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Research Paper

Somatic mutations in Interferon-γ signaling molecules in human uterine leiomyoscoma

Medicine

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ABSTRACT

Human uterine leiomyosarcoma (Ut-LMS) is neoplastic malignancy that typically arises in tissues of mesenchymal origin. The identification of novel molecular mechanism leading to human Ut-LMS formation and the establishment of new therapies has been hampered by several critical points. We earlier reported that mice with a homozygous deficiency for proteasome subunit beta type (PSMB) 9, an interferon (IFN)- inducible factor, spontaneously develop Ut-LMS. The use of research findings of the experiment with mouse model has been successful in increasing our knowledge and understanding of how alterations, in relevant oncogenic, tumor suppressive, and signaling pathways directly impact sarcomagenesis. The IFN- pathway is important for control of tumor growth and invasion and has been implicated in several malignant tumors. In this study, experiments with human tissues revealed a defective PSMB9 expression in human Ut-LMS that was traced to the IFN- pathway and the specific effect of somatic mutations of JAK1 molecule or PSMB9 gene promoter region on the PSMB9 gene transcriptional activation. Understanding the molecular mechanisms of human Ut-LMS may lead to identification of new diagnostic candidates or therapeutic targets in human Ut-LMS.

Introduction

Uterine mesenchymal tumors have been traditionally divided into benign tumor leiomyomas (LMA) and malignant tumor leiomyosarcomas (LMS) based on cytological atypia, mitotic activity and other criteria. Ut-LMS are relatively rare smooth muscle tumor, having an estimated annual incidence of 0.64 per 100,000 women 1-3. Gynecological tumor, for instance endometrial carcinomas, are strongly promoted by female hormones, but the rate of hormone receptor expression is reported to be significantly less in human Ut-LMS compared with normal myometrium. These low receptor expressions were found to not correlate with the promotion of initial disease development or with the overall survival of patients with Ut-LMS. As Ut-LMS is resistant to chemotherapy and radiotherapy, and thus surgical intervention is virtually the only means of treatment for this disease 4-6, however, molecular targeting therapies against tumors have recently shown remarkable achievements 7. It is noteworthy that, when adjusting for stage and mitotic count, Ut-LMS has a significantly worse prognosis than carcinosarcoma 8; developing an efficient adjuvant therapy is expected to improve the prognosis of the disease 9. Although typical presentations with hypercalcemia or eosinophilia have been reported, this clinical abnormality is not an initial risk factor for Ut-LMS. To the best of our knowledge, little is known regarding the biology of Ut-LMS; therefore, the risk factors that promote the initial development of Ut-LMS and regulate their growth in vivo remain poorly understood.

The mice with a targeted disruption of PSMB9, which is IFN-γ-inducible proteasome subunit, exhibited a defect in tissue- and substrate- dependent proteasome function, and female Psmb9-deficient mice shown to develop Ut-LMS, with a disease prevalence of 37% by 14 months of age 10,11. Defective PSMB9 expression is likely to be one of the risk factors for the development of human Ut-LMS, as it is in Psmb9-deficient mice 11. Recent report shows that stable PSMB9 expression contributes to cell proliferation, which directly correlates to the progressive deterioration with increasing stage of the tumor. As the importance and involvement of the IFN-γ signal pathway in the transcriptional regulation of the PSMB9 promoter have been established, the defective PSMB9 expression was reportedly attributable to G871E mutation in the ATP-binding region of JAK1 in SKN cell line, which is established from patient with Ut-LMS. We demonstrate that there are serious mutational defects in the factors on the IFN-γ-signaling pathway and PSMB9 promoter region, in human Ut-LMS tissues. The somatic mutational defects in the IFN-γ-signaling pathway may induce the initial development of Ut-LMS. Recent advances in our understanding of the biology of Ut-LMS have concentrated on the impaired IFN-γ signaling pathway. It is clear that mutations in key regulatory genes alter the behavior of cells and can potentially lead to the unregulated growth seen in malignant tumor. Therefore, continued improvement of our knowledge of the molecular biology of Ut-LMS may ultimately lead to novel therapies and improved outcome.

