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REVIEW

Molecular Mechanisms of Uterine Leiomyosarcomas: Involvement of Defective LMP2 Activation

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Abstract. Patients with uterine leiomyosarcoma (LMS) typically present with vaginal bleeding, pain, and a pelvic mass. Typical presentations with hypercalcemia or eosinophilia have been reported. Radiographic evaluation with combined positron emission tomography/computed tomography may assist in the diagnosis and surveillance of women with uterine LMS. Stage and tumor grade continue to appear to be valid prognostic indicators. A recently developed risk-assessment index is highly predictive of disease-specific survival. Ovarian preservation does not appear to negatively impact outcome, and the addition of adjuvant therapy after surgical treatment do not seem to improve survival. It is noteworthy that LMP2-deficient mice exhibit spontaneous development of uterine LMS with a disease prevalence of ~37% by 12 months of age. Furthermore, a recent report demonstrated the loss of ability to induce LMP2 expression, which is an interferon-γ (IFN-γ) inducible factor, in human uterine LMS tissues. We analyzed human uterine LMS for genetic mutation in the IFN-γ signal cascade and found serious mutations in three genes. Molecular experiments demonstrated cytokine differential expression, especially IL-8, which directly regulated tumor cell migration or invasion, by LMP2 expression. The discovery of this mutational activation of a key cell-signaling pathway and cytokine differential expressions may provide new targets for diagnostic approaches and therapeutic intervention.

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INTRODUCTION

Smooth muscle tumors (SMTs) have been traditionally divided into benign leiomyomas (LMA) and malignant leiomyosarcomas (LMS) based on cytological atypia, mitotic activity and other criteria. Uterine LMS, which are some of the most common neoplasms of the female genital tract, are relatively rare SMTs, having an estimated annual incidence of 0.64 per 100,000 women [1]. They account for approximately one-third of uterine sarcomas and 1.3% of all uterine malignancies, and are considered to be aggressive malignancies with a five-year survival rate of only 50% for tumors confined to the uterus [2,3]. Generally, patients with uterine LMS typically present with vaginal bleeding, pain, and a pelvic mass. It is noteworthy that, when adjusting for stage and mitotic count, LMS has a significantly worse prognosis than carcinosarcoma [4]. As uterine LMS is resistant to chemotherapy and radiotherapy, and thus surgical intervention is virtually the only means of treatment for this disease [5-7], developing an efficient adjuvant therapy is expected to improve the prognosis of the disease. Matrix metalloproteinases (MMP), which degrade components of the extracellular matrix, appear to participate in tumor invasion and metastasis. A trend towards prolonged disease-free survival is seen in patients with MMP-2 negative tumors [8, 9]. Although typical presentations with hypercalcemia or eosinophilia have been reported, this clinical abnormality is not an initial risk factor for uterine LMS. To the best of our knowledge, little is known regarding the biology of uterine leiomyosarcomas; therefore, the risk factors that promote the initial development of uterine LMS and regulate their growth *in vivo* remain poorly understood.

Our reports demonstrate that proteasome subunit LMP2-deficient mice exhibit spontaneous development of uterine LMS [10]. Defective LMP2 expression is likely to be one of the risk factors for the development of human uterine LMS, as it is in LMP2-deficient mice [11]. As there is no effective therapy for unrespectable human uterine LMS, our research findings may lead to specific molecular therapies to treat this disease.

LMP2-DEFICIENT MICE EXHIBIT THE SPONTANEOUS DEVELOPMENT OF UTERINE LMS

As a significant effect was achieved for several types of cancer including breast cancer using antibody therapies directed against tumor-specific antigens, molecular targeting is regarded as a promising strategy for the treatment of malignant tumors [12]. Although gynecological cancers, for instance breast cancer and endometrial carcinomas, are strongly promoted by female hormones, the rate of estrogen receptor and progesterone receptor expression is reported to be significantly less in uterine LMS compared with normal uterine smooth muscle (USM). These low receptor expressions were found to not correlate with the promotion of initial disease development or with overall survival [13]. As apoptotic mechanisms have also been implicated in many human cancers, investigating the dysregulation of the expression of apoptotic and/or cell cycle regulators in uterine LMS is required to identify molecular pathways that could possibly be important in the development of human uterine LMS. Though the significant differential expression of apoptotic and cell cycle regulatory factors including initiation factor in human uterine LMS, such as bcl-2, bax, p16, p21, p27, c-kit, MDM2 and p53, have all been reported and compared to normal USM, there exists no scientific evidence to show that abnormal expression of these factors directly correlates to the initiation and promotion of uterine LMS [14-21]. Unfortunately, the apoptotic and cell cycle regulatory factor expression profiles of uterine LMS are not yet useful for clinical prognostic purposes.

