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Development of Uterine Leiomyosarcoma During Follow-up After Caesarean Section in a Woman With Uterine Leiomyoma

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Abstract. *Background/Aim: During pregnancy, uterine leiomyomas may cause problems and treatment typically entails uterine conservation. However, for cases of leiomyomas larger than a particular size with some clinical symptoms, enucleation should be performed. In clinical practice, the importance of postpartum follow-up of pregnancies with uterine fibroids must be established. Patients and Methods: A 47-year-old female visited an obstetrics and gynecology clinic with a primary complaint of irregular bleeding. We suspected an 8.4×6.6 cm myoma uteri and recommended immediate surgery. During the next visit, a pregnancy test was positive and the patient requested a follow-up for her myoma uteri diagnosis. Because of a breech presentation, we performed an elective cesarean section (CS) at 38 weeks and 1 day. The patient's myoma uteri was stable throughout the pregnancy, and after delivery, we continued to follow her up as an outpatient. Results: Two years after the CS, the myoma uterus was 6 cm in size, and 6 months later, it had increased to 10 cm. Magnetic resonance imaging (MRI) supported a diagnosis of uterine leiomyosarcoma and she underwent surgery. Ultimately, she was pathologically diagnosed with uterine leiomyoma, uterine leiomyoma with bizarre nuclei, and uterine leiomyosarcoma following examination of the excised tissue by using molecular pathological examination with anti-cyclin E antibody and anti-Ki-67 antibody. Conclusion: Notably, this case demonstrated the usefulness of cyclin E and Ki-67 as biomarkers for the malignancy of uterine mesenchymal tumors. Presently, she is being monitored for tumor recurrence and metastases on a quarterly basis. In order to detect the rapid increase in uterine mesenchymal tumor, regular follow-up after birth is important.*

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Uterine smooth muscle tumors are broadly classified as either benign uterine smooth muscle tumors with a clinically benign course or uterine leiomyosarcomas (LMSs) with a malignant course. Most benign uterine smooth muscle tumors are uterine leiomyomas (UL), whereas some uterine smooth muscle tumors have a special pathological name. These specially named uterine smooth muscle tumors are rarely seen in clinical practice, so their biological characteristics remain unknown (1). Histopathological studies provide important information to the medical staff for the clinical treatment of uterine mesenchymal malignancies; however, cellular leiomyoma, epithelioid smooth muscle tumor, symplastic smooth muscle tumor, LMS with bizarre nuclei, and uterine smooth muscle tumors of unknown malignant potential can be difficult to distinguish from uterine LMS (2, 3). Magnetic resonance imaging (MRI) images are necessary to perform prior to clinical treatment including surgical treatment for uterine mesenchymal malignancies. Although MRI images include methods such as diffusion-weighted imaging and dynamic contrast-enhanced MRI, the ability to differentiate between uterine myoma and sarcoma or other uterine mesenchymal tumors is challenging (4, 5). Therefore, it is difficult to establish malignant diagnostic criteria for uterine smooth muscle tumors. Hayashi *et al*. investigated the usefulness of caveolin 1, cyclin B, cyclin E, LMP2/β1i, and Ki-67 as auxiliary diagnostic biomarkers for uterine mesenchymal tumors based on the results of the molecular pathological analysis of

uterine LMS spontaneously occurring in LMP2-deficient mice (6, 7).

ULs are benign gynecological tumors with increased incidence after the third decade of life. The effects of ULs on pregnancy and childbirth are particularly problematic in patients of advanced maternal age (8). The frequency of pregnancies with ULs ranges from 2.6% to 3.9% (9). Because ULs may increase and cause pain during pregnancy, inpatient treatment may be required. There may also be an increased incidence of perinatal complications such as preterm birth, fetal stunting, fetal position abnormalities, and placental abruption. Also, during childbirth, ULs impede labor progress; hence, there is a high probability that a cesarean section (CS) will be necessary. Herein we report a case of uterine mesenchymal tumors with a mixture of leiomyoma, leiomyoma with bizarre nuclei, and uterine LMS diagnosed in a pregnant woman. The tumor was considered to be a UL was monitored at follow-up 2 years and 6 months after delivery by CS. Additionally, we report the usefulness of cyclin E and Ki-67 as biomarkers of malignancy of uterine mesenchymal tumors.

Patients and Methods

Immunohistochemistry (IHC). IHC staining for cyclin E1 and Ki-67 was performed on serial human uterine mesenchymal tumor sections. The antibody for cyclin E1 (CCNE1/2460) was purchased from Abcam (Cambridge Biomedical Campus, Cambridge, UK), and Ki-67 (MIB-1) was purchased from Immunotech (Marseille, France). IHC was performed using the avidin–biotin complex method, as described previously (10). Briefly, one representative 5 mm tissue section was cut from a paraffin-embedded sample of a radical hysterectomy specimen from each patient with a uterine mesenchymal tumor.

