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Mouse models of sarcoma for understanding of the tumourigenesis

Takuma Hayashi | Chhatrapati Bhilai Institute of Technolgy ,Durg,(CG) 8966000053.

ABSTRACT

Sarcomas are neoplastic malignancies that typically arise in tissues of mesenchymal origin. The identification of novel molecular mechanisms leading to sarcoma formation and the establishment of new therapies and biomarker has been hampered by several critical factors. First, this type of cancer 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 (e.g. osteosarcoma, liposarcoma, and myosarcoma). The scarcity of clinical samples 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 treatment options available to patients as compared to other cancers. In order to glean insight into the pathobiology of sarcomas, scientists are now using in vivo 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 biomarker and therapeutic strategies.

KEYWORDS | Leimyosarcoma; Mouse model; 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 these 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 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 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 genomic instability plays an important role in many sarcomas.

Tumour suppressor and oncogenic pathways involved in sarcomagenesis:

Tumour protein 53 (TP53), tumour suppressor pathway is one of the most well characterized pathways in cancers.3 *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 cancers. In addition to loss of TP53 functions via inherited germline mutations, TP53 pathway is commonly disrupted by point mutations in *TP53* gene during sporadic sarcomagenesis.4,5 However, even though *TP53* gene alterations are widely regarded as having a significant impact on sarcomagenesis, 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 MDM2, a negative regulator of TP53 pathway, may result in TP53 inactivation.6,7 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.8 Additionally, deletion or silencing of *p19Arf* (*P14ARF* in human), an inhibitor of the MDM2-TP53 axis, often results in development of sarcomas. Together, these data indicate that while inactivation of the TP53 pathway is observed in the vast majority of human sarcomas, the mechanisms leading to disruption of the pathway can vary greatly.

RETINOBLASTOMA (RB) pathway represents a second major tumour suppressor pathway deregulated in many sarcomas. Individuals inheriting a germline *RB* mutation typically develop cancers of the eye early in life.9 However, in addition to retinal cancers, these children have a significantly higher propensity to develop sarcomas than the general population.10 While inheritance of a 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.11 Furthermore, *P16INK4A*, a negative regulator of the CDK-CYCLIN complexes that phosphorylate and activate RB, is often deleted in sarcomas.12 Together, these findings illustrate the importance of RB pathway in sarcomagenesis.

Oncogenic signalling and Leiomyosarcomas:

In addition to loss of tumour suppressor pathways, sarcomagenesis is also driven by aberrant oncogenic signal. The RAS signal pathway in particular is thought to be altered during sarcoma development.13 Deregulation of the RAS pathway aberrantly stimulates cellular proliferation, which in and of itself impinges on TP53 and RB pathways, collectively demonstrating the significant cross-talk between these three separate but overlapping pathways. Given the numerous signal 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. Since these experiments are nearly impossible in human patients, scientists and clinicians are now using mice genetically tailored for such studies. Below, we will highlight several well characterized genetically engineered mouse models harbouring common genetic alterations observed in sarcoma pathobiology.

Given the extreme heterogeneity of sarcomas with regards to tissue of origin, it is obvious that alterations to numerous genes, pathways, and signal complexes play an important role in the pathobiology of sarcomas. While this review does not cover all genetic alterations responsible for sarcoma de-

velopment, there are numerous additional genes that impact this disease. For example, alterations in expression of tumour suppressor genes, such as *NEUROFIBROMASIS* (*NF1* and *NF2*), likewise impact the etiology of some sarcomas. Mouse models that carry genomic deletions and/or tissue-specific Cre-mediated deletion of *NF1* result in neurofibromas.14 These NF1-dependent phenotypes are further exacerbated when NF1 is concomitantly deleted with other tumour suppressors (TP53 and P14ARF) resulting in more aggressive phenotypes as evidenced by malignant peripheral nerve sheath tumour formation.15 To further illustrate that loss of a single gene impacts sarcoma formation, mice harbouring an *Psmb9* deletion resulted in spontaneous uterine leiomyosarcomas.16 This provides evidence of its role as a tumour suppressor and a potential biomarker in human uterine leiomyosarcoma.16,17 In addition to loss of function alterations, overexpression of teratocarcinoma-derived growth factor 1, also known as CRIPTO, results in leiomyosarcomas by deregulation of the WNT pathway. 18,19

Conclusion:

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 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 interrogate how individual genes and mutations impact sarcomagenesis. Going forward, tumor analysis from multiple murine derived tumour types can be compared and contrasted in order to identify critical changes in specific sarcomas. These mouse models 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 leads to identify critical therapeutic approaches, which can be taken to lessen the impact of these debilitating diseases. First, we must recognize that these mouse models demonstrate the synergism between multiple pathways and thus combinatorial treatment strategies are needed to combat these sarcomas. For treatment of patients with translocations, one can envision a targeted therapeutic approach, like that which has been observed in the treatment of chronic myeloid leukemia. The addition of tyrosine kinase inhibitors (TKIs), such as Imatinib, which inhibits the activity of the BCR-ABL fusion gene, has reduced CML from a death sentence to a manageable and stable disease. The scientific/clinical community may design target drugs to the translocation events observed in sarcomas. The use of these mouse models may serve as an excellent preclinical platform for such studies.

