Review

Molecular Pathology and Novel Clinical Therapy for Uterine Leiomyosarcoma

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Abstract. Patients with uterine leiomyosarcoma (LMS) typically present with vaginal bleeding, pain, and a pelvic mass, with atypical presentations of hypercalcemia and eosinophilia also being reported. Radiographic evaluation with combined positron-emission tomography/computed tomography may assist in diagnosis and surveillance in women with uterine LMS; these are commonly used with stage and tumour grade as prognostic indicators and a recently developed risk-assessment index to predict disease-specific survival. Recent studies have shown that the addition of adjuvant therapy after surgical management does not seem to improve survival, and ovarian preservation does not appear to negatively impact outcome. Experimentally, it is noteworthy that proteasome subunit beta 9 (PSMB9)/ β 1i-deficient mice exhibit spontaneous development of uterine LMS, with a

disease prevalence of ~37% by 12 months of age. Furthermore, a recent report showed the loss of ability to induce PSMB9/ β 1i expression, that is up-regulated by interferon- γ (IFN γ), in human uterine LMS tissues. Here, we reviewed human uterine LMS for genetic mutations in the IFN γ signal cascade, and found serious mutations in three genes, Janus activated kinase 1 (JAK1), signal transducer and activator of transcription 1 (STAT1) and PSMB9/ β 1i promoter regions. Moreover, molecular experiments demonstrated differential expression of cyclin E and P27/KIP1, that regulate cell-cycle G₁ arrest via PSMB9/ β 1i expression. The discovery of this mutational activation of a key cell-signalling pathway may provide new targets for diagnostic approaches and therapeutic intervention.

Uterine mesenchymal tumours have been traditionally divided into benign leiomyomas (LMA) and malignant leiomyosarcomas (LMS), based on cytological atypia, mitotic activity, and other criteria. Globally, uterine LMSs, which are some of the most common neoplasms in the female genital tract, are relatively rare mesenchymal tumours, having an estimated annual incidence of approximately one per 160,000 women (1). They account for approximately one-third of uterine sarcomas and 1.3% of all uterine malignancies, and are considered aggressive malignancies, with a 5-year survival rate of only 50% for patients with tumours confined to the uterus (2, 3). It is noteworthy that when adjusting for

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stage and mitotic count, uterine LMS has a significantly worse prognosis than carcinosarcoma (4). Generally, patients with uterine LMS present with vaginal bleeding, pain, and a pelvic mass. Although atypical presentations of uterine LMS with hypercalcemia or eosinophilia have been reported, these clinical abnormalities are not considered initial risk factors for this disease (5, 6).

Since uterine LMS is resistant to chemotherapy and radiotherapy and surgical intervention is virtually the only means of treatment, the development of an efficient adjuvant therapy is expected to improve the prognosis of this disease (7-9). Matrix metallo-proteinases (MMPs), which degrade components of the extracellular matrix, appear to participate in tumour invasion and metastasis, and a trend towards prolonged disease-free survival has been seen in patients with MMP2-negative tumours (10, 11). To the best of our knowledge, little is known regarding the biology of uterine LMS. Therefore, the risk factors that promote the initial development of uterine LMSs and regulate their growth *in vivo* remain poorly understood. Earlier reports from our laboratory demonstrated that proteasome beta subunit (PSMB9)/ β 1i-deficient mice exhibit spontaneous development of uterine LMS (12). As such, defective PSMB9/ β 1i expression is likely to be one of the risk factors in the development of human uterine LMS, as it is in PSMB9/ β 1i-deficient mice (13). Because there is no effective therapy for unresectable uterine LMS, our research findings may lead to the development of specific molecular therapies to treat this disease.

PSMB9/ β 1i-deficient Mice Exhibit Spontaneous Development of Uterine LMS

In light of significant effects achieved using antibody therapies directed against tumour-specific antigens, molecular targeting is regarded as a promising strategy for the treatment of malignant tumours (14). Although gynaecological cancer, for instance breast cancer and endometrial carcinomas, are strongly promoted by female hormones, the rates of oestrogen and progesterone receptor expression is reported to be significantly less in uterine LMS compared to the normal myometrium, and were found not to correlate with the promotion of initial disease development or with overall survival (15). Since apoptotic mechanisms have also been implicated in many types of human cancer, investigation of apoptotic and cell-cycle regulator dysregulation in uterine LMS is required to identify molecular pathways that could be involved in the development of this disease. Although the significant differential expression of apoptotic and cell-cycle regulatory factors, such as sexual hormone receptors (oestrogen receptor and progesterone receptor), B-cell lymphoma-2 (BCL2); BCL2-associated X protein (BAX); P16 inhibiting cyclin-dependent kinase 4 (P16/INK4a); P21 cyclin-