The targeted disruption of LMP2 results in the impairment of tissue- and substrate-dependent proteasome activation [22]. We reported that LMP2$^{-/-}$ mice were prone to the development of uterine neoplasms [10] (Fig. 1). The percentage of mice with overt tumors increased with age after six months, with a cumulative prevalence of disease in female mice of 37% by 12 months of age and no apparent plateau at this late observation time. LMS was observed in LMP2-/- female mice but not in C57BL/6 mice

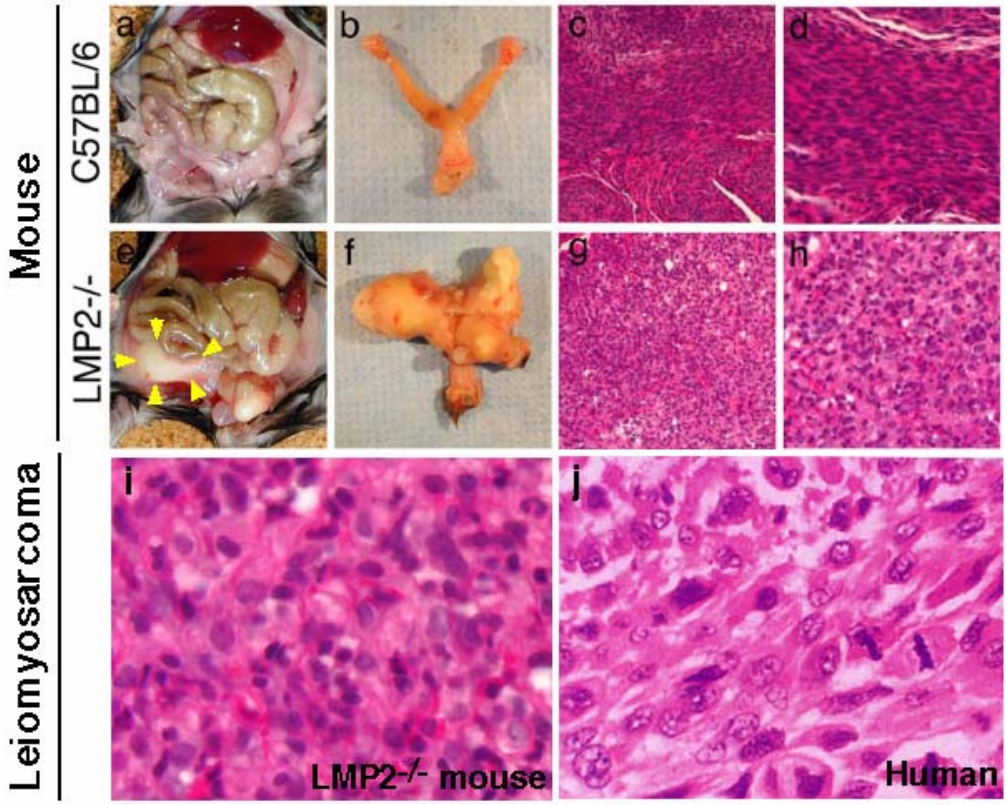


Figure 1 Development of uterine neoplasms in LMP2$^{-/-}$ mice. Uterine neoplasms in LMP2$^{-/-}$ mice. a and e, abdominal cavities of female C57BL/6 (a) and LMP2$^{-/-}$ (e) mice, showing a uterine tumor (outlined by yellow arrowheads) in the latter. b and f, female genital organs of C57BL/6 (b) and LMP2$^{-/-}$ (f) mice, showing a uterine neoplasm in the latter. c, d, g, h, histologycal analysis showing the normal smooth muscle cells of the uterus of C67BL/6 mice (c and d) and the abnormal cells of a leiomyosarcoma of the uterus of LMP2$^{-/-}$ mice (g and h). c and g, 200x. d and h, 400x. Comparison between human uterine leiomyosarcoma and LMP2$^{-/-}$ mice uterine leiomyosarcoma. I and j, histologycal analysis showing the abnormal cells of a leiomyosarcoma of LMP2-/-mice (i) and the uterus of human (j). i and j, 800x.

with the same genetic background as LMP2-/- mice (Fig. 1). Histological examinations of LMP2$^{-/-}$ uterine neoplasms revealed common characteristic abnormalities of uterine LMS (Fig. 1). The tumors lacked lymphoid infiltrates, which is a sign of immune recognition, and consisted of uniform elongated USM cells arranged into bundles. The nuclei of the tumor cells varied in size and shape; furthermore, mitosis was frequent. In contrast, the USM cells of C57BL/6 mice were normal in appearance, and relatively few Ki-67-positive cells, the proliferating cells of solid tumors, were observed in the basal cell layer of normal USM, whereas most of the basal cells vividly expressed Ki-67 in LMP2$^{-/-}$ mice [10]. These histopathological examinations indicate the abnormal proliferation of LMP2$^{-/-}$ USM cells in the basal cell layer of normal USM. In LMP2$^{-/-}$ mice, proteasomal peptidase activity against hydrophobic and basic substrates but not acidic substrates was lower in the spleen and liver from mutant mice compared with wild-type mice, but differences in the muscle and brain were not significant. Furthermore, flow cytometric analysis showed no difference in the expression of MHC class I molecules. Importantly, spontaneous uterine LMS was specially detected, but no other cancer progression was observed at high

