor is comprehensively made by findings such as (1) cell type, (2) fission (index), and (3) coagulation necrosis. A uterine smooth muscle tumor that does not satisfy the diagnostic criteria of leiomyosarcoma and cannot be determined neither malignant nor benign in histopathological diagnosis that is definite diagnosis method is called “smooth muscle tumor of uncertain malignant potential (STUMP)”. As a result of detailed histopathological examinations of the excised specimen, “cases of smooth muscle tumor with unknown malignancy”, cases involving metastasis/relapse to a part of it are included. Eleven cases of STUMP were negative for PSMB9/B1i. In addition, the expression level of PSMB9/B1i was also examined in the skeletal muscle metastasis from human uLMS, the histological diagnosis was consistent with metastatic LMS for skeletal muscle lesions. Pathological study of surgical human samples showed presence of a mass measuring 3 cm at largest diameter in lumbar quadrant muscle without a fibrous capsule. All lymph nodes were negative.

Most frequently, human uLMS have appeared in the uterus, retroperitoneum or extremities, and although histologically indistinguishable, they have different clinical courses and chemotherapeutic responses. The molecular basis for these differences remains unclear, in addition, physiological significance of mutational defect is reportedly associated with progression of malignant neoplasm. Therefore, the molecular examinations of the development of malignant neoplasms and ethnic background, so we conducted CGH experiments with tissue samples obtained from Japanese patients to obtain genome-level information. Our results showed that human uLMS having a clear functional loss at JAK1 (1p31-p32) and PSMB9/B1i (6p21.3) [14,15] has also been demonstrated that a correlation exists between the development of malignant neoplasms and ethnic background, for instance breast cancer and endometrial carcinomas, are strongly promoted by female hormones, but the rate of expression of receptors for estrogen or progesterone is reported to vary in human uLMS compared with normal myometrium. In case of elder patients, low receptor expressions were found to not correlate with the promotion of initial disease or with the overall survival of patients with uLMS; however, molecular targeting therapies against neoplasms have recently shown remarkable achievements [16]. To improve the expression of human uLMS, the research experiments were performed to identify the key role of pro- or anti-oncogenic factors that have an important function in their pathogenesis and that could serve as molecular targets for neoplasm treatment. For this purpose, several research facilities conducted a microarray procedure between human uLMS and normal myometrium and showed that several known pro-oncogenic factors, such as brain-specific polypeptide PEP-19 and a transmembrane tyrosine kinase receptor, c-KIT, may be associated with the pathogenesis of human uLMS [17]. However, in terms of the sarcomagenesis of human uLMS, merely comparing the expression of potential pro-oncogenic factors between normal and malignant tissues is not sufficient because the results obtained may be the consequence of malignant transformation and, therefore, not necessarily the cause. In addition, dysregulation of apoptotic activator, was elucidated in human uLMS. Nearly 21.7% (5/23) of human uLMS tissues unexpectedly had somatic mutations in the intermolecular region of STAT1, which is not yet reported to be important for biological function as transcriptional activation. No somatic mutation in the ATP-binding region and kinase-active site of JAK2 molecule was detected in human uLMS. MOTIF Search profiling [12] and NCBI’s Conserved Domain Database and Search Service, v2.17 analysis also revealed that somatic mutations, which were identified in the catalytic domains of these genes, resulted in impaired physiological functions of tyrosine kinases or transcriptional factor [13].