dependent kinase inhibitor 1 (P21/CIP1); P27 kinase inhibitor protein 1 (P27/KIP1); cellular v-KIT Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (c-KIT); mitogen-inducible gene-2 (MIG2); murine double minute 2 (MDM2); tumour protein 53 (TP53); as well as human uterine LMS growth initiation factor, have all been compared to myometrium and reported, there is no scientific evidence to show that abnormal expression of these factors directly correlates with the initiation and promotion of human uterine LMS (16-25). As such, the apoptotic and cell-cycle regulatory protein expression profiles of human uterine LMS are not yet useful for clinical prognostic purposes.

Previous reports have shown ATP-dependent proteasome function impairment in mice with a targeted disruption of PSMB9/ β 1i. In a recent study, we report how *Psmb9/ β 1i*^{-/-} mice are prone to the development of uterine neoplasms (12) (Figure 1). The percentage of animals with overt tumours increases with age after 6 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, which is the most common tumour of the uterus, was observed in the female *Psmb9/ β 1i*^{-/-} mice, but not in their parental C57BL/6 mice with the same genetic background (Figure 1). Histological examinations of the *Psmb9/ β 1i*^{-/-} uterine tumours revealed the common characteristic abnormalities seen in human uterine LMS: tumours lacked lymphoid infiltrates, which is a sign of immune recognition, and consisted of uniform elongated uterine smooth muscle cells arranged into bundles (Figure 1). The nuclei of the tumour cells varied in size and shape, and mitosis was frequent. In contrast, the uterine smooth muscle cells of C57BL/6 mice were normal in appearance. Whereas relatively few Ki-67-positive cells, the proliferating cells of solid tumours, were observed in the basal cell layer of normal myometrium, most of the basal cells vividly expressed Ki-67 in *Psmb9/ β 1i*^{-/-} mice. These histopathological examinations indicated abnormal proliferation of *Psmb9/ β 1i*^{-/-} uterine smooth muscle cells in the basal cell layer of normal myometrium. Additionally in *Psmb9/ β 1i*^{-/-} mice, proteasomal peptidase activity against hydrophobic and basic substrates, but not acidic substrates, was lower in the spleen and liver compared to wild-type mice. Differences in muscle and the brain were not significant. Although flow cytometric analysis showed no difference in the expression of major histocompatibility complex (MHC) class I molecules, immunological antigen-presenting cells from *Psmb9/ β 1i*^{-/-} mice were less able to stimulate T-cell hybridomas specific for a nucleoprotein (NP) envelope antigen of an influenza A virus. *Psmb9/ β 1i*^{-/-} mice also had less than half of the wild-type levels of CD8-positive cytotoxic T-lymphocytes, and generated much lower levels of cytotoxic T-cell precursors specific for NP, though not for ovalbumin (12, 26). Van Kaer *et al.* concluded that PSMB9/ β 1i selectively influences antigen

processing of MHC class I-restricted antigens (26). Most importantly, they found that spontaneous uterine LMS only was detected in *Psmb9/β1i*^{-/-} mice; no other cancer progression was observed at high or low incidence in both male and female animals. Therefore, expression of PSMB9/β1i, rather than providing an escape from immune surveillance, seems to play an important role in the spontaneous development of uterine LMS.