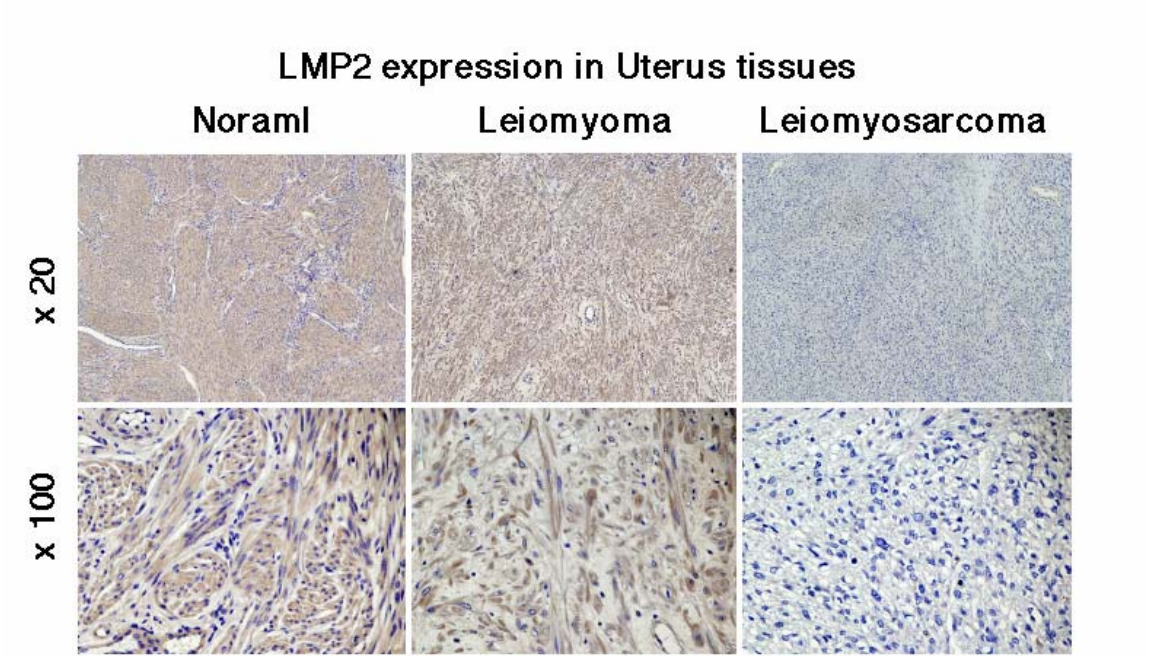


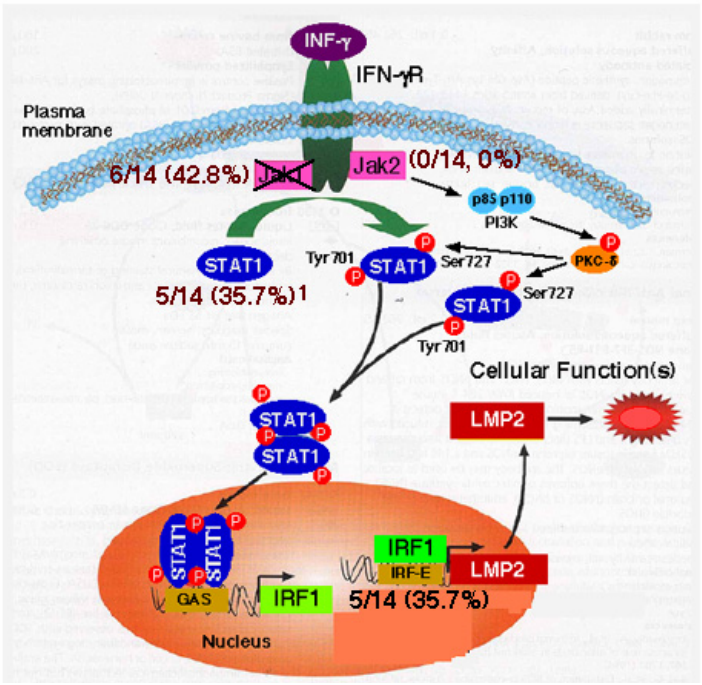
Figure 2 Defect in LMP2 expression in human uterine leiomyosarcoma tissue. Immunohistchemistry of LMP2 expression in human normal uterine smooth muscle, human uterine leiomyoma tissues and uterine leiomyosarcoma.

and low incidences in both male and female LMP2$^{-/-}$ mice; therefore, LMP2 expression, rather than providing an escape from immune surveillance, seems to play an important role in the spontaneous development of uterus LMS [10].

CORRELATION BETWEEN DEFECT IN LMP2 EXPRESSION AND HUMAN UTERINE LMS

Several reports, including experiments with LMP2$^{-/-}$ mice, suggest that INF-γ-induced restoration of antigen-processing machinery improves anti-tumor-specific antigen CTL recognition in some patients; thus, approaches to activate this pathway may be of benefit to patients with LMP2 deficiency. Furthermore, it must therefore be demonstrated whether human uterine LMS shows a weak expression of LMP2. The effects of IFN-γ on LMP2 expression were examined by immunoblotting with five cell lines [11]. LMP2 expression were not markedly induced by IFN-γ treatment in the human uterine LMS cell lines "SKN" and "RKN", although HeLa and HeLa.S3, cervix epithelial adenocarcinoma cell lines, and normal human uterus smooth muscle cells (Hu.USMC), underwent strong induction of LMP2 following IFN-γ treatment [11]. Furthermore, our experiments, which were individually performed at several medical facilities, revealed a serious loss in the ability to induce LMP2 expression in human uterine LMS tissues in comparison with normal USM tissues located in same tissue sections [11]; normal total: 21 cases, LMA total 24 cases, LMS total: 29 cases (Fig. 2) as well as the SKN human uterine LMS cell line treated with IFNγ, similar to other primary uterine LMS cells established from LMS patients in medical facilities [11]. In addition, immunohistochemistry shows marked LMP2 expression in cervix epithelial adenocarcinoma tissues as well as HeLa and HeLa.S3 cell lines treated with IFN-γ (Fig. 2).