In a recent report, a comparative genomic hybridization (CGH)-based analysis of human uLMS using a high-resolution genome-wide array gave genome-level information about the amplified and deleted regions that may play a role in the development and progression of human uLMS. Other reports showed that among the most intriguing changes in genes were losses of JAK1 (1p31-p32) and PSMB9/B1i (6p21.3) [14,15]. It has also been demonstrated that a correlation exists between the development of malignant neoplasms and ethnic background, for instance breast cancer and endometrial carcinomas, are strongly promoted by female hormones, but the rate of expression of receptors for estrogen or progesterone is reported to vary in human uLMS compared with normal myometrium. In case of elder patients, low receptor expressions were found to not correlate with the promotion of initial disease or with the overall survival of patients with uLMS; however, molecular targeting therapies against neoplasms have recently shown remarkable achievements [16]. To improve the expression of human uLMS, the research experiments were performed to identify the key role of pro- or anti-oncogenic factors that have an important function in their pathogenesis and that could serve as molecular targets for neoplasm treatment. For this purpose, several research facilities conducted a microarray procedure between human uLMS and normal myometrium and showed that several known pro-oncogenic factors, such as brain-specific polypeptide PEP-19 and a transmembrane tyrosine kinase receptor, c-KIT, may be associated with the pathogenesis of human uLMS [17]. However, in terms of the sarcomagenesis of human uLMS, merely comparing the expression of potential pro-oncogenic factors between normal and malignant tissues is not sufficient because the results obtained may be the consequence of malignant transformation and, therefore, not necessarily the cause. In addition, dysregulation of apoptotic
cascade has also been implicated in many human malignancies. Although the significant differential expression of apoptotic and cell cycle regulators in human uLMS, such as BCL-2-Associated X protein (BAX), B-cell Lymphoma-2 (BCL-2), cellular v-KIT Hardy-Zuckerman 4 Feline Sarcoma Viral Oncogene Homolog (c-KIT), mitogen-inducible gene-2 (MIG-2), p16 inhibits CDK4 (P16/INK4a), p21 cyclin-dependent kinase inhibitor 1 (P21/CIP1), p27 kinase inhibitor protein 1 (P27/KIP1), Murine Double Minute 2 (MDM2), which may mediate the initiation of liposarcoma, TP53, have all been reported and compared to normal myometrium, there exists no scientific evidence to show that abnormal expression of these factors directly correlates to the initiation and promotion of human uLMS. PSM9/B1i-deficient mice were reported to be prone to the development of uLMS, but not in their parental mice, C57BL/6 mice [10]. The histopathological experiments revealed serious loss in the ability to induce the expression of PSM9/B1i in human uLMS tissues in comparison with normal myometrium tissues located in same tissue sections.

About two-thirds of cancer-causing mutations were suggested by mathematical models due to errors that occurred when cells copied DNA. Calculated the relative contribution of the environment to genetic mutation causing cancer, random DNA replication error were calculated using data from the UK cancer database and genome wide researches were performed to identify the somatic mutations that are indicators of specific environmental exposures. Analyzer results of the cancer database revealed that the rate at which genetic mutations are involved in cancer development and tumorigenesis varies depending on the type of neoplasm. For example, in lung adenocarcinoma, which is a kind of lung cancer, 65% of all cancer-causing mutations were environmental or genetic factors, and replication error was 35%. In prostate, brain and bone cancers, more than 95% of cancer driver mutations have been caused by random errors that occur when DNA is replicated. Comprehensively, analysis results of 32 types of cancers revealed that about 66% of cancer-causing mutations are caused by random error in DNA replication, only 29% mutations are caused by environmental factors, 5% of the mutations were inherited from parents. IFN-γ treatment markedly induced the expression of PSM9/B1i, which alters the proteolytic specificity of proteasomes. Molecular analysis was performed to elucidate molecular mechanism, which result in the loss of IFN-γ responsiveness or inexpression of PSM9/B1i in the human uLMS [11]. Genetic alterations in tyrosine kinases have previously been firmly implicated in tumorigenesis, but only a few serine/threonine kinases are known to be mutated in human malignant neoplasms [18-21]. For instance, mice carrying homoygous deletion of Phosphatase and tensin homolog deleted from chromosome 10 (Pten) alleles developed widespread smooth muscle cell hyperplasia and abdominal LMS [22], and JUN oncogene amplification and over-expression block adipocytic differentiation in highly aggressive sarcomas. Most frequently, LMS have appeared in the uterus, retroperitoneum or extremities, and although histologically indistinguishable, they have different clinical courses and chemotherapeutic responses. The molecular basis for these differences remains unclear, therefore, the examination with 23 cases of human uLMS tissues was performed to detect somatic mutations in the IFN-γ signal molecules. In recent reports, high-resolution genome wide array CGH analysis of human uLMS cases gave gene-level information about the amplified and deleted regions that may play a role in the development and progression of human uLMS. Among the most intriguing genes, whose copy number sequence was revealed by CGH, were loss of JAK1 (1p31-p32) and PSM9/B1i (6p21.3) [14,15]. The discovery of these mutational defects in a key cell-signal pathway may be an important development in the pathogenesis of human uLMS. The growth of JAK1-deficient cell lines is reportedly unaffected; similarly, the cell cycle distribution pattern of freshly explanted neoplasm cells derived from JAK1-deficient neoplasms shows no response to IFN-γ signal [23]. In recent study, the growth of the original human uLMS cell line, which had defective JAK1 activity, was unaffected by IFN-γ treatment [11]. In contrast, the growth of JAK1-transfected human uLMS cell line, which had strong exogenous JAK1 activity, was prevented by IFN-γ treatment [11]. Interestingly, when PSM9/B1i-transfected human uLMS cell line, which have marked PSM9/B1i expression, were analyzed, exogenous PSM9/B1i expression resulted in cell growth inhibition [11]. Conversely, the growth of PSM9/B1i-transfected human uLMS cell line was unaffected by IFN-γ treatment [11]. Taken together, IFN-γ response to cell growth inhibition may be attributable to the IFN-γγ inducibility of PSM9/B1i.

