International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 4 Issue 5, August 2020 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Mystery of Uterine Leiomyosarcoma: Possible Reasons for the High Prevalence of Hematogenous Metastases

Takuma Hayashi[1,2,3], Kenji Sano[4], Ikuo Konishi[1,5]

[1]Cancer Medicine, National Hospital Organization Kyoto Medical Center, Kyoto, Japan
[2]School of Health Science, Baika Women's University, Ibaraki, Japan
[3]SIGMA-Aldrich Collaboration Laboratory, SIGMA-Aldrich Israel, Rehovot, Israel
[4]Department of Medical Laboratory, Shinshu University Hospital, Nagano, Japan
[5]Department of Obstetrics and Gynecology, Kyoto University Graduate School of Medicine, Kyoto, Japan

ABSTRACT

Uterine leiomyosarcoma is a refractory tumor that recurs and metastasizes repeatedly. The differential diagnosis between uterine leiomyoma (uterine fibroid) and uterine sarcoma that occurs in many adult women of all races is extremely difficult. In the current clinical management, treatment of uterine sarcoma is limited to surgical procedures. Therefore, it is desired to establish a molecular-targeted therapeutic method that has a life-prolonging effect. We reported that uterine sarcoma frequently occurs spontaneously in mice lacking the proteasome component low molecular protein 2/bl1 (LMP2/bl1). Therefore, we examined the expression status of LMP2/bl1 in biopsy tissues of various uterine mesenchymal tumors selected from pathological files by immunohistochemical staining. We reported that LMP2/bl1 expression was significantly attenuated in specifically uterine sarcoma. Malignant tumor stem cells have stronger antitumor drug resistance and radiation resistance than ordinary malignant tumor cells. Therefore, the presence of malignant tumor stem cells is considered to be a major cause of recurrence of malignant tumor cells after existing antitumor agents and radiotherapy. Currently, we have isolated stem-like cells from surgically excised human uterine sarcoma tissue, have been studying the biological characteristics of uterine sarcoma stem-like cells. From the results of our research to date, it was revealed that uterine sarcoma stem-like cells have a stronger hematogenous metastatic ability as compared with uterine sarcoma cells. The results of this research will provide useful medical information for the development of new treatments for uterine sarcoma with hematogenous metastatic potential.

KEYWORDS: *Leiomyosarcoma, tumor stem cell, angiogenesis, hematogenous metastasis*

How to cite this paper: Takuma Hayashi | Kenji Sano | Ikuo Konishi "Mystery of Uterine Leiomyosarcoma: Possible Reasons for the High Prevalence of Hematogenous Metastases" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-4 | Issue-5, August 2020, pp.426-429, URL: www.ijtsrd.com/papers/ijtsrd31886.pdf

IJTSRD31886

Introduction and Purpose

Uterine leiomyosarcoma (uterine sarcoma) is a refractory gynecologic tumor that repeats recurrence and metastasis, and there are many unclear points regarding the pathogenesis of uterine sarcoma [1,2]. In the clinical findings, uterine sarcoma is associated with uterine leiomyoma (uterine fibroid), onset of uterine sarcoma alone is rare [3,4]. Surgical treatment is the best treatment for uterine sarcoma and fibroids. From today's social background, uterine preservation is required for treatment. Clinical studies to date have not identified a treatment that would have an immediate survival benefit for sarcoma. Therefore, establishment of an effective treatment for these uterine mesenchymal tumor is urgent. We reported that uterine sarcoma frequently occurs spontaneously in mice lacking the proteasome component low molecular protein 2/bl1 (LMP2/bl1)[note1] [5]. Therefore, we examined the expression status of LMP2/bl1 in biopsy tissues of various uterine mesenchymal tumors selected from pathological files by immunohistochemical staining. We specifically reported that LMP2/bl1 expression was significantly attenuated in uterine sarcoma [5-7]. In other words, it was suggested that LMP2/bl1 expression was decreased as a biological characteristic of uterine sarcoma. Malignant tumor stem cells have stronger antitumor drug resistance and radiation resistance than ordinary malignant tumor cells. Therefore, it is considered that the major cause of the recurrence of malignant tumors after the existing antitumor drug treatment and radiotherapy is the presence of malignant tumor stem cells. We isolated three types of stem-like cells, normal human uterine smooth muscle cells, human uterine fibroids and human uterine sarcoma, from surgically excised tissue, and have been investigating the difference in biological characteristics of each stem-like cell. The purpose of this study is to apply the obtained research results to the development of new therapeutic methods for uterine mesenchymal tumors.