The defect was localized to JAK1 activation, which acts upstream in the IFN-γ signal pathway since IFN-γ-treatment could not strongly induce JAK1 kinase activity in SKN and RKN cells. Sequence analysis identified a serious G781E mutation in the ATP binding region, which is required for JAK1 kinase activation, and additional experiments demonstrated that the



Mutations in IFN-γ signalling pathway

	N	LMP2
Normal	21	++++
Leiomyoma	24	++++
Endometrial stromal sarcoma	6	+/-
Leiomyosarcoma	29	-

[1]Mutations are observed in the internal molecule of STAT1, which is not yet reported kinase activation domain, in 5 individual uterine leiomyosarcoma tissues.

Figure 3 **The interferon-γ signaling pathway and mutations in its components found in human uterine leiomyosarcoma. After binding of interferon-γ (IFN-γ) to the type II IFN receptor, Janus activated kinase 1 (JAK1) and JAK2 are activated and phosphorylate STAT1 (signal transducer and activator of transcription 1) on the tyrosine residue at position 701 (Tyr701). The tyrosine-phosphorylated form of STAT1 forms homodimers that translocate to the nucleus and bind GAS (IFN-γ-activated site) elements, which are present in the promoters of IFN-γ-regulated genes. The 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 phosphorylation of STAT1 on the serine residue at position 727 (Ser727). The phosphorylation of Ser727 is not essential for the translocation of STAT1 to the nucleus or for the binding of STAT1 to DNA, but it is required for full transcriptional activation. IFNGR1, IFN-γ receptor subunit 1; IFNGR2, IFN-γ receptor subunit 2. Investigation of human uterine LMS tissues (total of 14 cases of LMS tissue sections and normal tissue sections located in same tissue) for somatic mutations in the IFN-γ signal cascade, JAK1, JAK2, STAT1 and Lmp2 promoter region. Overall, nearly 42.9% (6/14) of uterine LMS tissues had serious mutations in kinase active domain of JAK1. Furthermore, 35.7% (5/14) of uterine LMS tissues had serious mutations in important sites of the Lmp2 promoter region. Nearly 36% (5/14) of uterine LMS tissues had mutations in the intermolecular region. of STAT1. A drastic loss in ability to induce LMP2 expression in human uterine LMS tissues compared with normal leiomyoma (LMA) tissues located in the same sections in a total of 21 normal cases, 24 LMA cases, 29 LMS cases, as well as in SKN cell lines treated with IFN-γ. As such, defective LMP2 expression is likely to be one of the risk factors in the development of human uterine LMS, as it is in the LMP2-deficient mice.**

loss of IFN-γ responsiveness in SKN cells was attributable to the inadequate kinase activity of JAK1 due to a G781E mutation [11].

MUTATIONS IN IFN-Γ SIGNALLING PATHWAY IN HUMAN LMS TISSUE

IFN-γ treatment markedly increased the expression of LMP2, 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) [23, 24]. Tyrosine-phosphorylated STAT1 forms homodimers that translocate to the nucleus and bind GAS (IFN-γ-activated site) elements, which are present in the promoters of IFN-γ-regulated

genes [23, 24]. IFN-γ-activated JAKs also regulate, through as-yetunknown 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 serine residue at position 727 (Ser727). The phosphorylation of Ser727 is not essential for the translocation of STAT1 to the nucleus or for the binding of STAT1 to DNA, but it is required for full transcriptional activation [23, 24].

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 cancers [25-31]; Hayashi et al. MHR 2:45 PM 07.2.21 therefore, we analysed human uterine LMS tissues (14 LMS tissue sections and normal tissue sections located in the same tissue) for somatic (tumor-specific) mutations in IFN-γ signal cascade, JAK1, JAK2, STAT1 and Lmp2 promoter region. Overall, nearly 42.9% (6/14) of uterine LMS tissues had serious mutations in the ATP binding region or kinase-specific active site of JAK1; furthermore, 35.7% (5/14) of uterine LMS tissues had serious mutations in important sites of the Lmp2 promoter region, which is required for transcriptional activation (Fig. 3). Nearly 35.7% (5/14) of uterine LMS tissues had mutations in the STAT1 intermolecular region, which is not yet reported to be important for biological function as transcriptional activation. Although the genetic approach already addressed that marked JAK2 activation causes myelo-and lymphoproliferative disease, polycythemia vera or myelogenous leukemia [32-38], no serious mutation in the ATP-binding region and kinase-specific active site of JAK2 was detected in uterine LMS (Fig. 3). In a recent report, high-resolution genome-wide array comparative genomic hybrodization (CGH) analysis of LMS cases gave gene-level information about the amplified and deleted region that may play a role in the development and progression of human uterine LMS. Among the most intriguing genes, whose copy number sequence was revealed to be altered by CGH, were JAK1 (1p31~p32) and LMP2 (6p21.3) [39, 40]. The discovery of these mutational defects of a key cell-signalling pathway may be an important development in the pathogenesis of human uterine LMS. Our research experiments first showed that JAK1 mutation correlated to the development of human uterine LMS.