**Conclusion**

In conclusion, in this challenging clinical group of diseases early recognition and diagnosis of human uLMS is critical to improve patient outcomes. The defective expression of major histocompatibility complex (MHC)-related molecules, including the TAP1 and PSM9/B1i genes, is one of the biological mechanisms for evading host immune surveillance by neoplasm cells [24]. Recently, the incidence of IFN-γ unresponsiveness in human neoplasm was examined in several malignant neoplasms and revealed that approximately 33% of each group exhibited a reduction in IFN-γ sensitivity [25]. Nevertheless, the expression of PSM9/B1i, rather than providing an escape from immune surveillance, seems to play key role in the tumor suppressor for human uLMS. Defective expression of PSM9/B1i is likely to be one of the risk factors for the sarcomagenesis of human uLMS. Our colleague has been studying to establish preoperative diagnostic method with needle biopsy [26]. This role of PSM9/B1i as a tumor suppressor may lead to new preoperative diagnostic and therapeutic targets in human uLMS.

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The results revealed that the IFN-γ signal metrium tissue sections derived from wild-type, IFN-γ-deficient, at least 24 h of stimulation [27].

**Figure 1:** Key role of the IFN-γ-pathway in PSMB9/β1i expression in normal myometrium.

(a) IFN-γ treatment markedly increased the expression of PSMB9/β1i, a subunit of the immunoproteasome, which alters the proteolytic specificity of proteasomes. After binding of IFN-γ to the type II IFN receptor, which is constructed by two components, IFN-γ receptor subunit 1 (IFNGR1) and IFN-γ receptor subunit 2 (IFNGR2), Janus-activated kinase 1 (JAK1) and JAK2 are activated and phosphorylate the signal transducer and activator of transcription 1 (STAT1) on the tyrosine residue at position 701 (Tyr701) and the serine residue at position 727 (Ser727) [27]. Tyrosine phosphorylated STAT1 forms homodimers that translocate to the nucleus and bind IFN-γ-activated site (GAS) elements in the promoters of IFN-γ-regulated genes [39,40]. IFN-γ activated JAKs also regulate, through as yet unknown intermediates, activation of the catalytic subunit (p110) of phosphatidylinositol 3-kinase (PI3K). The activation of PI3K ultimately results in downstream activation of protein kinase C-δ (PKC-δ), which in turn regulates the phosphorylation of STAT1 on the Ser727. The phosphorylation of Ser727 is not essential for the translocation of STAT1 to the nucleus or for the binding of STAT1 to GAS in enhancer/promoter region of targeted DNA, but it is required for full transcriptional activation [28,29]. Tumor necrosis factor (TNF)-α is a multifunctional proinflammatory cytokine that belongs to the TNF superfamily. It signals through two distinct cell surface receptors, TNFR1 and TNFR2. NFkB is a transcription regulator that is activated by various intra and extra cellular stimuli including cytokines like TNF-α. The TNF-α induced NFkB activity involves the five mammalian NF-κB/Rel proteins. In the absence of TNF-α stimulation, NF-κB is associated with the inhibitor of kappa B (IκB) in the cytoplasm. TNF-α-induced activation of NF-κB largely relies on phosphorylation dependent ubiquitination and degradation of IκB proteins. The inhibitor of kappa B kinase (IKK) complex, a multiprotein kinase complex is responsible for the TNF-α induced phosphorylation of IκB. The free NF-κB translocates to the nucleus and induces expression of certain genes. TRADD adaptor molecule interacts with TNFR1 and recruits the additional adaptor proteins like RIP, TRAF2, and FADD, which in turn recruit additional key components to TNFR1 responsible for initiating downstream events and mediating programmed cell death signaling and NF-κB activation. The steady state of Psmb9 mRNA in macrophages or T cells was followed as a function of time after stimulation with 10 ng/ml TNF-α. Increased levels of mRNA were not detected until at least 24 h of stimulation [27]. (b) IHC experiments with myometrium tissue sections derived from wild-type, IFN-γ-deficient, and TNF-α-deficient mice (2 months old) were carried out [29]. (magnification x100) The results revealed that the IFN-γ signaling cascade was required for basal PSMB9/β1i expression.

**References**


13. NCBI's Conserved Domain Database and Search Service, v2.17 http://motif.genome.jp