Correlation Between Defects in PSMB9/β1i Expression and Human Uterine LMS

Several reports, including experiments with *Psmb9/β1i*^{-/-} mice, suggest that interferon (IFN)-γ-induced restoration of antigen-processing machinery, such as transporter associated with antigen processing 1 (TAP1) and PSMB9/β1i, improves antitumour-specific antigen cytotoxic T-lymphocyte recognition in some patients; thus, approaches to activate this pathway may be of benefit to patients with TAP and PSMB9/β1i deficiencies. Therefore, we sought to demonstrate whether human uterine LMS exhibits weak expression of TAP1 and PSMB9/β1i by immunoblotting four human gynaecological tumour cell lines (13). TAP1 and PSMB9/β1i expression were not markedly induced by IFNγ treatment in the human LMS cell line SKN, although HeLa and HeLa.S3, both cervical epithelial adenocarcinoma cell lines, and a normal human uterus smooth muscle cell line, Hu.USMC, underwent strong induction of TAP1 and PSMB9/β1i following IFNγ treatment (13). Furthermore, our experiments, which were individually performed at several medical facilities, revealed a drastic loss of ability to induce PSMB9/β1i expression in human uterine LMS tissues compared with normal myometrial tissues located in the same sections in a total of 73 normal cases, 52 LMA cases, three bizarre LMA cases, 58 LMS cases, as well as in SKN human uterine LMS cell lines treated with IFNγ (13) (Figure 2). In addition, immunohistochemistry showed marked expression of PSMB9/β1i in cervical epithelial adenocarcinoma tissues and HeLa and HeLa.S3 cell lines treated with IFNγ (Figure 2). The defect was localized to JAK1 activation, which acts upstream in the IFNγ signalling pathway, since IFNγ treatment did not strongly induce JAK1 kinase activity in the SKN human uterine LMS cell line. 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 loss of IFNγ responsiveness in the SKN cells was attributable to the inadequate kinase activity of JAK1 due to this G781E mutation (13).

Mutations in the IFNγ Signaling Pathway in Human LMS Tissue

IFNγ treatment increases the expression of immuno-proteasome beta subunits, PSMB8/β5i and PSMB9/β1i, and

reduces expression of two proteasome beta subunits, named PSMB5/β5 and PSMB6/β1, which alters the proteolytic specificity of proteasomes. From the cDNAs encoding PSMB5/β5 and PSMB6/β1, Akiyama *et al.* showed that their proteins have high amino acid similarity to PSMB8/β5i and PSMB9/β1i, respectively (27). IFNγ may induce subunit replacement of PSMB5/β5 and PSMB6/β1 by PSMB8/β5i and PSMB9/β1i, respectively, producing proteasomes that may be more appropriate for the immunological processing of endogenous antigens. After binding of IFNγ to the type II IFN receptor (constructed of IFNγ receptor subunit 1 and 2), JAK1 and JAK2 are activated and phosphorylate signal transducer and activator of transcription 1 (STAT1) on the tyrosine residue at position 701 (28, 29). The tyrosine-phosphorylated form of STAT1 forms homodimers that translocate to the nucleus and bind IFNγ-activated site (GAS) elements, which are present in the promoters of IFNγ-regulated genes (28, 29). The IFNγ-activated JAKs also regulate, through as yet unknown intermediates, activation of the catalytic subunit (p110) of phosphatidylinositol 3-kinase (PI3K) (30). This activation of PI3K ultimately results in downstream activation of protein kinase C-δ (PKCδ), which in turn regulates phosphorylation of STAT1 on the serine residue at position 727. This phosphorylation 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 (28, 29).

Genetic alterations in tyrosine kinases have been firmly implicated in tumorigenesis, and only a few serine/threonine kinases are known to be mutated in human cancer (31-37). Therefore, we analyzed human uterine LMS tissues (a total of 23 cases of LMS tissue sections and normal tissue sections located in same tissue) for somatic (tumour-specific) mutations in the IFNγ signalling cascade, JAK1, JAK2, STAT1 and promoter region of *PSMB9/β1i* gene. Overall, nearly 43.5% (10/23) of human uterine LMS tissues had serious mutations in the ATP-binding region or kinase-specific active site of JAK1. Furthermore, 43.5% (10/23) of human uterine LMS tissues had serious mutations in important sites of the promoter region of *PSMB9/β1i* gene, which is required for transcriptional activation (Figure 3). Nearly 21.7% (5/23) of human uterine LMS tissues had mutations in the STAT1 intermolecular region, which is not yet reported to be important for biological function as a transcriptional activator. Although a genetic approach has already addressed the finding that marked JAK2 activation causes myelo- and lymphoproliferative disease, polycythemia vera, and myelogenous leukemia, no serious mutations in the ATP-binding region or kinase-specific active site of JAK2 were detected in human uterine LMS (38-44) (Figure 3). In a recent report, a high-resolution genome-wide array comparative genomic hybridization (CGH) analysis on human LMS cases provided gene-level information about the

