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

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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 or 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

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/LMP2 (2)) are separated by the side population (SP) method note1. We transplant SP/LMP2 (-) and No-SP/LMP2 (-) cells into the secondary 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 hematogenus 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.

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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 Hoechst33342 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 FACS Aria 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 EGGF/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 FACS Aria and contamination of the flow cell. The side population (SP) cell fraction was then gated and SP cells were sorted. 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.

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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 FACS Aria™ 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.
Figure 1 Xenografting: Intracutaneous injection with 1×10^6 cells of SP/LMP2(-) or No-SP/LMP2(-) at Second mammary fatpad left site of 10 weeks old-BALB/c nude mice under the standard maintenance condition. Date of xenografting: 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 performed 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 $1 \times 10^7$ cells of SP/LMP2(-) or No-SP/LMP2(-) at Second mammary fat pad left site of 10-weeks 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.