Materials and Methods

In clinical medicine, side population (SP) prescriptions that utilize specific stem cell markers (CD133, CD34, CD44) and ABC transporter dye selectivity are widely used as methods for selecting tumor stem cells. So far, we have reported that

hematogenous metastasis was observed in LMP2/bl1-deficient mice in which uterine sarcoma spontaneously developed (research cooperation by Professor Susumu Tonegawa, MIT) [5-8]. Our research results to date suggest that the expression of LMP2/bl1 is decreased as a biological characteristic of uterine sarcoma [5-7]. By the recent method previously reported, we isolated only uterine sarcoma cells (SK-LMS/LMP2 (-)) that do not express LMP2/bl1 from human uterine sarcoma cells SK-LMS established from human uterine sarcoma tissue [9-11]. From the isolated SK-LMS/LMP2 (-), uterine sarcoma stem-like cells (SP/LMP2 (-)) and other cells (No-SP/LMP 2 (-)) are separated by the side population (SP) method [note2]. We transplant SP/LMP2 (-) cells and No-SP/LMP2 (-) cells into the second mammary gland of immunodeficient mice (BALB/c nu/nu) to investigate the biological properties of SP/LMP2 (-).

Result

As a result of measuring the amount of various growth factors derived from SP/LMP2(-) and No-SP/LMP2(-), which respond to vascular endothelial cells or lymphatic endothelial cells, it was revealed that the vascular endothelial growth factor-A (VEGF-A) was significantly secreted from SP/LMP2(-) (Figure 1). From the results of transplantation experiments using nude mice, the tumorigenicity was recognized at almost the same value for both SP/LMP2(-) and No-SP/LMP2(-). Notably, micro metastases were significantly more abundant in the alveolar tissue of SP/LMP2(-)-transplanted mice compared to No-SP/LMP2(-)-transplanted mice (Figure 2). From these experimental results, it is considered that SP/LMP2 (-) as stem-like cells of uterine sarcoma deeply influences hematogenous metastasis of uterine sarcoma.

Discussion

Clinically, patients with uterine sarcoma have a higher prevalence of hematogenous metastases. On the other hand, the prevalence of lymphatic metastases in patients with the same tumor is extremely low. The medical reason for this clinical finding has not yet been clarified. It was revealed that uterine sarcoma stem-like cells have an ability of inducing neovascularization and high hematogenous metastatic ability as compared with uterine sarcoma cells. Since the characteristics of these uterine sarcoma stem-like cells may be applied to the development of new treatments and diagnostic methods for uterine sarcoma, we are conducting a more detailed study of the biological properties of uterine sarcoma stem-like cells. The grant to this study was used to manage basic and clinical studies aimed at establishing new diagnostic and therapeutic methods for uterine sarcoma, a refractory uterine mesenchymal tumor.

Joint research project with other facilities (National Hospital Organization Kyoto Medical Center, Shinshu University, Kyoto University, Tokyo University, Tohoku University, Osaka City University, National Cancer Center, Keio University, Hyogo Cancer Center, SIGMA-Aldrich)

Footnote

Low molecular protein 2/bl1 (LMP2/bl1) [note1]: Low molecular protein 2/bl1 (LMP2/bl1) (also called as proteasome subunit beta type-9(PSMB9)) as known as 20S proteasome subunit beta-1i is a protein that in humans is encoded by the *LMP2/b1i*(*PSMB9)* gene [12-15]. This protein is one of the 17 essential subunits (alpha subunits 1-7, constitutive beta subunits 1-7, and inducible subunits including beta1i, beta2i, beta5i) that contributes to the complete assembly of 20S proteasome complex. In particular, proteasome subunit beta type-5, along with other beta subunits, assemble into two heptameric rings and subsequently a proteolytic chamber for substrate degradation. This protein contains "Trypsin-like" activity and is capable of cleaving after basic residues of peptide [15]. The eukaryotic proteasome recognized degradable proteins, including damaged proteins for protein quality control purpose or key regulatory protein components for dynamic biological processes.

