

Short Communication

Biological Characterization of the Uterine Malignant Mesenchymal Tumours

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Abstract

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 biomarker has been hampered by several critical factors. First, mesenchymal malignant tumour is rarely observed in the clinic with fewer than 15,000 newly cases diagnosed each year in the United States. Another complicating factor is that 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 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 sarcomas. The use of these model organisms has been successful in increasing our knowledge and understanding of how alterations in relevant oncogenic, tumour suppressive, and signaling pathways directly impact sarcomagenesis. It is the goal of many in the biological 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.

Keywords: Mesenchymal tumour; Leiomyosarcoma; PSMB9; TUMOUR PROTEIN 53(TP53); RETINOBLASTOMA(RB).

Introduction

Sarcomas are a rare malignant tumour with less than 15,000 new cases diagnosed each year in the United States. Though rare, sarcomas are highly debilitating malignancies as they are often associated with significant morbidity and mortality. Sarcomas

are biologically very heterogeneous as evidenced by the fact that mesenchymal tumours 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 karyo type analyses have revealed two divergent genetic profiles in 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 sarcomas.

Proteasome beta subunit (PSMB) 9/ β 1i is encoded in the major histocompatibility complex (MHC) class region of the 20S proteasome, which is part of the 26s complex that degrades ubiquitin-conjugated proteins. A study done by Hayashi et al. reported that defective expression of PSMB9/ β 1i may initiate the development of spontaneous human uterine leiomyosarcoma (Ut-LMS) [3]. As human mesenchymal tumours including Ut-LMS

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are resistant to chemotherapy and radiotherapy, and thus surgical intervention is virtually the only means of treatment, developing an efficient adjuvant therapy is expected to improve the prognosis of the sarcoma. The identification of a risk factor associated with the development of mesenchymal tumours would significantly contribute to the development of diagnostic biomarkers, preventive and therapeutic treatments.

IFN- γ -inducible factor, PSMB9/ β 1i correlates to uterine mesenchymal transformation

The proteasomal degradation is essential for many cellular processes, including the cell cycle, the regulation of gene expression and immunological function [4-6]. Interferon (IFN)- γ induces the expression of large numbers of responsive genes, subunits of proteasome β -ring, i.e., proteasome beta subunit (PSMB)9/ β 1i, PSMB5/ β 5i, and PSMB10/multicatalytic endopeptidase complex-like (MECL)-1/ β 2i [7,8]. A molecular approach to study the correlation of IFN- γ with tumour cell growth has drawn attention. Homozygous mice deficient in PSMB9/ β 1i show tissue- and substrate-dependent abnormalities in the biological functions of the proteasome [7-9]. Ut-LMS reportedly occurred in female PSMB9/ β 1i-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/ β 1i-lacking human uterine mesenchymal tumours have revealed characteristic abnormalities of Ut-LMS [3,10]. In recent studies, experiments with mouse uterine tissues and human clinical materials revealed a defective expression of PSMB9/ β 1i in human Ut-LMS that was traced to the IFN- γ pathway and the specific effect of somatic mutations in molecule of JANUS KINASE 1 (JAK1), which is also important for transducing a signal by type I (IFN- α/β) and type II (IFN- γ) interferons, on the PSMB9/ β 1i transcriptional activation [11]. Furthermore, analysis of several human Ut-LMS cell lines clarified the biological significance of PSMB9/ β 1i in malignant myometrium transformation, thus implicating PSMB9/ β 1i as an anti-tumorigenic candidate [10,11].

Biological significance of TP53 in human sarcomagenesis

Tumour protein 53 (TP53), tumour suppressor pathway is one of the most well characterized pathways in malignant tumours [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 malignant tumours. In addition to loss of *TP53* functions via inherited germline mutations, *TP53* pathway is commonly disrupted by somatic mutations in *TP53* gene during sporadic sarcoma genesis [13,14]. However, even though *TP53* gene alterations are widely regarded as having a significant impact on sarcoma genesis, many 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, a negative regulator of *TP53* pathway, may result in *TP53* inactivation [15,16]. Furthermore, both mice and humans with elevated levels of MDM2 due to a high frequency single nucleotide

polymorphism in the *MDM2* promoter (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 sarcomas. To increase the incidence of uterine mesenchymal tumour, 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. 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 data indicate that while inactivation of the *TP53* pathway is observed in the vast majority of human sarcomas except for Ut-LMS, the mechanisms leading to disruption of the pathway can vary greatly.

Correlation between biological function of RB and human sarcoma genesis

RETINOBLASTOMA (RB) is an embryonic malignant neoplasm of retinal origin. It almost always presents in early childhood and is often bilateral. The retinoblastoma 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 G1 phase and G2 phase [19]. RB pathway 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 [20]. While inheritance of germ line *RB* alterations increases sarcoma risk, there are also numerous examples of sporadic sarcomas harbouring spontaneous mutations and deletions of *RB*, particularly osteosarcomas and rhabdomyosarcomas [21]. Furthermore, *P16^{INK4A}*, a negative regulator of the CDK-CYCLIN complexes that phosphorylate and activate *RB*, is often deleted in human sarcomas [22]. Clinical experiment suggests increased risk of Ut-LMS in hereditary *RB* patients [23]. Together, these findings illustrate the importance of *RB* pathway 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 mesenchymal tumours. 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 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 [18,23,24]. Human mesenchymal tumours 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 mesenchymal tumours.

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