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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 31months. 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/? 1i in human uLMS that was traced to the IFN-γ pathway and the specific effect of somatic mutations of Janus kinase (JAK) 1molecule and/or promoter region on the locus cording 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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Abbreviations: LMA: Leiomyoma; uLMS: Uterine Leiomyosarcioma; PSMB9: Proteasome Beta Subunit 9; IFN-γ: interferon-γ, JAK: Janus Kinase, MMP-2: Matrix Metalloproteinase-2, TAP: Transporter Associated With Antigen Processing, ATP: Adenosine Triphosphate, IHC: Immunohistochemistry, ER: Estrogen Receptor, PR: Progesterone Receptor, TP53: Tumor Protein 53, STAT1: Signal Transducer and Activator of Transcription 1, CGH: Comparative Genomic Hybridization, BAX: BCL-2-Associated X protein, BCL: B-cell Lymphoma-2, c-KIT: Cellular v-KIT Hardy-Zuckerman 4 Feline Sarcoma Viral Oncogene Homolog, MIG-2: Mitogen-Inducible Gene-2, P16/INK4a: p16 Inhibits CDK4, P21/CIP1: p21 Cyclin-Dependent Kinase Inhibitor 1, P27/KIP1: p27 Kinase Inhibitor Protein 1, PTEN: Phosphatase And Tensin Homolog Deleted From Chromosome 10, MHC: Major Histocompatibility Complex

Introduction

Uterine sarcoma is a particularly poor prognostic neoplasm among gynecological neoplasms, and standard treatment is not established. One reason for this is that the frequency of occurrence is low, and it is difficult to conduct clinical trials. Most of the sarcoma occurs in the uterine body, and in the examination of 2,677 cases of uterine sarcoma that occurred in patients over the age of 35 years, uterine sarcoma was shown to account for 8% of the total uterine body malignancy [1]. About half of the sarcomas are carcinosarcoma, the majority of the remainder are occupied by leiomyosarcoma, endometrial stromal sarcoma, adenosarcoma. In domestic reports, 43 to 46% of uterine body sarcoma was carcinosarcoma, 36 to 38% was leiomyosarcoma, and 13 to 19% was endometrial stromal sarcoma [2]. Peak age of onset of leiomyosarcoma and endometrial stromal sarcoma are around 50 years old, whereas carcinosarcoma is relatively older at age 60 and older [2]. Fifty % survival time is 76 months for endometrial stromal sarcoma, while carcinosarcoma and leiomyosarcoma are 28 months and 31 months respectively [2]. In the case of uterine sarcoma, the frequency of onset is low and various forms are observed even with the same tissue type, so that histopathologic diagnosis of uterine sarcoma is often accompanied with great difficulty. However, since treatment plan decisions and prognostic predictions are largely responsible for histological diagnosis, it is important to confirm diagnosis by sharing information between gynecologists, radiologists and pathologists. Uterine leiomyosarcoma (uLMS) is the most common histological subtype of uterine sarcoma originating in the smooth muscles of the myometrium. It accounts for only 1% of all uterine malignancies; however, it contributes to a considerable proportion of uterine cancer deaths [3]. With poor biological characteristics, the overall 5-year survival rate for uLMS is only 25% [4]. The diagnostic criteria advocated by the group of Hendrickson and Kempson are widely used for histopathological diagnosis of leiomyosarcoma. In other words, (1) cell type, (2) fission (index), and (3) coagulation necrosis are evaluated comprehensively. In the case of excisable cases, primary treatment of leiomyosarcoma is basically based on abdominal simple hysterectomy and bilateral adnexectomy. There is no clear evidence that expansion surgery or addition of lymph node dissection will improve prognosis. In the Phase III study, the effectiveness of radiation therapy or chemotherapy as a postoperative treatment has not been shown at present [5]. At a relatively early stage, leiomyosarcoma is susceptible to hematogenous metastasis to the lungs and liver. However, the metastasis to the retroperitoneal lymph node is as low as 6 to 11% compared with endometrial cancer, carcinosarcoma, and endometrial stromal sarcoma. Targeted therapy has been developed rapidly in recent years and it is expected to be a promising treatment for uLMS [5,6]. Thus, it is necessary to explore the molecular aetiology and pathogenesis of uLMS and to search for therapeutic molecular targets.

