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Sarcomagenesis in *Psmb9*-deficient mice; involvement of defective IRF1 activation.

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ABSTRACT

Uterine leiomyosarcoma (Ut-LMS) is a highly metastatic smooth muscle neoplasm for which CALPONIN h1 is suspected to play a biological role as a tumor suppressor. We previously reported that proteasome β-subunit (Psmb)9-deficient mice spontaneously developed Ut-LMS through malignant transformation of the myometrium, which implicated this protein as an anti-oncogenic candidate. We also suggested that PSMB9 may negatively regulate Ut-LMS independently of its role in the proteasome. Interferon regulatory factor (IRF)1 was the first member of the IRF family to be identified. Initially described as a transcription factor that was able to activate the expression of Interferon (IFN)-γ responsible genes, Irf-1 has been shown to play roles in the immune response, regulation of apoptosis, and tumor suppression. The aim of this study was to elucidate the molecular mechanism of sarcomagenesis using the samples of Ut-LMSs from Psmb9-deficient mice and tissue sections of human Ut-LMSs and normal myometrium from patients. The expressions of the IFN-γ signal molecules, IRF1, IRF2, STAT1, PSMB5/β5i, PSMB9/β1i, PSMB8/β3i, PSMB10/β2i and β-ACTIN were examined with the tissue samples of mouse and human clinical materials. Physiological significant of IRF1 in sarcomagenesis was demonstrated by xenograft studies. In the present study, several lines of evidence indicated that although treatments with IFN-γ strongly induced the activation of STAT1 as a transcriptional activator, its target molecule, IRF1, was not clearly produced in Psmb9-deficient uterine smooth muscle cells (Ut-SMCs). Defective IRF1 expression may result in the malignant transformation of Ut-SMCs. The modulation of PSMB9 may lead to new therapeutic approaches in human Ut-LMS. (251 words)

KEYWORDS : PSMB9, IRF1, uterine leiomyosarcoma, smooth muscle cell, diagnostic biomarker

Introduction

The uterus is composed of three layers, the uterine endometrium, which serves as a bed for the embryo; the myometrium of the wall, which protects the embryo; and a serious membrane enveloping the uterus. The term uterine tumor generally refers to an epithelial malignant tumor of the uterus, which is roughly classified as a tumor of the uterine cervix or uterine body. Due to the prevalence of medical checkups, the rate of mortality from uterine cervix malignant tumors is now decreasing, and is commonly detected at a very early stage. In contrast, the mortality rate from malignant uterine tumors is increasing, and is rarely detected at the initial stages. While most uterine tumors are adenocarcinomas, uterine cervix tumors are classified into squamous cancer and adenocarcinomas. Tumors of uterine smooth muscle cells (Ut-SMCs), which develop in the myometrium, have traditionally been divided into benign leiomyoma (LMA) and malignant human uterine leiomyosarcoma (Ut-LMS) based on cytological atypia, mitotic activity, and other criteria. Ut-LMS is relatively rare, having an estimated annual incidence of 0.64 per 100,000 women [1]. Ut-LMS accounts for between 2% and 5% of tumors in the uterine body and develops more frequently in the muscle layer of the uterine body than in the uterine cervix. As Ut-LMS is resistant to chemotherapy and radiotherapy, surgical intervention is virtually the only means of treatment [2,3]. The prognosis of human Ut-LMS is poor, with the five-year survival rate being approximately 35%, although this has been shown to depend on the disease stage [2,3]. When adjusted for stage and mitotic count, human Ut-LMS has a significantly worse prognosis than carcinosarcoma [4]. Since human Ut-LMS is resistant to chemotherapy and radiotherapy, surgical intervention is virtually the only means of treatment; therefore, the development of efficient adjuvant therapies is expected to improve the prognosis of this disease [5-7]. The identification of risk factors associated with the development of human Ut-LMS may significantly contribute to the development of preventive and therapeutic treatments.