Result and Discussion

Defective PSMB9 expression of human Ut-LMS

The effects of IFN-γ on PSMB9 expression was examined using five cell lines 12. PSMB9 expression were not markedly induced by IFN-γ treatment in human Ut-LMS cell lines, although cervical epithelial adenocarcinoma cell lines and normal human myometrium cells underwent strong induction of PSMB9 following IFN-γ treatment 12. Furthermore, the immunohistochemistry (IHC) experiments revealed a serious loss in the ability to induce PSMB9 expression in human Ut-LMS tissues in comparison with normal myometrium tissues located in same tissue sections and other 4 mesenchymal tumor types. Of 56 Ut-LMS, 48 cases were negative for PSMB9, 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 ER, PR, TP53, and CALPONIN h1. In addition, PSMB9 expression level was also examined in the skeletal muscle metastasis from Ut-LMS, the histological diagnosis was consistent with metastatic Ut-LMS for skeletal muscle lesions. Pathological examination of surgical samples showed presence of a mass measuring 3 cm at largest diameter in lumbar quadrate muscle without a fibrous capsule. In western blotting experiments and RT-PCR experiments, PSMB9 was expressed in normal myometrium, LMA, but not in human Ut-LMS. The both research experiments strongly supported the research findings obtained from IHC experiments.

Somatic mutations in IFN-γ signaling pathway in human Ut-LMS tissues

IFN-γ treatment markedly increased the expression of PSMB9, which alters the proteolytic specificity of proteasomes. After binding of IFN-γ to the type II IFN receptor, JAK1 and JAK2 are activated and phosphorylate STAT1 on the tyrosine residue at position 701 and the serine residue at position 727 13,14. Tyrosine phosphorylated STAT1 forms homodimers that translocate to the nucleus and bind GAS (IFN-γ-activated site) elements in the promoters of IFN-γ-regulated genes 13,14. IFN-γ activated JAKs also regulate, through as yet unknown intermediates, activation of the catalytic subunit (p110) of PI3K. The activation of PI3K ultimately results in downstream activation of PKC-δ, which in turn regulates the phosphorylation of STAT1 on the Ser727. The phosphorylation of Ser727 is required for full transcriptional activation 15.

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 human Ut-LMS cell lines.

Sequence analysis demonstrated that the loss of IFN-γ responsiveness in the human Ut-LMS cell line was attributable to the inadequate kinase activity of JAK1 due to a G781E mutation in the ATP-binding region 1213. 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 16-1817-19. For instance, mice carring homozygous deletion of Pten alleles developed wide spread SMC hyperplasia and abdominal LMS 17, and JUN oncogene amplification and overexpression block adipocytic differentiation in highly aggressive sarcomas 18.

Most frequently, Ut-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 of human Ut-LMS tissues (23 Ut-LMS tissue sections and normal tissue sections located in the same tissue) was performed to detect somatic mutations in the IFN-γ signaling cascade, JAK1, JAK2, STAT1 and PSMB9 promoter region. As the catalytic domains of these genes are most likely to harbour mutations that activate the gene product, we focused on stretches containing the kinase domains, transcriptional activation domains and enhancer/promoter region. Over all, nearly 43.5% (10/23) of Ut-LMS tissues had serious mutations in the ATP binding region or kinase-specific active site of JAK1; furthermore, 43.5% (10/23) of Ut-LMS tissues had serious mutations in essential sites of the PSMB9 promoter region, which is required for PSMB9 gene transcriptional activation. No somatic mutation in essential sites, Tyr701 and Ser727, which are required for STAT1 transcriptional activation, was elucidated in Ut-LMS. Nearly 21.7% (5/23) of Ut-LMS tissues unexpectedly had mutations in the STAT1 intermolecular region, 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 was detected in Ut-LMS.

Conclusion

Defective PSMB9 expression is likely to be one of the risk factors for the development of human uterine neoplasm, as it is in the Psmb9-deficient mouse. Thus, gene therapy with PSMB9 expression vectors may be a new treatment for Ut-LMS that exhibits a defect in PSMB9 expression. Because there is no effective therapy for unresectable Ut-LMS, our results may bring us to specific molecular therapies to treat this disease.

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