Figure 1. Development of uterine neoplasms in proteasome beta subunit 9 (PSMB9)/ $\beta 1i^{-/-}$ mice. A: Uterine neoplasms in PSMB9/ $\beta 1i^{-/-}$ mice. Abdominal cavities of female C57BL/6 (a) and PSMB9/ $\beta 1i^{-/-}$ (e) mice, showing a uterine tumor (outlined by yellow arrowheads) in the latter. Companion of the female genital organs of C57BL/6 (b) and PSMB9/ $\beta 1i^{-/-}$ (f) mice, showing a uterine neoplasm in the latter. Histological analysis showing the normal smooth muscle cells of the uterus of C57BL/6 mice (c and d) and the abnormal cells of a leiomyosarcoma of the uterus of PSMB9/ $\beta 1i^{-/-}$ mice (g and h). Original magnification: c and g: $\times 200$; d and h, $\times 400$. B. Comparison of histological analysis of uterine leiomyosarcoma in humans (left) and PSMB9/ $\beta 1i^{-/-}$ mice (right); showing the abnormal cells of leiomyosarcoma of the uterus. Original magnification: $\times 800$.

amplified and deleted regions that may play a role in the development and progression of human LMS. Among the most intriguing genes whose copy number sequence was revealed to be altered by CGH, were JAK1 (1p31-p32) and PSMB9/ $\beta 1i$ (6p21.3) (45,46) (Figure 4). The discovery of these mutational defects of a key cell-signaling pathway may be an important step in the understanding of the pathogenesis of human uterine LMS, in addition to our own research showing that somatic mutations of *JAK1* correlate to the development of human uterine LMS.

It is probable that the list of new elements involved in IFN-mediated signaling will continue to grow during the next few years, and the contributions of known pathways might need to be re-evaluated. At present, it seems that activation of more than one signaling pathways is required for the generation of the different biological properties of IFNs, and no one signaling cascade alone is sufficient for generation of any given biological end-point. For example,

the combined biological functions of the nuclear factor-kappa B, STAT, and P38 signalling pathways are required for the antiviral and antitumor effects of IFNs, but activation of any pathway alone is not sufficient to elicit such responses (1). Such a requirement for multiple signaling pathways also seems to be the case for IFN γ -dependent antiproliferative responses, and might reflect the synergistic effects of various signals at the level of gene transcription and mRNA translation. Therefore, additional genetic analysis is required to completely elucidate the mutational activation of key cell-signalling pathways in human uterine LMS.

Potential Anti-oncogenic Function of PSMB9/ $\beta 1i$ in Human Uterine LMS

Whereas the growth of cell lines that have JAK1 kinase activity have been demonstrated to be strongly inhibited by IFN γ treatment, the growth of JAK1-deficient cell lines is

Figure 2. Immunohistochemistry of Proteasome beta subunit 9 (PSMB9)/ $\beta 1i$ expression in human tissues of normal uterine smooth muscle (A) and uterine leiomyosarcoma (B), showing defective PSMB9/ $\beta 1i$ expression in uterine leiomyosarcoma tissue. Immunohistochemistry of PSMB9/ $\beta 1i$ expression in normal human cervical epithelium (C) and cervical epithelial carcinoma tissues (D).

unaffected by IFN γ . Similarly, the cell-cycle distribution pattern of freshly explanted tumor cells derived from JAK1-deficient tumors showed no response to IFN γ treatment (49). In our study, the growth of the original SKN cells, which had defective JAK1 activity, was unaffected by IFN γ treatment [population doubling time (PDT)=15.2 h] (13). In contrast, the growth of JAK1-transfected SKN cells, which had strong exogenous JAK1 activity, was slowed by IFN γ treatment (PDT=18.1 h). Interestingly, when PSMB9/ $\beta 1i$ -transfected SKN cells, which have marked PSMB9/ $\beta 1i$ expression, were analyzed, exogenous PSMB9/ $\beta 1i$ expression resulted in cell growth arrest (PDT=17.9 h), and the growth of PSMB9/ $\beta 1i$ -transfected SKN cells was unaffected by IFN γ treatment (PDT=8.0 h) (13). In SKN-PSMB9/ $\beta 1i$ transfectants, there was correlation between levels of exogenous PSMB9/ $\beta 1i$ expression and the degree of suppression of the transformed phenotype, demonstrating the revertant-inducing activity of PSMB9/ $\beta 1i$ on SKN cells (Figure 5).

