Biological characterization of soft tissue sarcomas

Takuma Hayashi[1,2,3], Akiko Horiuchi[4], Kenji Sano[5], Yae Kanai[6,7], Nobuo Yaegashi[8], Hiroyuki Aburatani[9], Ikuo Konishi[10]

[1]Department of Immunology and Infectious Disease, Shinshu University School of Medicine, Nagano, Japan; [2]Promoting Business using Advanced Technology, Japan Science and Technology Agency (JST), Tokyo, Japan; [3]Sigma-Aldrich Collaboration Laboratory, Rehovot 76100, Israel; [4]Horiuchi Ladies Clinic, Nagano, Japan; [5]Department of Laboratory Medicine, Shinshu University Hospital, Nagano, Japan; [6]Department of Pathology, Keio University Graduate School of Medicine, Tokyo, Japan; [7]The International Human Epigenome Consortium (IHEC) and CREST, Japan Science and Technology Agency (JST), Saitama, Japan; [8]Department of Obstetrics and Gynecology, Tohoku University Graduate School of Medicine, Miyagi, Japan; [9]The Cancer System Laboratory, Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan; [10]Department of Obstetrics and Gynecology, Kyoto University Graduate School of Medicine, Kyoto, Japan

Correspondence to: Takuma Hayashi. Department of Immunology and Infectious Disease, Shinshu University Graduate School of Medicine, 3-1-1, Asahi, Matsumoto, Nagano 390-8621, Japan. Email: yoyoyo224@hotmail.com.

Abstract: Soft tissue sarcomas are neoplastic malignancies that typically arise in tissues of mesenchymal origin. The identification of novel molecular mechanisms leading to mesenchymal transformation and the establishment of new therapies and diagnostic biomarker has been hampered by several critical factors. First, malignant soft tissue sarcomas are rarely observed in the clinic with fewer than 15,000 newly cases diagnosed each year in the United States. Another complicating factor is that soft tissue sarcomas are extremely heterogeneous as they arise in a multitude of tissues from many different cell lineages. The scarcity of clinical materials coupled with its inherent heterogeneity creates a challenging experimental environment for clinicians and scientists. Faced with these challenges, there has been extremely limited advancement in clinical treatment options available to patients as compared to other malignant tumours. In order to glean insight into the pathobiology of soft tissue sarcomas, scientists are now using mouse models whose genomes have been specifically tailored to carry gene deletions, gene amplifications, and somatic mutations commonly observed in human soft tissue sarcomas. The use of these model organisms has been successful in increasing our knowledge and understanding of how alterations in relevant oncogenic and/or tumour suppressive signal cascades, i.e., interferon-γ (IFN-γ), tumour protein 53 (TP53) and/or retinoblastoma (RB) pathway directly impact sarcomagenesis. It is the goal of many in the physiological community that the use of several mouse models will serve as powerful *in vivo* tools for further understanding of sarcomagenesis and potentially identify new diagnostic biomarker and therapeutic strategies against human soft tissue sarcomas.

Keywords: Sarcoma; sarcomagenesis; proteasome beta subunit 9/β1i (PSMB9/β1i); tumour protein 53 (TP53); retinoblastoma (RB)

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Introduction

Soft tissue sarcomas are rare malignant mesenchymal tumours with less than 15,000 new cases diagnosed each year in the United States. Though rare, soft tissue sarcomas are highly debilitating malignancies as they are often associated with significant morbidity and mortality. Soft tissue sarcomas are biologically very heterogeneous as evidenced by the fact that soft tissue sarcomas arise from a plethora of different tissues and cell types. They are classically defined by their tissue of origin and are additionally stratified by their histopathology or patient's age at clinical diagnosis (1). While these classifications have proven useful, modern pathobiological and clinical techniques have the ability to further stratify sarcomas based on their genetic profile (2). Cytogenetic and karyotype

analyses have revealed two divergent genetic profiles in soft tissue sarcomas. The first and most simple genetic profile is the observation of translocation events in sarcomas with an otherwise normal diploid karyotype. On the other hand, most sarcomas display a more complex genetic phenotype, suggesting that genomic instability plays an important role in many soft tissue sarcomas.