Cytoplasmic proteins are mostly degraded by a protease complex, which has many substrates consisting of twenty-eight 20- to 200-kDa subunits, referred to as the 20S proteasome [8,9]. Proteasomal degradation is essential for many cellular processes, including the cell cycle, regulation of gene expression, and immunological function [10]. Interferon (IFN)-γ induces the expression of large numbers of responsive genes, including interferon regulatory factor (IRF)1 and proteasome subunits, i.e., proteasome β-subunit (PSMB)5/β5i, PSMB9/b1i, PSMB8/b3i and PSMB10/b2i [10]. A molecular approach to investigating the relationship between IFN-γ and tumor cell growth has been attracting attention. Homozygous mice deficient in *Psmb9* show tissue- and substrate-dependent abnormalities in the biological functions of the proteasome [11]. Ut-LMS was reportedly detected in female *Psmb9*-deficient mice at 6 months of age or older, and its incidence at 14 months of age was approximately 40% [12]. Histological studies of *Psmb9*-lacking uterine tumors identified the characteristic abnormalities of Ut-LMS [12,13]. These tumors consisted of uniform elongated Ut-SMCs arranged into bundles. The nuclei of the tumor cells varied in size and shape, and mitosis was frequently observed. Marked reductions in body weight have been reported in *Psmb9*-deficient mice that developed Ut-LMS, and these mice died by 14 months of age. *Psmb9*-deficient mice also developed skeletal muscle metastasis from Ut-LMS. Therefore, *Psmb9*-deficient mice with Ut-LMS may die as a result of the tumor mass and metastasis.

In the present study, we investigated the molecular mechanisms underlying sarcomagenesis in mouse Ut-LMS involving the defective expression of PSMB9. Biological and histological findings showed that although the expression of STAT1 was clearly induced by the treatment with IFN-γ, IRF1 was not detected in Ut-SMCs derived from *Psmb9*-deficient mice. IRF1 contributed to cell proliferation, which directly correlated with tumor progression. Thus, a deficiency in PSMB9 resulted in the defective function of IRF1 as an anti-tumor factor in *Psmb9*-deficient Ut-SMCs in an anti-oncogenic manner. Continued improvements in our knowledge of the molecular biology of mouse Ut-LMS may ultimately lead to novel diagnoses and therapies and better outcomes.

Discussion

Ut-LMS mainly develops in the myometrium or endometrial stroma, and menstrual anomalies, such as hypermenorrhea and prolonged menstruation, and symptoms, such as abnormal hemorrhage, hypogastric pain, lumbar pain, and abdominal strain, have been reported previously [4]. In the case of gynecological cancers, such as breast cancer, a female hormonal imbalance is often a risk factor for the development of tumors. However, as is also the case for uterine LMA, the relationship between the development of human Ut-LMS and female hormones and their receptors has yet to be elucidated [14]. Unfortu-

nately, it currently remains unclear whether the estrous cycle with the defective expression of PSMB9 is involved in the onset of Ut-LMS. Human Ut-LMS often appears to develop in individuals exposed to radiation in the pelvis. Risk factors for the development of human Ut-LMS have not yet been identified because of the absence of a suitable animal model. The *Psmb9*-deficient mouse was established as the first mouse model of spontaneous Ut-LMS [12]. To establish whether PSMB9 can be used as a potential biomarker to distinguish human Ut-LMS from LMA, we are now investigating the reliability and characteristics of PSMB9 as a diagnostic biomarker with several clinical research facilities. This research is ongoing and large scale clinical studies must also be conducted. Studies using gene-expression profiling revealed differential expression of several known pro-oncogenic factors and previously reported factors i.e. brain-specific polypeptide PEP-19, CALPONIN h1, and the transmembrane tyrosine kinase receptor, c-kit, which were associated with the pathogenesis of Ut-LMS [15-19]. Since the spontaneous development of Ut-LMS has not been reported in *Irf1*-, *Calponin h1*-deficient mice or heterozygous *Rb* mice, the lack of PSMB9 has been strongly associated with the expression of other known or unknown cell-cycle regulatory factors. Further studies are needed to demonstrate the correlative functions of PSMB9 and other anti-oncogenic factors with CALPONIN h1 in human Ut-LMS sarcomagenesis. Human Ut-LMS is refractory to chemotherapy and has a poor prognosis. Molecular biological and cytological information obtained from *Psmb9*-deficient mice will markedly contribute to the development of preventative methods against, potential diagnostic biomarkers for, and new therapeutic approaches to treat Ut-LMS.

Conflicts of interest: We do not have any conflicts of interest.

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