It is noteworthy that, when adjusting for stage and mitotic count, human uLMS 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 shown to develop uLMS-like neoplasms, with a disease prevalence of 37% by 14 months of age [9,10]. Histopathological examinations of PSMB9/β 1i-deficient uterine neoplasms revealed common characteristic abnormalities of human uLMS. Defective expression of PSMB9/β 1i 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/β 1i 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/β 1i expression in human carcinoma cell lines and human lymphocytes (Figure 1). However, the essential signaling pathway for PSMB9/β 1i 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/β 1i 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/β 1i 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/β 1i. 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/β 1i* 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/β1i 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/β1i following IFN-γ treatment [11]. Furthermore, the IHC experiments revealed a serious loss in the ability to induce expression of PSMB9/β1i 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/β1i, 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/β1i. In addition, the expression level of PSMB9/β1i 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 quadrate 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 neoplasms. Therefore, the molecular examinations of 23 human uLMS tissue regions and normal tissue regions located in the tissue sections obtained from individual patients were performed to detect somatic mutations in the IFN-γ pathway, i.e. JAK1, JAK2, signal transducer and activator of transcription 1 (STAT1) and promoter region of *PSMB9/*β*1i* gene (Figure 1). As the catalytic domains of these IFN-γ signal molecules are most likely to harbor mutations that inactivate the gene product, we focused on stretches (exons) containing the kinase domains, transcriptional activation domains and enhancer/promoter region. Over all, nearly 43.5% (10/23) of human uLMS tissues had serious mutations in the ATP binding region or kinase-specific active site of JAK1; furthermore, 43.5% (10/23) of human uLMS tissues had serious mutations in transcriptional activation sites of the promoter region of *PSMB9/*β*1i* gene, which is required for transcriptional activation of *PSMB9/*β*1i* gene. No somatic mutation in essential sites, e.g. Tyr701 and Ser727, which are required for physiological function of STAT1 as transcriptional 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/β1i (6p21.3) [14,15]. It has also been demonstrated that a correlation exists between 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/β1i (6p21.3) also harbored one nonsense mutation and one deletion, suggesting a possible homozygous loss of function. The discovery of these mutational defects in a key signal pathway may be important in understanding the pathogenesis of human uLMS.

All current therapies for uLMS have some limitations because of its high rates of recurrence and metastasis. Therefore, the recently emerging targeted therapy shows importance. Bioinformatics analysis of data from high-throughput sequencing and microarrays can accurately reveal the potential molecular mechanisms of the development of uLMS and predict therapeutic targets by comparing sarcoma lesions with normal tissues. At present, surgical intervention is virtually the only means of treatment for uLMS [5,6]. Although adjuvant pelvic irradiation appears to decrease the rate of local recurrence, adjuvant therapy does not appear to significantly improve survival. Furthermore, gynecological cancer, 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 prognosis 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. PSMB9/β1i-dificient 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 PSMB9/β1i 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 PSMB9/β1i, 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 PSMB9/β1i 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 homozygous 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 PSMB9/β1i (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 PSMB9/β1i-transfected human uLMS cell line, which have marked PSMB9/β1i expression, were analyzed, exogenous PSMB9/β1i expression resulted in cell growth inhibition [11]. Conversely, the growth of PSMB9/β1i-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 PSMB9/β1i.

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 *PSMB9/β1i* 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 neoplasms 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 PSMB9/β1i, rather than providing an escape from immune surveillance, seems to play key role in the tumor suppressor for human uLMS. Defective expression of PSMB9/β1i 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 PSMB9/β1i as a tumor suppressor may lead to new preoperative diagnostic and therapeutic targets in human uLMS.