Proteasome beta subunit (PSMB) 9/β1i is encoded in the major histocompatibility complex (MHC) class region of the 20S proteasome, which is component of the 26S complex that degrades ubiquitin-conjugated proteins. Studies done by Hayashi *et al*. reported that defective expression of PSMB9/β1i may initiate the development of spontaneous human malignant uterine mesenchymal tumour, i.e., uterine leiomyosarcoma (Ut-LMS) (3). As human soft tissue sarcomas including Ut-LMS are resistant to chemotherapy and radiotherapy, and thus surgical intervention is virtually the only means of clinical treatment, developing an efficient adjuvant therapy is expected to improve the prognosis of the soft tissue sarcoma. The identification of risk factors associated with the development of soft tissue sarcomas would significantly contribute to the development of diagnostic biomarkers, preventive and therapeutic treatments.

PSMB9/β1i correlates to uterine myometrium transformation

The proteasomal degradation is essential role for many cellular processes, including the cell cycle, the regulation of gene expression and immunological functions (4-6). Interferon (IFN)-γ induces the expression of large numbers of responsive genes, subunits of proteasome *β-ring*, i.e., PSMB9/β1i, PSMB5/β5i, and PSMB10/multicatalytic endopeptidase complex-like (MECL)-1/β2i (7,8). A molecular approach to study the correlation of IFN-γ with tumour cell growth has drawn attention. Homozygous mice deficient in PSMB9/β1i show tissue- and substrate-dependent abnormalities in the biological functions of the proteasome (7-9). Ut-LMS reportedly occurred in female *PSMB9/β1i*-deficient mice at age 6 months or older, and the incidence at 14 months of age was about 40% (3,10). Histological studies of PSMB9/β1i-lacking uterine soft tissue sarcomas have revealed characteristic abnormalities of human or mouse Ut-LMS (3,10). In recent studies, experiments with mouse uterine tissues and human clinical materials demonstrated a defective expression of PSMB9/β1i in human Ut-LMS that was traced to the IFN-γ signal cascade and the specific effect of somatic mutations in molecule of Janus kinase 1 (JAK1), which is also important for transducing a signal by IFN type I (IFN-α/β) and type II (IFN-γ), on the transcriptional activation of *PSMB9/β1i* gene (11). Furthermore, analysis of several established human Ut-LMS cell lines clarified the physiological significance of PSMB9/β1i in malignant myometrium transformation, thus implicating PSMB9/β1i as an anti-tumorigenic candidate (10,11).

Biological significance of tumour suppressor, TP53 in human sarcomagenesis

Tumour protein 53 (TP53), tumour suppressor pathway is one of the most well characterized pathways in malignant soft tissue sarcomas (12). *TP53* gene encodes a transcription factor required for the activation of numerous DNA damage-dependent checkpoint response and apoptotic genes, and thus its activities are often ablated in many soft tissue sarcomas. In addition to loss of TP53 functions via inherited germ line mutations, TP53 pathway is commonly disrupted by somatic mutations in *TP53* gene during sporadic sarcomagenesis (13,14). However, even though *TP53* gene alterations are widely regarded as having a significant impact on sarcomagenesis, many soft tissue sarcomas retain wild type *TP53*, yet phenotypically display a loss of TP53 function. These findings suggest that changes in other components of TP53 pathway; such as amplification of mouse double minute (MDM) 2 homolog, which is a negative regulator of TP53 signal cascade, may result in TP53 inactivation (15,16). Furthermore, both mice and humans with elevated expression levels of MDM2 due to a high frequency single nucleotide polymorphism in the *MDM2* promoter region (Mdm2SNP309) are more susceptible to sarcoma formation (17). Additionally, deletion or silencing of $P19^{Arf}$ ($P14^{ARF}$ in human), an inhibitor of the MDM2-TP53 axis, often results in development of soft tissue sarcomas. To increase the incidence of uterine malignant soft tissue sarcomas, i.e., Ut-LMS, and for better assessment of the role of the systemic expression of transform related protein 53 (TRP53) in response to the initiation of mouse Ut-LMS tumorigenesis, *Psmb9*-deficient mice were bred with *Trp53*-deficient mice (18). These breeding created $Psmb9^{-/-}Trp53^{-/-}$ mice and closely matched control $Psmb9^{-/-}Trp53^{+/+}$ mice (18). However, no significant differences were observed in Ut-LMS incidence between these three genetically modified mouse groups. Genetic susceptibility to young onset osteosarcoma is distinct from older adult onset osteosarcoma, with a high frequency of

Li-Fraumeni syndrome (LFS)-associated (3.8%) and rare exonic TP53 variants (5.7%) (19). The relationship between the onset of human Ut-LMS and TP53 was not clarified from the clinical data or experimental results obtained from these mice. Together, these experimental data indicate that while inactivation of the TP53 signal cascade is observed in the vast majority of human soft tissue sarcomas except for Ut-LMS, the mechanisms leading to disruption of the pathway can vary greatly.

