Short Communication

Biological Characterization of the Uterine Malignant Mesenchymal Tumours

Takuma Hayashi1,2*, Akiko Horiuchi3, Kenji Sano4, Yare Kanai4,5, Nobuo Yaegashi2, Hiroyuki Aburatani6 and Ikuo Konishi7

1Department of Immunology and Infectious Disease, Shinshu University School of Medicine, Nagano, Japan
2Horiuchi Ladies Clinic, Nagano, Japan
3Department of Laboratory Medicine, Shinshu University Hospital, Nagano, Japan
4Department of Pathology, Keio University Graduate School of Medicine, Tokyo, Japan
5Department of Obstetrics and Gynecology, Keio University Graduate School of Medicine, Miyagi, Japan
6The Cancer System Laboratory, Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan
7Department of Obstetrics and Gynecology, Kyoto University Graduate School of Medicine, Kyoto, Japan
8Promoting Business using Advanced Technology, Japan Science and Technology Agency (JST), Tokyo, Japan
9The International Human Epigenome Consortium (IHEC) and CREST, Japan Science and Technology Agency (JST), Saitama, Japan

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 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) 9i 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 PSMB9i may initiate the development of spontaneous human uterine leiomyosarcoma (Ut-LMS) [3]. As human mesenchymal tumours including Ut-LMS

*Corresponding author: Takuma Hayashi, Dept. of Immunology and Infectious Disease, Shinshu University Graduate School of Medicine, 3-1-1, Asahi, Matsumoto, Nagano 390-8621, Japan. Tel: 81-263-37-2611; E-mail: yoyoyo224@hotmail.com

Rec Date: September 30 2015, Acc Date: October 16 2015, Pub Date: October 19 2015.

Citation: Takuma Hayashi, Akiko Horiuchi, Kenji Sano, Yare Kanai, Nobuo Yaegashi, et al. (2015) Biological Characterization of the Uterine Malignant Mesenchymal Tumours. BAOJ Cancer Res Ther 1: 013.

Copyright: © 2015 Takuma Hayashi, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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, preventative and therapeutic treatments.

**IFN-γ-inducible factor, PSMB9/β1i correlates to uterine mesenchymal transformation**

The proteasomal degradation is essential for many cellular processes, including the cell cycle, the regulation of many 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 (PSMB9/β1i, PSMB5/β5i, and PSMB10/multicatalytic endopeptidase complex-like (MECL)-1/β2i [7,8]). A molecular approach to study the correlation of IFN-γ with tumour cell growth has drawn attention. Homozygous mice deficient in PSMB9/β1i show tissue- and substrate-dependent abnormalities in the biological functions of the proteasome [7-9]. Ut-LMS reportedly occurred in female PSMB9/β1i-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/β1i-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/β1i 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/β1i transcriptional activation [11]. Furthermore, analysis of several human Ut-LMS cell lines clarified the biological significance of PSMB9/β1i in malignant myometrium transformation, thus implicating PSMB9/β1i 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 p19ARF (P14ARF 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 transforn 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, P16INK4A, 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.

Acknowledgments

We sincerely thank Professor Susumu Tonegawa (Picower Institute for Learning and Memory, M.I.T.) and Professor Luc Van Kaer (Vanderbilt University Medical Center) for their research assistance. These studies were supported in part by grants from the Ministry of Education, Culture, Science and Technology, The Foundation of Osaka Cancer Research, The Ichiro Kanehara Foundation of the Promotion of Medical Science and Medical Care, The Foundation for the Promotion of Cancer Research, The Kanzawa Medical Research Foundation, The Shinshu Medical Foundation, and The Takeda Foundation for Medical Science.

References

1. Lasota J, Fanburg-Smith JC (2007) Genetics for the diagnosis and treatment of mesenchymal tumors. Semin. Musculoskelet Radiol 11(3): 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(8): 541-557.
3. Hayashi T, Faustman DL (2002) Development of spontaneous uterine tumours in low molecular mass polypeptide-2 knockout mice. Cancer Res 62(1): 24-27.
4. 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(10): 7709-7718.
5. Lodish H, Berk A, Matsudaira P, Kaiser CA, Krieger M, et al. (2004) “3”. Molecular Biology of the Cell (5th ed.). New York: W.H. Freeman and CO. S: 66-72.
6. Konstantinova IM, Tsimokha AS, Mittenberg AG (2008) Role of proteasomes in cellular regulation. Intl Rev Cell Mol Biol 267: 59-124.
7. Wang J, Maldonado MA (2006) The Ubiquitin-proteasome system and its role in inflammatory and autoimmune diseases. Cell Mol Immunol 3(4): 255-261.
8. Van Kaer L, Ashton-Rickardt PG, Eichelberger M, Gaczyńska M, Nagashima K, et al. (1994) Altered peptidase and viral-specific T cell response in LMP2 mutant mice. Immunity 1(7): 533-541.
9. Hayashi T, Kodama S, Faustman D (2000) LMP2 expression and proteasome activity in NOD mice. Nature Medicine 6(10): 1064-1066.
10. Hayashi T, Horiiuchi 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.
11. Hayashi T, Horiiuchi 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(13): 1824-1831.
12. Vogelstein B, Lane D, Levine AJ (2000) Surfing the p53 network. Nature 408(6810): 307-310.
13. Raycroft L, Wu HY, Lozano G (1990) Transcriptional activation by wild-type but not transforming mutants of the p53 anti-oncogene. Science 249(4972): 1049-1051.
14. Wang LL (2005) Biology of osteogenic sarcoma. Cancer J 11(4): 294–305.
15. 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(6381): 80-83.
16. 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(6423): 857-860.
17. Ito M, Barys L, O’Reilly T, Young S, Gorbacheva 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(3): 416-426.
18. Hayashi T, Horiiuchi A, Sano K, Yaegashi N, Konishi I (2015) Uterine leiomyosarcoma tumorigenesis in Lmp2-deficient Mice: Involvement of Impaired Anti-oncogenic Factor IRF1. Anticancer Research 35(9): 4665-4679.
19. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100(1): 57-70.
20. Deshpande A, Hinds PW (2006) The retinoblastoma protein in osteoblast differentiation and osteosarcoma. Curr Mol Med 6(7): 809-817.
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(6211): 156-158.
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 myoid/round cell liposarcoma: p53 gene alterations and reduced p14(ARF) expression both correlate with poor prognosis. J Pathol 207(4): 410-421.
23. Hernando E, Charytonowicz E, Dudas ME, Menendez S, Matushansky I, et al. (2007) The AKT-mTOR pathway plays a critical role in the development of leiomyosarcomas. Nat Med 13(6): 748-753.
24. Mariani O, Brennetot C, Coindre JM, Gruel N, Ganem C, et al. (2007) JUN. Oncogene amplification and overexpression block adipocytic differentiation in highly aggressive sarcomas. Cancer Cell 11(4): 361–374.