IOSR Journal Of Pharmacy *www.iosrphr.org*
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 6, Issue 1 (January 2016), PP. 41-44

Molecular approaches of sarcomagenesis for establishment of clinical therapy

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Abstract: 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 newly cases being 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 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 sarcomas than in those for patients with other cancers. 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 point 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, tumor 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)*

I. INTRODUCTION

Sarcomas are a rare malignant tumor with less than 15,000 new cases being 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 these tumors arising 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 diagnosis [1]. While these classifications have proven useful, modern pathobiological and clinical techniques have the ability to further stratify sarcomas based on their genetic profiles [2]. Cytogenetic and karyotype analyses have revealed two divergent genetic profiles in sarcomas. The first and most simple genetic profiles are 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.

II. IFN-□-INDUCIBLE FACTOR, LMP2/□1I 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)-□ induces the expression of large numbers of responsive genes, subunits of proteasome □-*ring*, i.e., low-molecular mass polypeptide (LMP)2/□1i, LMP7/□5i, and LMP10/multicatalytic endopeptidase complex-like (MECL)-1/□2i [7]. A molecular approach to investigating the relationship between IFN-γ and tumor cell growth has been attracting increasing attention. Homozygous mice deficient in LMP2/□1i show tissue- and substrate-dependent abnormalities in the biological functions of the proteasome [7]. Uterine leiomyosarcoma (Ut-LMS) reportedly occurred in female *LMP2/□1i*-deficient mice at age 6 months or older, and the incidence at 12 months of age was about 37% [8]. Histological studies on LMP2/□1i-lacking uterine tumors have revealed the characteristic abnormalities of Ut-LMS [8]. Recent study, experiments with human and mouse uterine tissues revealed a defective LMP2/□1i expression in human Ut-LMS that was traced to the IFN-□ pathway and the specific effect of JANUS KINASE 1 (JAK1) somatic mutations on the LMP2/□1i transcriptional activation 9. Furthermore, an analysis of a human Ut-LMS cell line clarified the biological significance of LMP2/□1i in malignant myometrium transformation, thereby implicating LMP2/□1i as an anti-tumorigenic candidate [9,10].

III. TUMOR SUPPRESSOR AND ONCOGENIC PATHWAYS INVOLVED IN SARCOMAGENESIS

Tumor protein 53 (TP53), tumor suppressor pathway is one of the most well characterized signal cascades in tumorigenesis [11]. *TP53* gene encodes a transcription regulator required for the activation of numerous DNA damage-dependent checkpoint response and apoptotic genes, and thus its activities are often ablated in many malignant tumors. In addition to the loss of TP53 functions via inherited germline mutations, the TP53 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 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 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 sarcomas. However studies with genetically modified mice and human clinical materials have not clearly demonstrated biological significance of TP53 or TPR53 in uterine sarcomagenesis [17]. Together, these findings indicate that while inactivation of the TP53 pathway is observed in the vast majority of human sarcomas except uterine leiomyosrcoma, the mechanisms leading to disruption of the pathway vary greatly.

The RETINOBLASTOMA (RB) pathway represents a second major tumor suppressor pathway that is deregulated in many 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 sarcomas than the general population [18]. 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 [19]. Furthermore, $P16^{INK4A}$, a negative regulator of the CDK-CYCLIN complexes that phosphorylate and activate RB, is often deleted in sarcomas [20]. Together, these findings illustrate the importance of RB pathway in sarcomagenesis.

IV. CONCLUSIONS

The prominent differences in the cellular origins of sarcomas, the lack of availability of tumor specimens, and the heterogeneity inherent within individual tumors has impeded our ability to fully understand the biology of 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 determine the impact of individual genes and mutations on sarcomagenesis. Going forward, tumor analysis from multiple murine-derived tumor 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 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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