It is probable that the list of new elements involved in IFNs-mediated signaling will continue to grow during the next few years, whereas the contributions of known pathways might need to be reevaluated. At present, it seems that the activation of more than one signaling pathway is required for the generation of different biological properties of IFNs, and no signaling cascade alone is sufficient for the generation of any given biological end-point. For example, the biological functions of the STAT-, NF-κB-and p38-signalling pathways are required for the antiviral effects or anti-tumor effects of IFNs, but the activation of these pathways alone is not sufficient to elicit an antiviral or anti-tumor response [41, 42]. Such a requirement for multiple signaling pathways also seems to be the case for IFNs-dependent anti-proliferative responses, and it might reflect the synergistic effects of various signals at the levels of gene transcription and mRNA translation; therefore, more additional genetic analysis is required to completely elucidate the mutational activation of a key cell-signaling pathway in human uterine LMS.

POTENTIAL ROLE OF ANTI-ONCOGENIC FUNCTION BY LMP2

The growth of cell lines with JAK1 kinase activity has been demonstrated to be strongly inhibited by IFN-γ treatment, whereas the growth of JAK1-deficient cell lines is unaffected [43]. Similarly, the cell cycle distribution pattern of freshly explanted tumor cells derived from Jak1-deficient tumors shows no response to IFN-γ treatment [43]. In our study, the growth of original SKN cells, which had defective JAK1 activity, was unaffected by IFN-γ treatment (population doubling time (P.D.T.) = 15.2 hrs) [11]. In contrast, the growth of JAK1-transfected SKN cells, which had strong exogenous JAK1 activity, was prevented by IFN-γ treatment (P.D.T. = 18.1 hrs). Interestingly, when LMP2-transfected SKN cells, which have marked LMP2 expression, were ana-

lyzed, exogenous LMP2 expression resulted in cell growth arrest (P.D.T. = 17.9 hrs) [11]. Conversely, the growth of LMP2-transfected SKN cells was unaffected by IFN-γ treatment (P.D.T. = 18.0 hrs). In SKN-Lmp2 transfectants, there is a correlation between the levels of exogenous LMP2 expression and the degrees of suppression of the transformed phenotype including migration/invasion. The biological function of LMP2 with revertant-inducing activity on SKN cells has been demonstrated.

Microarray analysis provides insight into the gene expression changes associated with malignant transformation. To investigate whether stable LMP2 expression causes cell growth arrest and loss of migration in SKN cells, we compared the gene expressions (using Affymetrix human GeneChip HG U133 Plus2.0) of SKN cells and LMP2-transfected SKN cells. We analyzed the expression profile of SKN cells transfected with plasmid without insert (pCEP9) compared with LMP2 coding DNA (pCEP9-LMP2). Microarray analysis results show that LMP2 expression influences the expression pattern of cytokines and chemokines, especially the down-regulation of interleukin-8 (IL-8). IL-8, which is one of a family of 13 human CXC chemokines, induces the migration/invasion of tumor cells from the primary site breakdown of the basal membrane, and then tumor cells enter the surrounding tissues. Taken together, it is likely that LMP2 deficiency results in marked IL-8 expression and then induces cell migration/invasion. In breast cancer, the alterations in IL-8 expression become progressively worse with increasing stage and grade of the tumor [44, 45]. We therefore investigated IL-8 as a determinant of the virulence and metastatic potential of human uterine LMS. Levels of total IL-8 expression in uterine LMS correlated strongly with survival and malignancy in patients with uterine LMS, suggesting its potential use as a prognostic marker in uterine LMS as well as breast cancer.

The down-regulation of MHC expression, including the *lmp2* gene, is one of the biological that mechanisms tumor cells use to evade host immune surveillance [46,47]. Recently, the incidence of IFN-γ unresponsiveness in human tumors was examined in several cancers, and revealed that approximately 33% of each group exhibited a reduction in IFN-γ sensitivity [48]. Nevertheless, LMP2 expression, rather than providing an escape from immune surveillance, seems to play an important role in the negative regulation of uterine LMS cell growth. Defective LMP2 expression is likely to be a risk factor for the development of human uterine LMS, as it is in LMP2-deficient mice.

CONCLUSION

Uterine LMS is a disease with extremely poor prognosis, highly aggressive, and resistant to chemotherapy. At present, surgical intervention is virtually the only means of treatment for uterine LMS [49, 50]. 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 estrogen receptor and progesterone receptor expression is reported to be significantly less in human uterine LMS compared with normal USM cells. These low receptor expressions were found to not correlate with the promotion of initial disease development or with the overall survival of patients with uterine LMS; however, molecular targeting therapies against tumors have recently shown remarkable achievements [51]. To improve the prognosis of human uterine LMS, recent research experiments were performed to search for the key role of pro-oncogenic factors or anti-oncogenic factors that play an important function in their pathogenesis and that could serve as molecular target for tumor treatment. For this purpose, several research facilities conducted a cDNA microarray procedure between human uterine LMS and normal USM and showed that several known pro-oncogenic factors and other factors, such as brain-specific polypeptide PEP-19 and a transmembrane tyrosine kinase receptor, c-kit, may be associated with the pathogenesis of human uterine LMS [52-54]. However, in terms of the tumorgenesis of human uterine LMS, 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 mechanisms has also been implicated in many human cancers. Although the significant differential expression of apoptotic and cell cycle regulatory factors in human uterine LMS have all been reported and compared to normal USM cells, there exists no scientific evidence to show that abnormal expression of these factors directly correlates to the initiation and promotion of human uterine LMS.