Microarray analysis provides insight into these gene-expression changes associated with malignant transformation. To investigate whether stable expression of PSMB9/ $\beta 1i$ causes cell-growth arrest and loss of migration

in SKN cells, we compared mRNA gene expression (using Affymetrix human GeneChip HG U133 Plus2.0) between SKN cells and PSMB9/ $\beta 1i$ -transfected SKN cells, and analyzed the expression profile of SKN cells transfected with plasmids (pCEM9) with and without PSMB9/ $\beta 1i$ -coding DNA inserts (pCEM9-PSMB9/ $\beta 1i$). Our results showed that PSMB9/ $\beta 1i$ expression influences the expression pattern of cell cycle-regulating factors, especially down-regulation of cyclin E and up-regulation of P27/KIP1, depending on the stability of PSMB9/ $\beta 1i$ expression. High levels of P27/KIP1 present in quiescent (G_0) cells have been shown to decline upon mitogen induction, and P27/KIP1 activation is down-regulated by cyclin E/CDK2 (50-53). Furthermore, a recent report demonstrated that cyclin E-deficient cells actively proliferate in conditions of continuous cell cycling, but are unable to re-enter the cell cycle from G_0 , and are resistant to oncogenic transformation (54-55). Thus, IFN γ responses to cell-growth arrest may be attributable to the differential cyclin E and P27/KIP1 expressions by IFN γ inducibility of PSMB9/ $\beta 1i$. Taken together, it is likely that PSMB9/ $\beta 1i$ deficiencies result in defects in cyclin E-dependent cell-cycle arrest and

	Age, years	n	PSMB9/β1i expression			
			-	-/+	Focal+	+++
Normal	32-83	73				73
Leiomyoma (Cellular leiomyoma) (Ordinary leiomyoma) (Tumor of uncertain malignant potential)	33-83	52 (10) (30) (12)				52
Bizarre leiomyoma	44,49,55	3				3
Leiomyosarcoma	32-83	58	49	3	4	2

From results of immuno-staining experiments, -/+ : partially positive (5% to 10% of cells stained), focal/+ : local-positive (focal or sporadic staining with less than 5% of cells stained), +++ : diffuse-positive (homogeneous distribution with more than 90% of cells stained), - : negative (no stained cells).

Figure 3. The interferon- γ signaling pathway and mutations in its components found in human uterine leiomyosarcoma (LMS). After binding of interferon- γ (IFN γ) to the type II IFN receptor, Janus activated kinase 1 (JAK1) and JAK2 are activated and phosphorylate signal transducer and activator of transcription 1 (STAT1) on the tyrosine residue at position 701 (Tyr701). The tyrosine-phosphorylated form of STAT1 forms homodimers that translocate to the nucleus and bind IFN γ -activated site (GAS) elements, which are present in the promoters of IFN γ -regulated genes. 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. Investigation of human uterine LMS tissues (total of 23 cases of LMS tissue sections and normal tissue sections located in same tissue) for somatic mutations in the IFN γ signalling cascade, JAK1, JAK2, STAT1 and promoter region of proteasome beta subunit 9 (PSMB9)/ β 1i gene showed that overall, nearly 43.5% (10/23) of LMS tissues had serious mutations in kinase active domain of JAK1. Furthermore, 43.5% (10/23) of tissues had serious somatic mutations in important sites of the PSMB9/ β 1i promoter region. A total of 21.7% (5/23) of human uterine LMS tissues had somatic mutations in the intermolecular region of STAT1. A drastic loss in ability to induce the expression of PSMB9/ β 1i was found in human uterine LMS tissues compared to leiomyoma (LMA) tissues located in the same sections in a total of 73 normal cases, 52 LMA cases, 58 LMS cases, as well as in SKN cell lines treated with IFN γ . As such, defective expression of PSMB9/ β 1i is likely to be one of the risk factors in the development of human uterine LMS, as it is in PSMB9/ β 1i-deficient mice. IFNGR1: IFN γ receptor subunit 1; IFNGR2: IFN γ receptor subunit 2; TRFE: TBP-related factor element; IRF1: Interferon regulatory factor 1; TAP1: Transporter associated with antigen processing 1; PSMB9/ β 1i: proteasome beta subunit 9/ β 1i.

resistance to oncogenic transformation. In breast cancer, alterations in cyclin E expression become progressively worse with increasing stage and grade of tumor, prompting us to investigate cyclin E as a determinant of the aggressiveness and metastatic potential of human uterine LMS. Levels of total cyclin E expression correlated strongly with survival and malignancy in patients with uterine LMS, suggesting its potential use as a prognostic marker in uterine LMS, as it is in breast cancer (52, 55-61).