The side population method [note2]: Many stem cells have a high excretion capacity for the DNA fluorescent dye Hoechst33342. These stem cells are called side population (SP) cells because their fluorescence intensity is located lower than the G0/G1 phase population. Hoechst33342 is excreted from cells due to ABCG2 pump activity; however, a treatment with reserpine, an ABCG2 pump inhibitor, inhibits Hochst33342 excretion. Since the SP cell population is not recognized by the reserpine treatment, the reserpine treatment is used to confirm SP cells. The SP cell group contains cancer stem cells.

1. Stem cell culture medium (4 mL) was placed in a 352063 tube to receive the sorted cells, and several tubes were prepared.
2. BD FACSAria was launched, and the delay time of all lasers was optimized at a medium flow rate. Two-dimensional dot plot screens, such as FSC/SSC, Hoechst Red/Hoechst Blue, and EGFP/PI, were drawn. Furthermore, each parameter of the instrument setting was input. In our experiments, FACS was set up to isolate cancer stem-like cells as follows.
3. The flow rate of cells was low at 1.0-1.2, Violet laser-like parameters were optimized. The value of the parameter differed depending on the characteristics of each machine of BD FACSAria and contamination of the flow cell. The side population (SP) cell fraction was then gated and SP cells were sorted.
4. The major population (MP) fraction located above the SP cell fraction was gated and MP cells were sorted.

Disclosure of potential conflicts of interest

The authors declare no potential conflicts of interest.

Acknowledgments

This study was supported in part by grants from the Japan Ministry of Education, Culture, Science and Technology (No. 24592510, No. 15K1079, and No. 19K09840), 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, and The Takeda Foundation for Medical Science.

Author Contributions

T. H., and K. S. performed most of the experiments and coordinated the project; T. H., and K. S. performed cell sorting and the flow cytometric analysis by BD FACSAria™ III; T. H. performed xenograft studies for the micro metastasis model; T. H., and K. S. were involved in molecular pathology assessments and the detection of tumor stem-like cells; T. H. conceived the study and wrote the manuscript. T. H. and I. K. gave information on clinical medicine and oversaw the entire study.

References

[1] Wu TI, Chang TC, Hsueh S, Hsu KH, Chou HH, Huang HJ, Lai CH: Prognostic factors and impact of adjuvant chemotherapy for uterine leiomyosarcoma. Gynecol Oncol 100, 166-172, 2006.

[2] Leitao MM, Soslow RA, Nonaka D, Olshen AB, Aghajanian C, Sabbatini P, Dupont J, Hensley M, Sonoda Y, Barakat RR, Anderson S: Tissue microarray immunohis-tochemical expression of estrogen, progesterone, and androgen receptors in uterine leiomyomata and leiomyosarcoma. Cancer 101, 1455-1462, 2004.

[3] Kurma RJ: Pathology of the Female Genital Tract, 4 th ed. Springer-Verlag 4, 499, 2001.

[4] Diagnostic Criteria for LMS, Adapted from 2003 WHO Guidelines. World Health Organization Classification of Tumours: Pathology and Genetics, Pathology and Genetics of Tumours of the Breast and Female Genital Organs. IARC Press（France), 2003.

[5] Hayashi T, Faustman D: Development of spontaneous uterine tumors in low molecular mass polypeptide-2 knockout mice. Cancer Research 62, 24-27, 2002.

[6] Hayashi T, Kobayashi Y, Kohsaka S, Sano K: The mutation in ATP-binding region of JAK 1 , identified in human uterine leiomyosarcomas, results in defective interferon-γinducibility of TAP 1 and LMP 2 . Oncogene 25, 4016-4026, 2006.