Correlation between biological function of RB and human sarcomagenesis

Retinoblastoma (RB) is an embryonic malignant neoplasm of retinal origin. It almost always presents in early childhood and is often bilateral. The RB gene (*RB1*) was the first tumor suppressor gene cloned. It is a negative regulator of the cell cycle through its ability to bind the transcription factor E2F and repress transcription of genes required for synthesis phase (S phase), which is the part of the cell cycle in which DNA is replicated, occurring between cell cycle G1 phase and G2 phase (20). Many DNA tumor viruses appear to have transforming activity due, in part, to their ability to bind and inactivate the product of the *RB1* gene (21). RB cascade represents a second major tumour suppressor pathway deregulated in many sarcomas. Individuals inheriting a germline *RB* mutation typically develop malignant tumours of the eye early in life. However, in addition to retinal malignant tumours, these children have a significantly higher propensity to develop sarcomas than the general population (22). While inheritance of germline *RB* alterations increases sarcoma risk, there are also numerous examples of sporadic sarcomas harbouring spontaneous mutations and deletions of *RB*, particularly osteosarcomas and rhabdomyosarcomas (23). Furthermore, $P16^{INK4A}$, a negative regulator of the CDK-CYCLIN complexes that phosphorylate and activate RB, is often deleted in human soft tissue sarcomas (24). Francis *et al*. reported on the increased risk of human Ut-LMS in hereditary RB patients and provide a perspective for patient management relative to these clinical findings (25). Together, these findings illustrate the importance of RB signal cascade in sarcomagenesis.

Conclusions

The vast differences in the cellular origins of sarcomas, the lack of availability of tumour specimens, and the heterogeneity inherent within individual tumours has impeded our ability to fully understand the biological characterizations of soft tissue sarcomas. However, given the availability of numerous genetic knock-outs, knock-ins, and conditional alleles coupled with the bevy of tissue-specific Cre-recombinase expressing mouse lines, we now have the ability to systematically and prospectively interrogate how individual genes and mutations impact sarcomagenesis. Going forward, tumour analysis from multiple murine derived tumour types can be compared and contrasted in order to identify critical changes in specific soft tissue sarcomas. The molecular approaches have clearly demonstrated that while there are driver mutations/translocations, sarcomagenesis is, in fact, a multi-hit disease. The use of several mouse models mimicking the human disease symptom leads to identify critical therapeutic approaches, which can be taken to lessen the impact of these debilitating diseases (18,26-28). Human soft tissue sarcomas including Ut-LMS is refractory to chemotherapy and has a poor prognosis. The molecular biological and cytological information obtained from mouse tissues and human clinical materials will contribute remarkably to the development of preventive methods, a potential diagnostic biomarker, and new therapeutic approaches against human soft tissue sarcomas.

Summary

Soft tissue sarcomas are neoplastic malignancies that typically arise in tissues of mesenchymal origin. The identification of novel molecular mechanisms leading to sarcoma transformation and the establishment of new therapies has been hampered by several critical factors. Sarcomagenesis is also driven by aberrant oncogenic signaling and/or tumor suppressor pathways. Deregulation of oncogenic pathway aberrantly stimulates cellular proliferation, which in and of itself impinges on the IFN-γ, TP53 and/or RB pathways, collectively demonstrating the significant cross-talk between these signaling cascades but overlapping pathways. Given the numerous signaling pathways potentially disrupted in sarcomas, there has been a critical need to interrogate how each of these genes and divergent pathways impact sarcomagenesis in a prospective manner. The biological information will contribute remarkably to the development of preventive methods, a potential diagnostic biomarker, and new therapeutic approaches against human soft tissue sarcomas.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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