LMP2-deficient mice exhibit the development of uterine LMS with a disease prevalence of 37% by 12 months of age [10]. Furthermore, our experiments, which were individually performed at several medical facilities, revealed a serous loss in the ability to induce LMP2 expression in human uterine LMS tissues in comparison with normal USM tissues. The discovery of serious mutational defects of the IFN-γ-signaling pathway, which is the key cell-signaling pathway for LMP2 expression, may be an important development in the pathogenesis of human uterine LMS. It is noteworthy that stable LMP2 expression contributes to normal cell cycle regulation and migration due to the down-regulation of IL-8 expression, which directly mediates tumor migration/invasion and metastasis. Recent advances in our understanding of the biology of uterine LMS have concentrated on serious mutations in the IFN-γ-signaling pathway and defects in LMP2 expression. Loss of LMP2 expression may directly contribute to tumor invasion and metastasis. The continued improvement in our knowledge of the molecular biology of uterine LMS may ultimately lead to novel therapies and improved outcome.

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REFERENCES

1. **Zaloudek C, Hendrickson MR** [2002] Mesenchymal tumors of the uterus, in Kurman RJ (editor): Blaustein's Pathology of the Female Genital Tract (ed 5). New York, Springer-Verlag, pp. 561-578.
2. **Gadducci A, Landoni F, Sartori E, Zola P, Maggino T, Lissoni A, Bazzurini L, Arisio R, Romagnolo C, Cristofani R** [1996] Uterine leiomyosarcoma: analysis of treatment failures and survival. Gynecol Oncol 62: 25-32.
3. **Nordal R, Thoresen S** [1997] Uterine sarcomas in Norway 1956-1992: incidence, survival and mortality. Eur J Cancer 33: 907-911.
4. **Miettinen M, Fetsch JF** [2006] Evaluation of biological potential of smooth muscle tumours. Histopathology 48: 97-105.
5. **Brooks SE, Zhan M, Cote T, Baquet CR** [2004] Surveillance, epidemiology, and end results analysis of 2677 cases of uterine sarcoma 1989-1999. Gynecol Oncol 93: 204-208.
6. **Dusenbery KE, Potish RA, Judson P** [2004] Limitations of adjuvant radiotherapy for uterine sarcomas spread beyond the uterus. Gynecol Oncol 94: 191-196.
7. **Wu TI, Chang TC, Hsueh S, Hsu KH, Chou HH, Huang HJ, Lai CH** [2006] Prognostic factors and impact of adjuvant chemotherapy for uterine leiomyosarcoma. Gynecol Oncol 100: 166-172.
8. **Bodner-Adler B, Bodner K, Kimberger O, Czerwenka K, Leodolter S, Mayerhofer K** [2003] MMP-1 and MMP-2 expression in uterine leiomyosarcoma and correlation with different J Soc Gynecol Investig 10: 443-446.
9. **Bodner-Adler B, Bodner K, Kimberger O, Czerwenka K, Leodolter S, Mayerhofer K** [2004] Expression of matrix metalloproteinases in patients with uterine smooth muscle tumors: An immunohistochemical analysis of MMP-1 and MMP-2 protein expression in leiomyoma, uterine smooth muscle tumor of uncertain malignant potential, and leiomyosarcoma. J Soc Gynecol Investig 11: 182-186.
10. **Hayashi T, Faustman DL** [2002] Development of spontaneous uterine tumors in low molecular mass polypeptide-2 knockout mice. Cancer Res 62, 24-27.
11. **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-gamma inducibility of TAP1 and LMP2. Oncogene 25: 4016-4026.