[7] Hayashi T, Horiuchi A, Sano K, Hiraoka N, Kasai M, Ichimura T, Nagase S, Ishiko O, Kanai Y, Yaegashi N, Aburatani H, Shiozawa T, Konishi I: Potential role of LMP 2 as tumor-suppressor defines new targets for uterine leiomyosarcoma therapy. Scientific Reports 1, 180, 2011.

[8] Hayashi T, Horiuchi A, Sano K, Hiraoka N, Kasai M, Ichimura T, Nagase S, Ishiko O, Kanai Y, Yaegashi N, Aburatani H, Shiozawa T, Tonegawa S, Konishi I: Potential role of LMP 2 as an anti-oncogenic factor in human uterine leiomyosarcoma: morphological significance of calponin h 1. FEBS Letters 586, 1824-1831, 2012.

[9] Hayashi T, Ichimura T, Yaegashi N, Shiozawa T, Konishi I: Expression of CAVEOLIN 1 in uterine mesenchymal tumors: no relationship between malignancy and CAVEOLIN 1 expression. Biochem Biophys Res Commun 463, 982-987, 2015.

[10] Hayashi T, Horiuchi A, Sano K, Hiraoka N, Ichimura T, Sudo T, Ishiko O, Yaegashi N, Aburatani H, Konishi I: Potential diagnosticbiomarkers: LMP2/β1i and Cyclin B 1 differential expression in human uterine mesenchymal tumors. TUMORI 100, 509-516, 2014.

[11] Hayashi T, Sano K, Ichimura T, Gur G, Yaish P, Zharhary D, Kanai Y, Tonegawa S, Yaegashi N, Konishi I. Candidate molecules as diagnostic biomarker for human uterine mesenchymal tumors. Annals of cytology and pathology. 5(1), 54-57, 2020.

[12] Kelly A, Powis SH, Glynne R, Radley E, Beck S, Trowsdale J. Second proteasome-related gene in the human MHC class II region. Nature. 353(6345), 667-668, 1991. doi:10.1038/353667a0.

[13] Bodmer JG, Marsh SG, Albert ED, Bodmer WF, Dupont B, Erlich HA, Mach B, Mayr WR, Parham P, Sasazuki T. Nomenclature for factors of the HLA system, 1991. WHO Nomenclature Committee for factors of the HLA system. Tissue Antigens. 39(4), 161-73, 1991. doi:10.1111/j.1399-0039.1992.tb01932.x.

[14] Entrez Gene: PSMB9 proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2).

[15] Coux O, Tanaka K, Goldberg AL. Structure and functions of the 20S and 26S proteasomes. Annual Review of Biochemistry. 65, 801-47, 1996. doi:10.1146/annurev.bi.65.070196.00410