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Figures

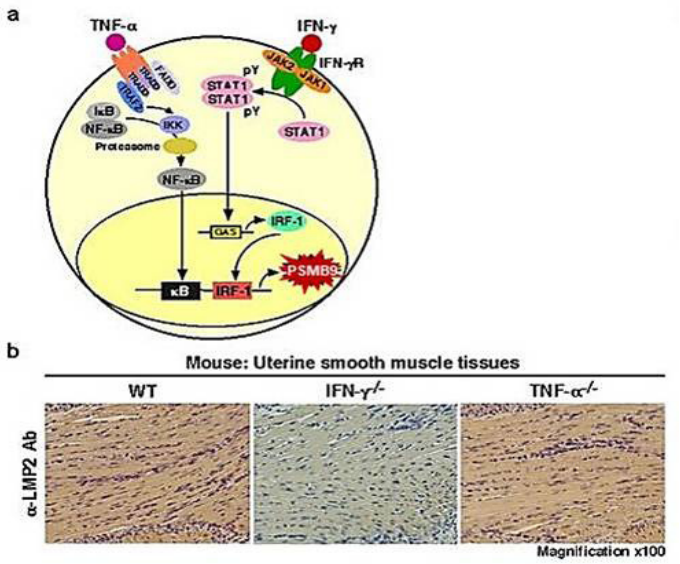

Figure 1: Key role of the IFN-γ-pathway in PSMB9/β1i expression in normal myometrium.
(a) IFN-γ treatment markedly increased the expression of PSMB9/β1i, a subunit of the immunoproteasome, which alters the proteolytic specificity of proteasomes. After binding of IFN-γ to the type II IFN receptor, which 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 translocate 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

1. Brooks SE, Zhan M, Cote T, Baquet CR. Surveillance, epidemiology, and end results analysis of 2,677 cases of uterine sarcoma 1989-1999. Gynecol Oncol. 2004; 93: 204-208.
2. Fujita H, Adachi S, Kikkawa J, Sugiyama T, Takeuchi S. Clinicopathological examination of uterine sarcoma - KCOG retrospective study-. Progress of obstetrics and gynecology. 2004; 56: 463-465.
3. Ricci S, Stone RL, Fader AN. Uterine leiomyosarcoma: Epidemiology, contemporary treatment strategies and the impact of uterine morcellation. Gynecol Oncol. 2017; 145: 208-216.
4. Park JY, Lee JW, Lee HJ, Lee JJ, Moon SH, et al. Prognostic significance of preoperative ^{18}F-FDG PET/CT in uterine leiomyosarcoma. J Gynecol Oncol. 2017; 28:e28.
5. Cui RR, Wright JD, Hou JY. Uterine leiomyosarcoma: A review of recent advances in molecular biology, clinical management and outcome. BJOG. 2017; 124: 1028-1037.
6. Wu TI, Chang TC, Hsueh S, Hsu KH, Chou HH, et al. Prognostic factors and impact of adjuvant chemotherapy for uterine leiomyosarcoma. Gynecol Oncol. 2006; 100: 166-172.
7. Miettinen M, Fetsch JF. Evaluation of biological potential of smooth muscle tumours. Histopathology. 2006; 48: 97-105.
8. Bodner-Adler B, Bodner K, Czerwenka K, Kimberger O, Leodolter S, et al. Expression of p16 protein in patients with uterine smooth muscle tumors: an immunohistochemical analysis. Gynecol Oncol. 2005; 96: 62-66.
9. Van Kaer L, Ashton-Rickardt PG, Eichelberger M, Gaczynska M, Nagashima K, et al. Altered peptidase and viral-specifi c T cell response in LMP2 mutant mice. Immunity. 1994; 1: 533-541.
10. Hayashi T, Faustman DL. Development of spontaneous uterine tumors in low molecular mass polypeptide-2 knockout mice. Cancer Res. 2002; 62: 24-27.
11. Hayashi T, Kobayashi Y, Kohsaka S, Sano K. The mutation in the ATP binding region of JAK1, identified in human uterine leiomyosarcomas, results in defective interferon-gamma inducibility of TAP1 and LMP2. Oncogene. 2006; 25: 4016-4026.
12. MOTIF Search profiling. http://motif.genome.jp
13. NCBI's Conserved Domain Database and Search Service, v2.17 analysis. http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml
14. Larramendy ML, Kaur S, Svarvar C, Bo¨hling T, Knuutila S. Gene copy number profiling of soft-tissue leiomyo- sarcomas by array comparative genome hybridization. Cancer Genet Cytogen. 2006; 169: 94-101.
15. Svarvar C, Larramendy ML, Blomqvist C, Gentile M, Koivisto-Korander R, et al. Do DNA copy number changes differentiate uterine from nonuterine leiomyosarcomas and predict metastasis? Modern Pathol. 2006, 19: 1068-1082.
16. Hayashi T, Horiuchi A, Sano K, Hiraoka N, Ichimura T, et al. Potential diagnostic biomarkers: LMP2/β1i and Cyclin B1 differential expression in human uterine mesenchymal tumors. Tumori. 2014; 100: 509-516.
17. Ylisaukko-oja SK, Kiuru M, Lehtonen HJ, Lehtonen R, Pukkala E, et al. Analysis of fumarate hydratase mutations in a population-based series of early onset uterine leiomyosarcoma patients. Int J Cancer. 2006; 119: 283-287.
18. Jones PA. Baylin SB. The epigenomics of cancer. Cell. 2007; 128: 683-692.

