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Short Communication

Biological Analyses for Characterization of the Uterine Sarcoma Using Mouse Model

Abstract

Uterine sarcomas are neoplastic malignancies that typically arise in tissues of a mesenchymal origin in uterine body. The identification of novel molecular mechanisms leading to sarcoma formation, and the establishment of new therapies and biomarkers has been hampered by several critical factors. Uterine leiomyosarcoma (U-LMS), which is the most common sarcoma, 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 U-LMS 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 U-LMS compared with those for patients with other malignant tumors. In order to glean insight into the pathobiology of U-LMS, 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, tumor suppressive, and signaling pathways directly impact sarcomagenesis. It is the aim of many in the biological community that the use of these genetically modified mouse models will serve as powerful *in vivo* tools to further our understanding of sarcomagenesis and potentially identify novel biomarkers and develop therapeutic strategies.

Introduction

Uterine sarcomas comprise a group of rare tumors with differing tumor biology, natural history and response to treatment. Clinical diagnosis is often made following surgical approach for presumed benign disease. Currently pre-operative imaging does not reliably distinguish between benign leiomyomas and other malignant pathology. Uterine leiomyosarcoma (U-LMS) is the most common sarcoma but other subtypes include endometrial stromal sarcoma (low grade and high grade), undifferentiated uterine sarcoma and adenosarcoma.

U-LMSs are a rare malignant mesenchymal tumor with less than 15,000 new cases being diagnosed each year in the United States. Though rare, U-LMSs are highly debilitating malignancies as they are often associated with significant morbidity and mortality. U-LMSs 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 soft tissue sarcomas based on their genetic profiles [2]. Cytogenetic and karyotype analyses have revealed two divergent genetic profiles in U-LMSs. The first and most simple genetic profiles are the observation of translocation events in U-LMSs with an otherwise normal diploid karyotype. On the other hand, most U-LMSs display a more complex genetic phenotype, suggesting that genomic instability plays an important role in many U-LMSs. Aim is to understand the molecular mechanisms of human U-LMS, which may lead to identification of new diagnostic candidates or therapeutic targets against human U-LMS.

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

The proteasome is a key regulator of cellular protein

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homeostasis and is a clinically validated anticancer target. The immunoproteasome, a subtype of proteasome expressed mainly in hematopoietic cells, was initially recognized for its role in antigen presentation during the immune response. Recently, the immunoproteasome has been implicated in several disease conditions including cancer and autoimmune disorders, but many of the factors contributing to these pathological processes remain unknown [3-5]. 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 tumor 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 [7]. Uterine mesenchymal tumors 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]. Histobiological studies on LMP2/β1i-lacking uterine mesenchymal tumors have revealed the characteristic abnormalities of human U-LMS [8].

Recent studies with human clinical materials and mouse uterine tissues revealed a defective LMP2/β1i expression in human U-LMS that was traced to the IFN-γ pathway and the specific effect of somatic mutations of JANUS KINASE 1 (JAK1) molecule on the LMP2/β1i transcriptional activation [9]. Furthermore, an analysis of a human U-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]. LMP2/β1i is frequently expressed in colon and pancreatic cancers, but the codon 60 LMP2/β1i polymorphism has no significant impact on the catalytic activity of LMP2/β1i expressed in multiple types of cancer cell lines [11]. In a recent report, a comparative genomic hybridization (CGH)-based analysis of LMS using a high resolution genome-wide array gave gene-level information about the amplified and deleted regions that may play a role in the development and progression of human U-LMS. Other reports showed that among the most intriguing changes in genes were losses of JAK1 (1p31-p32) and PSMB9/β1i (6p21.3) [12]. The functionally inactivated K33A mutant of LMP2/β1i, which cannot have incorporation to proteasome complex, has the same cellular morphology *in vitro* as the LMP2/β1i-wt transfectant, suggesting that the physiological action of LMP2/β1i is not only through its role in immunoproteasomes, but also as a single subunit molecule [9]. Single LMP2/β1i molecule with other cellular factors reportedly regulates tissue-specific tumorigenesis, i.e. uterine myometrium cell transformation and/or sarcomagenesis [9,11].

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 [13]. *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 somatic mutations, the TP53 pathway is commonly disrupted by somaric mutations in the *TP53* gene during sporadic sarcomagenesis [14,15]. 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 pathway, may result in inactivation of TP53 [16,17]. 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 [18]. 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. However, normal physiological function of TP53 is observed in human U-LMS at high stage malignancy [19]. Together, these findings indicate that while inactivation of the TP53 pathway is detected in the vast majority of human U-LMS, the mechanisms leading to disruption of the pathway vary greatly. TP53 might not play key role on sarcomagenesis of human U-LMS

The RETINOBLASTOMA (RB) pathway represents a second major tumour suppressor pathway that is deregulated in many soft tissue sarcomas. Individuals inheriting germline *RB* somatic mutations 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 soft tissue sarcomas than the general population [20]. 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 [21]. Furthermore, $P16^{INK4A}$, a negative regulator of the cyclin dependent kinase (CDK)-CYCLIN complexes that phosphorylate and activate RB, is often deleted in soft tissue sarcomas [22]. Together, these findings illustrate the importance of RB pathway in sarcomagenesis.

Conclusions

The prominent differences in the cellular origins of soft tissue sarcomas including U-LMS, the lack of availability of tumor specimens, and the heterogeneity inherent within individual tumors 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, tumor analysis from multiple murine-derived tumor 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 somatic mutations/translocations, sarcomagenesis is, in fact, a multi-hit disease. The use of these mouse models mimicking

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the human disease condition will lead to critical therapeutic approaches, which may lessen the impact of these debilitating diseases.