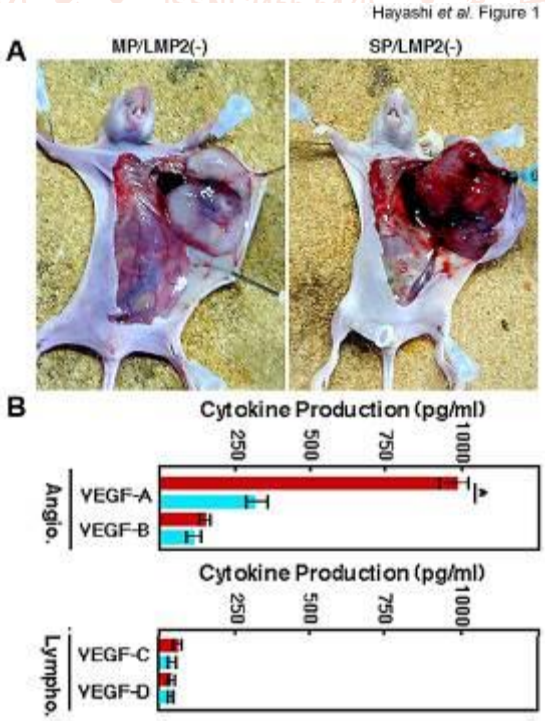

Figure 1 Xenografting: Intracutaneous injection with 1×10⁶ cells of SP/LMP2(-) or No-SP/LMP2(-) at Second mammary fatpad left site of 10weeks old-BALB/c nude mice under the standard maintenance condition. Date of Xenograft of SP or No-SP cells: February 18, 2013. Mice were sacrificed for pathological examinations at April 18, 2012. Date of the pathological studies: June 16, 17, 2015. ELISA with tumor extracts collected from BAL B/c nude mice were perfonmed using OptEIA TM Set, human VEGF-A, VEGF-B, VEGF-C, and VEGF-D, (BD PharMingen, CA, USA). Date of operation: ELISA, June 26-28, 2019.

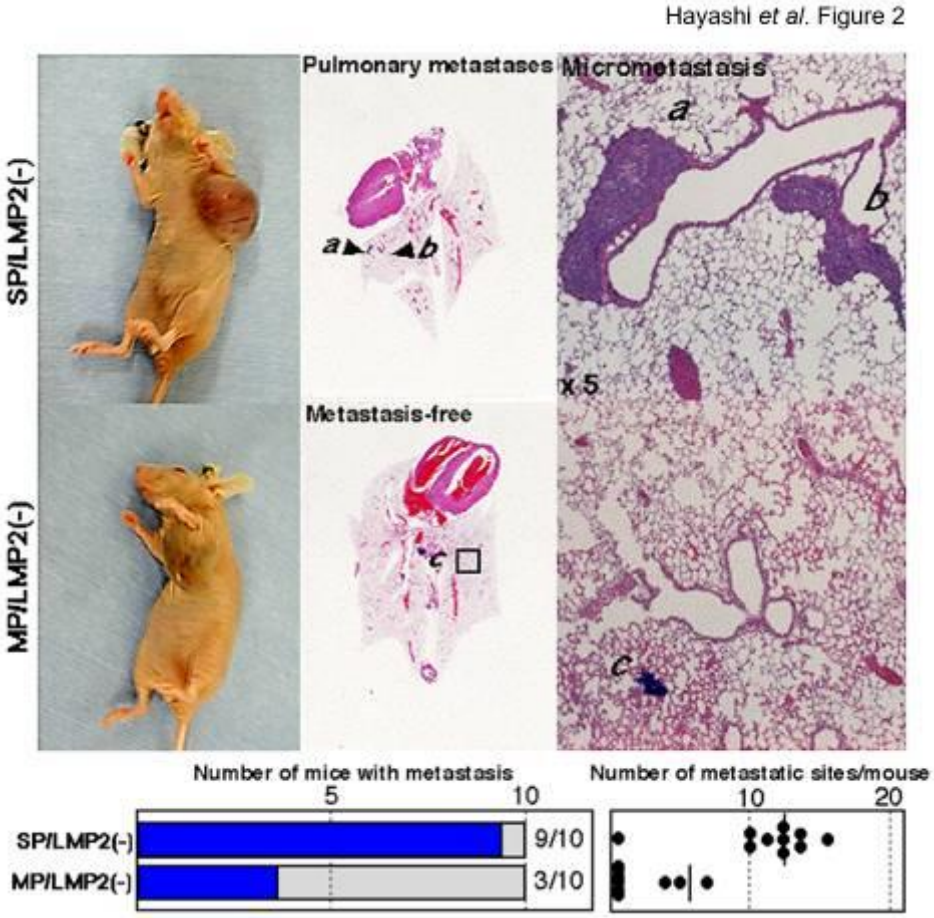

Figure 2 Xenografting: Intracutaneous injection with 1×10^7 cells of SP/LMP2(-) or No-SP/LMP2(-) at Second mammary fat pad left site of 10weeks old-BALB/c nude mice under the standard maintenance condition. Date of Xenograft: February 18, 2015. Mice were sacrificed for pathological examinations at April 18, 2015. Date of the pathological studies: June 16, 17, 2019.