Treating and alleviating the disease process in sarcomas with complex genetics may prove more difficult than identifying targeted therapies. However, given that many groups have identified the importance of specific pathways in sarcomagenesis, such as the TP53 pathway, we have a starting point. Preclinical chemical drugs like PRIMA1 have been shown to restore mutant TP53 activities. These drugs could be rapidly screened for efficacy in mutant TP53 sarcoma models. Moreover, TP53 pathway is also inactivated by dysregulation of its protein partners, MDM2 and $P14^{Arf}$. The employment of inhibitor against MDM2-TP53 interaction, such as Nutlin-3 and RITA may prove efficacious in reactivating TP53 pathway and thus provide a therapeutic benefit. Also, loss of $P14^{ARF}$ due to promoter methylation is a common event in sarcomagenesis. Therefore, these animal models may prove useful in examining the impact of hypomethylating agents, such as Azacytidine or SPRYCEL, in sarcomas. While mouse models can not completely predict the outcome of each disease, they can provide valuable and critical information, particularly in exceedingly rare types of sarcomas or when low penetrate single nucleotide polymorphisms confound data analysis.

REFERENCES

1. Lasota J, Fanburg-Smith JC: Genetics for the diagnosis and treatment of mesenchymal tumors. Semin Musculoskelet Radiol 2007; 11(3): 215-230. | 2. Taylor BS, et al: Advances in sarcoma genomics and new therapeutic targets. Nat Rev Cancer 2011; 11(8): 541-557. | 3. Vogelstein B, Lane D, Levine AJ: Surfing the p53 network. Nature 2000; 408(6810): 307-310. | 4. Raycroft L, Wu HY, Lozano G: Transcriptional activation by wild-type but not transforming mutants of the p53 anti-oncogene. Science 1990; 249(4972): 1049-1051. | 5. Wang LL: Biology of osteogenic sarcoma. Cancer J 2005; 11(4): 294–305. | 6. Oliner JD, et al: Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature 1992; 358(6381): 80-83. | 7. Oliner JD, et al: Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. Nature 1993; 362(6423): 857-860. | 8. Ito M, et al: Comprehensive Mapping of p53 Pathway Alterations Reveals an Apparent Role for Both SNP309 and MDM2 Amplification in Sarcomagenesis. Clin Cancer Res 2011; 17(3): 416-426. | 9. Knudson AG Jr: Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A 1971; 68(4): 820-823. | 10. Deshpande A, Hinds PW: The retinoblastoma protein in osteoblast differentiation and osteosarcoma. Curr Mol Med 2006; 6(7): 809-817. | 11. Toguchida J, et al: Preferential mutation of paternally derived RB gene as the initial event in sporadic osteosarcoma. Nature 1989; 338(6211): 156-158. | | 12. Oda Y, et al: Frequent alteration of p16(INK4a)/p14(ARF) and p53 pathways in the round cell component of myxoid/round cell liposarcoma: p53 gene alterations and reduced p14(ARF) expression both correlate with poor prognosis. J Pathol 2005; 207(4): 410-421. | 13. Bourdeaut F, et al: Mosaicism for oncogenic G12D KRAS mutation associated with epidermal nevus, polycystic kidneys and rhabdomyosarcoma. J Med Genet 2010; 47(12): 859-862. | 14. Brossier NM, Carroll SL: Genetically engineered mouse models shed new light on the pathogenesis of neurofibromatosis type I-related neoplasms of the peripheral nervous system. Brain Res Bull 2012; 88(1): 58-71. | 15. Buchstaller J, McKeever PE, Morrison SJ: Tumorigenic cells are common in mouse MPNSTs but their frequency depends upon tumor genotype and assay conditions. Cancer Cell 2012; 21(2): 240-252. | 16. Hayashi T, Faustman DL: Development of spontaneous uterine tumors in low molecular mass polypeptide-2 knockout mice. Cancer Res 2002; 62(1): 24-27. | 17. Hayashi T, et al: Molecular Approach to Uterine Leiomyosarcoma: LMP2-Deficient Mice as an Animal Model of Spontaneous Uterine Leiomyosarcoma. Sarcoma 2011; 476-498. | 18. Strizzi L, et al: Development of leiomyosarcoma of the uterus in MMTV-CR-1 transgenic mice. J Pathol 2007; 211(1): 36-44. | 19. Hayashi T, et al: Potential role of LMP2 as tumor-suppressor defines new targets for uterine leiomyosarcoma therapy. Sci Rep 2011; 1:180. |