References

1. Lasota J, Fanburg-Smith JC (2007) Genetics for the diagnosis and treatment of mesenchymal tumors. Semin. Musculoskelet Radiol 11: 215-230.

2. Taylor BS, Barretina J, Maki RG, Antonescu CR, Singer S, et al. (2011) Advances in sarcoma genomics and new therapeutic targets. Nat Rev Cancer 11: 541-557. Link: https://goo.gl/eCgJtq

3. Peters JM, Franke WW, Kleinschmidt JA. (1994) Distinct 19 S and 20 S subcomplexes of the 26 S proteasome and their distribution in the nucleus and the cytoplasm. J Biol Chem 269: 7709-7718. Link: https://goo.gl/R3Z3mP

4. Lodish H, Berk A, Matsudaira P, Kaiser CA, Krieger M, et al. (2004) Mol Cell Biol (5th ed.). New York: WH. Freeman and CO 5: 66-72.

5. Konstantinova IM, Tsimokha AS, Mittenberg AG (2008) Role of proteasomes in cellular regulation. Intl. Rev. Cell Mol. Biol 267: 59-124. Link: https://goo.gl/73avDE

6. Wang J, Maldonado MA (2006) The Ubiquitin-Proteasome System and Its Role in Inflammatory and Autoimmune Diseases. Cell Mol Immunol 3: 255-261. Link: https://goo.gl/00iCpw

7. Van KL, Ashton-Rickardt PG, Eichelberger M, Gaczynska M, Nagashima K, et al. (1994) Altered peptidase and viral-specifi c T cell response in LMP2 mutant mice. Immunity 1: 533-541. Link: https://goo.gl/ClHVE6

8. Hayashi T, Faustman DL (2002) Development of spontaneous uterine tumors in low molecular mass polypeptide-2 knockout mice. Cancer Res 62: 24-27. Link: https://goo.gl/EgTiSw

9. Hayashi T, Horiuchi A, Sano K, Hiraoka N, Kasai M, et al. (2011) Potential role of LMP2 as tumor-suppressor defines new targets for uterine leiomyosarcoma therapy. Sci Rep 1: 180. Link: https://goo.gl/xuvpRg

10. Hayashi T, Horiuchi A, Sano K, Hiraoka N, Kasai M, et al. (2012) Potential role of LMP2 as an anti-oncogenic factor in human uterine leiomyosarcoma: morphological significance of calponin h1. FEBS Lett 586: 1824-1831. Link: https://goo.gl/feXNss

11. Park JE, Ao L, Miller Z, Kim K, Wu Y, et al. (2013) PSMB9 Codon 60 Polymorphisms Have No Impact on the Activity of the Immunoproteasome Catalytic Subunit B1i Expressed in Multiple Types of Solid Cancer. Plos One 8: 1-7. Link: https://goo.gl/qxlwgP

12. Hayashi T, Kawano M, Ichimura T, Kasai M, Ida K, et al. (2016) Gene analysis of Interferon-g signal molecules in human uterine leiomyosarcoma. WULFENIA J 23: 1-18.

13. Vogelstein B, Lane D, Levine AJ (2000) Surfing the p53 network. Nature 408: 307-310. Link: https://goo.gl/oqMEJZ

14. Raycroft L, Wu HY, Lozano G (1990) Transcriptional activation by wild-type but not transforming mutants of the p53 anti-oncogene. Science 249: 1049-1051. Link: https://goo.gl/7UwWeu

15. Wang LL (2005) Biology of osteogenic sarcoma. Cancer J 11: 294-305. Link: https://goo.gl/ElThQB

16. Oliner JD, Kinzler KW, Meltzer PS, George DL, Vogelstein B (1992) Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature 358: 80-83. Link: https://goo.gl/uwvEBE

17. Oliner JD, Pietenpol JA, Thiagalingam S, Gyuris J, Kinzler KW, et al. (1993) Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. Nature 362: 857-860. Link: https://goo.gl/9E59oU

18. Ito M, Barys L, O'Reilly T, Young S, Gorbatcheva B, et al. (2011) Comprehensive Mapping of p53 Pathway Alterations Reveals an Apparent Role for Both SNP309 and MDM2 Amplification in Sarcomagenesis. Clin. Cancer Res 17: 416-426. Link: https://goo.gl/o708JZ

19. Hayashi T, Kawano M, Ichimura T, Ida K, Ando H, et al. (2016) Molecular Pathology and Novel Clinical Therapy for Uterine Leiomyosarcoma. Anticancer Research 36: 4997-5007. Link: https://goo.gl/TXCP2W

20. Deshpande A, Hinds PW (2006) The retinoblastoma protein in osteoblast differentiation and osteosarcoma. Curr Mol Med 6: 809-817. Link: https://goo.gl/JJhgQl

21. Toguchida J, Ishizaki K, Sasaki MS, Nakamura Y, Ikenaga M, et al. (1989) Preferential mutation of paternally derived RB gene as the initial event in sporadic osteosarcoma. Nature 338: 156-158. Link: https://goo.gl/K6mKm0

22. Oda Y, Yamamoto H, Takahira T, Kobayashi C, Kawaguchi K, et al. (2005) 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 207: 410-421. Link: https://goo.gl/SIEK0w

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