12. **Dusenbery KE, Potish RA, Argenta PA, Judson PL** [2005] On the apparent failure of adjuvant pelvic radiotherapy to improve survival for women with uterine sarcomas confined to the uterus. Am J Clin Oncol 28: 295-300.
13. **Leitao MM, Soslow RA, Nonaka D, Olshen AB, Aghajanian C, Sabbatini P, Dupont J, Hensley M, Sonoda Y, Barakat RR, Anderson S** [2004] Tissue microarray immunohistochemical expression of estrogen, progesterone, and androgen receptors in uterine leiomyomata and leiomyosarcoma. Cancer 101: 1455-1462.
14. **Zhai YL, Kobayashi Y, Mori A, Orii A, Nikaido T, Konishi I, Fujii S** [1999] Expression of steroid receptors, Ki-67, and p53 in uterine leiomyosarcomas. Int J Gynecol Pathol 18: 20-28.
15. **Miyajima K, Tamiya S, Oda Y, Adachi T, Konomoto T, Toyoshiba H, Masuda K, Tsuneyoshi M** [2001] Relative quantitation of p53 and MDM2 gene expression in leiomyosarcoma; real-time semi-quantitative reverse transcription-polymerase chain reaction. Cancer Lett 164: 177-188.
16. **Kato K, Shiozawa T, Mitsushita J, Toda A, Horiuchi A, Nikaido T, Fujii S, Konishi I** [2004] Expression of the mitogen-inducible gene-2 (mig-2) is elevated in human uterine leiomyomas but not in leiomyosarcomas. Hum Pathol 35: 55-60.
17. **Kelley TW, Borden EC, Goldblum JR** [2004] Estrogen and progesterone receptor expression in uterine and extrauterine leiomyosarcomas: an immunohistochemical study. Appl Immunohistochem Mol Morphol 12: 338-341.
18. **Raspollini MR, Pinzani P, Simi L, Amunni G, Villanucci A, Paglierani M, Taddei GL, et al.** [2005] Uterine leiomyosarcomas express KIT protein but lack mutation(s) in exon 9 of c-KIT. Gynecol Oncol 98, 334-335.
19. **Alhopuro P, Ylisaukko-Oja SK, Koskinen WJ, Bono P, Arola J, Järvinen HJ, Mecklin JP, Atula T, Kontio R, Mäkitie AA, Suominen S, Leivo I, Vahteristo P, Aaltonen LM, Aaltonen LA** [2005] The MDM2 promoter polymorphism SNP309T→G and the risk of uterine leiomyosarcoma, colorectal cancer, and squamous cell carcinoma of the head and neck. J Med Genet 42: 694-698.
20. **Leiser AL, Anderson SE, Nonaka D, Chuai S, Olshen AB, Chi DS, Soslow RA** [2005] Apoptotic and cell cycle regulatory markers in uterine leiomyosarcoma. Gynecol Oncol 101: 86-91.
21. **Anderson SE, Nonaka D, Chuai S, Olshen AB, Chi D, Sabbatini P, Soslow RA** [2006] p53, epidermal growth factor, and platelet-derived growth factor in uterine leiomyosarcoma and leiomyomas. Int J Gynecol Cancer 16: 849-853.
22. **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.
23. **Parmar S, Platanias LC** [2005] Interferons. Cancer Treat Res 126: 45-68.
24. **Platanias LC** [2005] Mechanisms of type-I- and type-II-interferon-mediated signalling. Nature Rev Immunol 5: 375-386.
25. **Blume-Jensen P, Hunter T** [2001] Oncogenic kinase signalling. Nature 411: 355-365.
26. **Bardelli A, Parsons DW, Silliman N, Ptak J, Szabo S, Saha S, Markowitz S, Willson JK, Parmigiani G, Kinzler KW, Vogelstein B, Velculescu VE** [2003] Mutational analysis of the tyrosine kinome in colorectal cancers. Science 300: 949.
27. **Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA** [2002] Mutations of the BRAF gene in human cancer. Nature 417: 949-954.
28. **Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, Rahman N, Stratton MR** [2004] A census of human cancer genes. Nature Rev. Cancer 4: 177-183.
29. **Stephens P, Hunter C, Bignell G, Edkins S, Davies H, Teague J, Stevens C, O'Meara S, Smith R, Parker A, Barthorpe A, Blow M, Brackenbury L, Butler A, Clarke O, Cole J, Dicks E, Dike A, Drozd A, Edwards K, Forbes S, Foster R, Gray K, Greenman C, Halliday K, Hills K, Kosmidou V, Lugg R, Menzies A, Perry J, Petty R, Raine K, Ratford L, Shepherd R, Small A, Stephens Y, Tofts C, Varian J, West S, Widaa S, Yates A, Brasseur F, Cooper CS, Flanagan AM, Knowles M, Leung SY, Louis DN, Looijenga LH, Malkowicz B, Pierotti MA, Teh B, Chenevix-Trench G, Weber BL, Yuen ST, Harris G, Goldstraw P, Nicholson AG, Futreal PA, Wooster R, Stratton MR** [2004] Lung cancer: intragenic ERBB2 kinase mutations in tumours. Nature 431: 525-526.
30. **Wang Z, Shen D, Parsons DW, Bardelli A, Sager J, Szabo S, Ptak J, Silliman N, Peters BA, van der Heijden MS, Parmigiani G, Yan H, Wang TL, Riggins G, Powell SM, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE** [2004] Mutational analysis of the tyrosine phosphatome in colorectal cancers. Science 304: 1164-1166.
31. **Parsons DW, Wang TL, Samuels Y, Bardelli A, Cummins JM, DeLong L, Silliman N, Ptak J, Szabo S, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Lengauer C, Velculescu VE** [2005] Colorectal cancer: Mutations in a signalling pathway. Nature 436: 792.
32. **Schwaller J, Parganas E, Wang D, Cain D, Aster JC, Williams IR, Lee CK, Gerthner R, Kitamura T, Frantsve J, Anastasiadou E, Loh ML, Levy DE, Ihle JN, Gilliland DG** [2000] Stat5 is essential for the myelo- and lymphoproliferative disease induced by TEL/JAK2. Mol Cell 6: 693-704.
33. **Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, Vassiliou GS, Bench AJ, Boyd EM, Curtin**

N, Scott MA, Erber WN AND Green AR; Cancer Genome Project [2005] Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 365: 1054-1061.