The down-regulation of MHC expression, including *TAP1* and *PSMB9/β1i* genes, is one of the biological mechanisms

tumour cells use to evade host immune-surveillance (62, 63). Recently, the incidence of IFN γ unresponsiveness was examined in several types human malignant tumours, revealing that approximately 33% of each group exhibited a reduction in IFN γ sensitivity (64). Nevertheless, expression of PSMB9/ β 1i, rather than providing an escape from immune surveillance, seems to play an important role in the negative regulation of uterine LMS cell growth. Defective expression of PSMB9/ β 1i is likely to be one of the risk factors for the development of human uterine LMS, as it is in the PSMB9/ β 1i-deficient mouse.

Figure 4. Gene copy number and somatic mutations in leiomyosarcoma. *JAK1/2*: Janus kinase 1/2; *PSMB9/β1i*: proteasome beta subunit 9/β1i; *STAT1*: signal transducers and activator of transcription 1.

Figure 5. Morphology of the SKN transfectants. Phase-contrast micrographs of SKN colonies obtained after transfection of control pCEM9 plasmid (A and B) into SKN cells, and of the primary flat-revertant SKN cells obtained after transfection of pCEM9- proteasome beta subunit 9 (PSMB9)/β1i plasmid into SKN cells (C and D). Bars indicate 200 μm. Growth rates of the SKN-PSMB9/β1i transfectants were generally lower than those of control SKN-pCEM9 transfectants. PDT: Population doubling time.

Future Directions

Uterine LMS is a disease with an extremely poor prognosis, which is highly aggressive, and is resistant to chemotherapies. Following a reported 23% response rate (RR) for mitomycin, doxorubicin, and cisplatin (MAP), and preliminary data suggesting a superior RR for dacarbazine plus MAP plus sargramostim, the Gynecologic Oncology Group conducted a phase II trial of dacarbazine plus MAP plus sargramostim in patients with advanced uterine LMS. This combination produced a 27.8% RR, but its complexity and toxicity precluded further investigation, and the study was closed after the first stage of accrual (65). At present, surgical intervention is virtually the only means of treatment for this disease (6, 66, 67). Although adjuvant pelvic irradiation appears to reduce the rate of local recurrence, adjuvant therapy does not appear to significantly improve survival. Furthermore, gynaecological cancer, for instance breast cancer and endometrial carcinomas, are strongly promoted by female hormones, but the rate of oestrogen receptor and progesterone receptor expression is reported to be significantly less in human uterine LMS compared with normal myometrial cells. These low receptor expressions were found not to correlate with promotion of initial disease development, nor with overall survival of patients with human uterine LMS. Prospective multicentre trials are necessary to clarify the role of chemotherapy, selection criteria, and optimal chemotherapy regimen for human uterine LMS.

Recently, molecular targeting therapies have shown remarkable achievements against tumours (1). To improve the prognosis of human uterine LMS, research is being performed to determine the role of pro- and anti-oncogenic factors that play an important function in human uterine LMS pathogenesis and could serve as molecular targets for tumour treatment. To this end, several research facilities have conducted cDNA microarray procedures with human uterine LMS and normal myometrium, showing that several known pro-oncogenic factors, as well as other factors such as brain-specific polypeptide PEP-19, the transmembrane tyrosine kinase receptor, C-KIT and mutations in fumarate hydratase, may be associated with the pathogenesis of human uterine LMS (69-71). However, in terms of LMS tumorigenesis, 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, not necessarily its cause. In addition, dysregulation of apoptotic mechanisms has also been implicated in many types of human cancer. Although the significant differential expression of apoptotic and cell cycle regulatory factors in uterine LMS, such as BCL2, BAX, P16/INK4a, P19/ARF, C-KIT, MDM2, and TP53, have all been reported and compared to normal uterine smooth muscle cells, there is no scientific evidence to show

that abnormal expression of these factors directly correlates with the initiation and promotion of human uterine LMS (22, 24). The discovery of PSMB9/ β 1i deficiency and the mutational activation of a key cell-signaling pathway may provide new approaches for understanding the molecular mechanism of human uterine LMS development.

Conclusion

Recent advances in our understanding of the biology of uterine LMS will continue to focus on serious somatic mutations in the IFN γ signal pathway and defects in PSMB9/ β 1i expression, and may ultimately lead to novel therapies and improved outcome in patients with human uterine LMS.

Disclosure

The Authors report no conflicts of interest.

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