19. Pajares MJ, Ezponda T, Catena R, Calvo A, Pio R, et al. Alternative splicing: an emerging topic in molecular and clinical oncology. Lancet Oncol 2007; 8: 349-357.

20. Ludmil B, Alexandrov LB, Ju YS, Haase K, Van Loo P, Martincorena I, et al. Mutational signatures associated with tobacco smoking in human cancer. Science. 2016; 354: 618-622.

21. Hayashi T, Kawano M, Ichimura T, Kasai M, Ida K, et al. Gene analysis of Interferon-γ signal molecules in human uterine leiomyosarcoma. WULFENIA Journal. 2016; 23: 01-18.

22. Post SM. Mouse models of sarcomas: critical tools in our understanding of the pathology. Clinical Sarcoma Res 2012; 2: 20.

23. Sexl V, Kovacic B, Piekorz R, Moriggl R, Stoiber D, et al. Jak1 deficiency leads to enhanced Abelson-induced B-cell tumor formation. Blood. 2003; 101: 4937-4943.

24. Cabrera CM, Jimenez P, Cabrera T, Esparza C, Ruiz-Cabello F, et al. Total loss of MHC class I in colorectal tumors can be explained by two molecular pathways: beta2-microglobulin inactivation in MSI-positive tumors and LMP7/TAP2 downregulation in MSI-negative tumors. Tissue Antigens. 2003; 61: 211-219.

25. Kaplan DH, Shankaran V, Dighe AS, Stockert E, Aguet M, et al. Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. Proc Natl Acad Sci USA. 1998; 95: 7556-7561.

26. Murakami M, Ichimura T, Kasai M, Matsuda M, Kawamura N, Fukuda T, Sumi T. Examination of the use of needle biopsy to perform laparoscopic surgery safely on uterine smooth muscle tumors. Oncol Lett. 2018; 15: 8647-8651.

27. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol. 2005; 5: 375-386.

28. Futreal PA, Coin L, Marshall M, Down T, Hubbard T, et al. A census of human cancer genes. Nat Rev Cancer. 2004; 4: 177-183.

29. Hayashi T, Horiuchi A, Sano K, Hiraoka N, Kanai Y, et al. Potential role of LMP2 as tumor-suppressor defines new targets for uterine leiomyosarcoma therapy. Scientific Reports. 2011; 1: 180.