34. **Campbell PJ, Scott LM, Buck G, Wheatley K, East CL, Marsden JT, Duffy A, Boyd EM, Bench AJ, Scott MA, Vassiliou GS, Milligan DW, Smith SR, Erber WN, Bareford D, Wilkins BS, Reilly JT, Harrison CN, Green AR, United Kingdom Myeloproliferative Disorders Study Group, Medical Research Council Adult Leukaemia Working Party, Australasian Leukaemia and Lymphoma Group** [2005] Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. Lancet 366: 1945-1953.
35. **James C, Ugo V, Le Couédic JP, Staerk J, Delhommeau F, Lacout C, Garçon L, Raslova H, Berger R, Bennaceur-Griscelli A, Villeval JL, Constantinescu SN, Casadevall N, Vainchenker W** [2005] A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 434: 1144-1148.
36. **Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, Tichelli A, Cazzola M, Skoda RC** [2005] A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 352: 1779-1790.
37. **Tefferi A, Lasho TL, Gilliland G** [2005] JAK2 mutations in myeloproliferative disorders. N Engl J Med. 353: 1416-1417.
38. **Lee JW, Kim YG, Soung YH, Han KJ, Kim SY, Rhim HS, Min WS, Nam SW, Park WS, Lee JY, Yoo NJ, Lee SH** [2006] The JAK2 V617F mutation in de novo acute myelogenous leukemias. Oncogene 25: 1434-1436.
39. **Larramendy ML, Kaur S, Svarvar C, Böhling T, Knuutila S** [2006] Gene copy number profiling of soft-tissue leiomyosarcomas by array-comparative genomic hybridization. Cancer Cenet Cytogene 169: 94-101.
40. **Svarvar C, Larramendy ML, Blomqvist C, Gentile M, Koivisto-Korander R, Leminen A, Bützow R, Böhling T, Knuutila S** [2006] Do DNA copy number changes differentiate uterine from non-uterine leiomyosarcomas and predict metastasis? Mod Pathol 19: 1068-1082.
41. **Ramana CV, Gil MP, Schreiber RD, Stark GR** [2002] Stat1-dependent and -independent pathways in IFN-gamma-dependent signaling. Trend Immunol 23: 96-101.
42. **Sizemore N, Agarwal A, Das K, Lerner N, Sulak M, Rani S, Ransohoff R, Shultz D, Stark GR** [2004] Inhibitor of kappaB kinase is required to activate a subset of interferon gamma-stimulated genes. Proc Natl Acad Sci USA. 101: 7994-7998.
43. **Sexl V, Kovacic B, Piekorz R, Moriggl R, Stoiber D, Hoffmeyer A, Liebminger R, Kudlacek O, Weisz E, Rothammer K, Ihle JN** [2003] Jak1 deficiency leads to enhanced Abelson-induced B-cell tumor formation. Blood 101: 4937-4943.
44. **Kim SW, Hayashi M, Lo JF, Fearns C, Xiang R, Lazennec G, Yang Y, Lee JD** [2005] Tid1 negatively regulates the migratory potential of cancer cells by inhibiting the production of interleukin-8. Cancer Res 65: 8784-8791.
45. **Melnikova VO, Bar-Eli M** [2006] Bioimmunotherapy for melanoma using fully human antibodies targeting MCAM/MUC18 and IL-8. Pigment Cell Res 19: 395-405.
46. **Singal DP, Ye M, Ni J, Snider DP** [1996] Markedly decreased expression of TAP1 and LMP2 genes in HLA class I-deficient human tumor cell lines. Immunol Lett 50: 149-154.
47. **Cabrera CM, Jiménez P, Cabrera T, Esparza C, Ruiz-Cabello F AND Garrido F** [2003] Total loss of MHC class I in colorectal tumors can be explained by two molecular pathways: β_2-microglobulin inactivation in MSI-positive tumors and LMP7/TAP2 down regulation in MSI-negative tumors. Tissue Antigens 61: 211-219.
48. **Kaplan DH, Shankaran V, Dighe AS, Stockert E, Aguet M, Old LJ, Schreiber RD** [1998] Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. Proc Natl Acad Sci USA 95: 7556-7561.
49. **Dusenbery KE, Potish RA, Judson P** [2004] Limitations of adjuvant radiotherapy for uterine sarcomas spread beyond the uterus. Gynecol Oncol 94: 191-196.
50. **Giuntoli RL, Bristow RE** [2004] Uterine leiomyosarcoma: present management. Curr Opin Oncol 16: 324-327.
51. **Perez EA, Pusztai L, Van de Vijver M** [2004] Improving patient care through molecular diagnostics. Semin Oncol 31: 14-20.
52. **Kanamori T, Takakura K, Mandai M, Kariya M, Fukuhara K, Kusakari T, Momma C, Shime H, Yagi H, Konishi M, Suzuki A, Matsumura N, Nanbu K, Fujita J, Fujii S** [2003] PEP-19 overexpression in human uterine leiomyoma. Mol Hum Reprod 9: 709-717.
53. **Wang L, Felix JC, Lee JL, Tan PY, Tourgeman DE, O'Meara AT, Amezcua CA** [2003] The proto-oncogene c-kit is expressed in leiomyosarcomas of the uterus. Gynecol Oncol 90: 402-406.
54. **Ylisaukko-oja SK, Kiuru M, Lehtonen HJ, Lehtonen R, Pukkala E, Arola J, Launonen V, Aaltonen LA** [2006] Analysis of fumarate hydratase mutations in a population-based series of early onset uterine leiomyosarcoma patients. Int J Cancer 119: 283-287.