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Biological Analyses for Understanding of The Uterine Sarcomagenesis

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ABSTRACT

Soft tissue sarcomas are neoplastic malignancies that typically arise in tissues of a mesenchymal origin. The identification of novel molecular mechanisms leading to sarcoma formation and the establishment of new therapies and biomarkers have been hampered by several critical factors. This type of cancer is rarely observed in clinical settings, with fewer than 15,000 new cases being 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 samples coupled with its inherent heterogeneity creates a challenging experimental environment for clinicians and scientists. Faced with these challenges, there have been extremely limited advances in treatment options available to patients with soft tissue soft tissue sarcomas compared with those for patients with other cancers. 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 point 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, tumour suppressive, and signaling pathways directly impact sarcomagenesis. It is the goal of many in the biological community that the use of these mouse models will serve as powerful in vivo tools to further our understanding of sarcomagenesis and potentially identify new biomarkers and develop therapeutic strategies.

KEYWORDS : Leiomyosarcoma; LMP2; TUMOUR PROTEIN 53 (TP53); RETINOBLASTOMA (RB)

Introduction

Soft tissue sarcomas are a rare malignant tumour with less than 15,000 new cases being 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 these tumours arising from a plethora of different tissues and cell types. The prognosis of patients with uterine leiomyosarcoma (Ut-LMS) is poor, and the 5-year survival rate is approximately 35% [1]. Uterine leiomyoma (LMA) may occur in 70-80% of women by the age of 50 years [1]. They are classically defined by their tissue of origin and are additionally stratified by their histopathology or patient's age at diagnosis [1]. While these classifications have proven useful, modern pathobiological and clinical techniques have the ability to further stratify soft tissue sarcomas based on their genetic profiles [2]. Cytogenetic and karyotype analyses have revealed two divergent genetic profiles in soft tissue sarcomas. The first and most simple genetic profiles are the observation of translocation events in soft tissue sarcomas with an otherwise normal diploid karyotype. On the other hand, soft tissue sarcomas display a more complex genetic phenotype, suggesting that genomic instability plays an important role in many soft tissue sarcomas. Difficulties have been reported in distinguishing Ut-LMS from other uterine mesenchymal tumors, and a diagnosis generally requires surgery and cytoscopy. Diagnostic categories for uterine mesenchymal tumors and morphological criteria are used to assign cases. The non-standard subtypes of uterine mesenchymal tumors such as the epithelioid and myxoid types are classified in a different manner using these features; therefore, a diagnostic method needs to be established that can identify non-standard smooth muscle differentiation.

IFN-γ-inducible factor, LMP2/β1i correlates to uterine mesenchymal transformation

Proteasomal degradation is essential for many cellular processes, including the cell cycle, the regulation of gene expression, and immunological functions [3,4,5]. Interferon (IFN)-γ induces the expression of large numbers of responsive genes, subunits of proteasome β-*ring*, i.e., low-molecular mass polypeptide (LMP)2/β1i, LMP7/β5i, and LMP10/multicatalytic endopeptidase complex-like (MECL)-1/β2i [6,7]. A molecular approach to investigating the relationship between IFN-γ and tumour cell growth has been attracting increasing attention. Homozygous mice deficient in LMP2/β1i show tissue- and substrate-dependent abnormalities in the biological functions of the proteasome [6,7]. Ut-LMS reportedly occurred in female *LMP2/β1i*-deficient mice at age 6 months or older, and the incidence at 12 months of age was about 37% [8]. Histological studies on LMP2/β1i-lacking uterine tumours have revealed the characteristic abnormalities of Ut-LMS [8]. Recent study, experiments with human and mouse uterine tissues revealed a defective LMP2/β1i expression in human Ut-LMS that was traced to the IFN-γ pathway and the specific effect of JANUS KINASE 1 (JAK1) somatic mutations on the LMP2/β1i transcriptional activation [9]. Furthermore, an analysis of a human Ut-LMS cell line clarified the biological significance of LMP2/β1i in malignant myometrium transformation, thereby implicating LMP2/β1i as an anti-tumourigenic candidate [9,10].

Tumour suppressor and oncogenic pathways involved in sarcomagenesis

Tumour protein 53 (TP53), tumour suppressor pathway is one of the most well characterized signal cascades in tumourigenesis [11]. *TP53* gene encodes a transcription regulator required for the 1activation of numerous DNA damage-dependent checkpoint response and apoptotic genes, and thus its activities are often ablated in many malignant tumours. In addition to the loss of TP53 functions via inherited germline mutations, the TP53 signaling pathway is commonly disrupted by point mutations in the *TP53* gene during sporadic sarcomagenesis [12,13]. However, even though *TP53* gene alterations are widely regarded to have a significant impact on sarcomagenesis, many soft tissue sarcomas retain wild-type TP53, but phenotypically display a loss of TP53 function. These research findings suggest that changes in other components of TP53 signal cascade; such as amplification of MDM2, a negative regulator of TP53 signaling pathway, may result in TP53 inactivation [14,15]. Furthermore, mice and humans with elevated levels of MDM2 due to a high frequency single nucleotide polymorphism in the *MDM2* promoter (Mdm2SNP309) are both more susceptible to sarcoma formation [16]. 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. Together, these findings indicate that while inactivation of the TP53 signaling pathway is observed in the vast majority of human soft tissue sarcomas, the mechanisms leading to disruption of the pathway vary greatly.

The RETINOBLASTOMA (RB) signaling pathway represents a second major tumour suppressor pathway that is deregulated in many soft

tissue sarcomas. Individuals inheriting a germline *RB* mutation typically develop cancers of the eye early in life. However, in addition to retinal cancers, these children have a significantly higher propensity to develop soft tissue sarcomas than the general population [17]. While the inheritance of germline *RB* alterations increases the risk of sarcoma, there are also numerous examples of sporadic sarcomas harboring spontaneous mutations and deletions in RB, particularly osteosarcomas and rhabdomyosarcomas [18]. Furthermore, *P16^{INK4A}*, a negative regulator of the CDK-CYCLIN complexes that phosphorylate and activate RB, is often deleted in soft tissue sarcomas [19]. These findings may illustrate the importance of RB signaling pathway in sarcomagenesis. Although we previously demonstrated that the abnormal expression of TP53, Ki-67 and mutations in TP53 were frequently associated with Ut-LMS, the defective expression of LMP2/β1i appeared to be more characteristic of human Ut-LMS than these factors.

Conclusions

The prominent differences in the cellular origins of soft tissue sarcomas, the lack of availability of tumour specimens, and the heterogeneity inherent within individual tumours has impeded our ability to fully understand the biology of soft tissue sarcomas. However, given the availability of numerous genetic knock-outs, knock-ins, and conditional alleles coupled with the numerous of tissue-specific Cre-recombinase expressing mouse lines, we now have the ability to systematically and prospectively determine the impact of individual genes and mutations on 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 these mouse models mimicking the human disease condition will lead to critical therapeutic approaches, which may lessen the impact of these debilitating diseases.

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Competing Interests

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of this manuscript.

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