Next, the sections were incubated with a biotinylated secondary antibody (Dako, Santa Clara, CA, USA) and then incubated with a streptavidin complex (Dako). The completed reaction was developed by 3, 39-diaminobenzidine, and the slide was counterstained with hematoxylin. Normal myometrium portions in the specimens were used as positive controls. Negative controls comprised tissue sections incubated with normal rabbit IgG instead of the primary antibody. These experiments were approved at Shinshu University according to internal guidelines (approval no. M192). Expression of cyclin E and Ki-67 by DAB staining is shown as a brown color. Normal rabbit or mouse antiserum was used as a negative control for the primary antibody. The entire DAB-stained tissue was scanned with a digital microscope BZ-X800 (Keyence, Osaka, Osaka, Japan). The expression of cyclin E and Ki-67 is indicated by black dots.

DNA transfection and isolation of transfected clones. Transfection of Scr.shRNA or cyclin E.shRNA (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was conducted using FuGENE6 Transfection Reagent (Promega Corporation, Madison, WI, USA) according to the manufacturer's recommendations with 5 μg of plasmid DNA, 5×10^5 SK-LMS-1 cells [American Type Culture Collection (ATCC), Manassas, VA, USA], and 5×105 HeLa cells (from Dr. Y. Adachi, Shinshu University, Nagano, Japan) plated into six-well tissue culture dishes (CORNING Incorporated, Corning, NY, USA) on the previous day. We purchased the established human LMS primary cell line, SK-LMS (ATCC® HTB-88™), from the ATCC. Forty-eight hours after transfection, cells were treated with trypsin and replated into 100 mm dishes with 15 ml of growth medium containing 1 mg of G418 per milliliter (SIGMA-Aldrich Co. LLC., St. Louis, MO, USA). The cells were incubated at 37˚C for an additional 6-8 days with medium changes on day 1 and day 3 or 4. The number of G418-resistant colonies was counted. Transfected G418-resistant colonies from 2 to 10 dishes were pooled after trypsinizing and aliquots of 1×10^5 cells were subjected to one of the following enrichment procedures: (i) Adhesion enrichment: This procedure selects for cells with increased attachment to an Ultra-Low attachment dish (Coster, New York, NY, USA). Cells were plated onto 100 mm tissue culture dishes (Nunc, New York, NY, USA) with 10 ml of G418 medium and incubated for 4 days at 37˚C. Cells that were weakly adherent to the plastic were mechanically removed by sharply blowing off the medium with a pipette onto a cell sheet. The cultures were washed extensively with phosphate-buffered saline without Ca^{2+} or Mg^{2+}. (ii) Soft agar enrichment: This procedure selects cells that are unable to grow in a soft agar medium. Suspended cells were mixed with 4 ml of melted agar medium, which comprised a growth medium containing 0.33% agarose (SIGMA-Aldrich Co. LLC.) and G418 as described above. The cells in the agar medium were poured onto a base layer (4 ml per 60 mm bacterial dish) comprising a growth medium containing 0.8% agar and G418. After 48 h of incubation at 37˚C, the top agar portion was transferred to a 15 ml tube and suspended in 5 ml of a serum-free medium after passing several times through a G21 needle. The cells together with the small agar blocks were then recovered by centrifugation at 200 × *g* for 15 min, rinsed twice with 10 ml of serum-free medium, resuspended, plated with 5 ml of growth medium into 60 mm tissue culture dishes, and incubated overnight at 37˚C.

After each of the above treatments, the cultures were re-fed with G418 medium and incubated for an additional 5-7 days. One or more cycles of adhesion enrichment were applied to further facilitate the detection of small flat colonies among the vast majority of large transformed colonies. The number of cells was measured by the Giemsa staining method.

Xenograft studies. Nude mice (BALB/cSlc-nu/nu, female, 7-8 weeks old, Japan SLC, Shizuoka, Japan) were injected subcutaneously with 1×10^7 cells of the SK-LMS-1 Scr.shRNA clones (five clones) and SK-LMS-1 cyclin E.shRNA clones (five clones) with BD Matrigel Matrix (BD Biosciences, Franklin Lakes, NJ, USA) in 5 mg/ml of culture medium containing 15% FCS plus SmGM-2 SingleQuots (CAMBREX, MD, USA) at a volume of 100 μl. Nude mice (BALB/cSlc-nu/nu, female, 7-8 weeks old, Japan SLC) were also injected subcutaneously with 1×10^7 cells of the HeLa-Scr.shRNA clones (five clones), HeLa-cyclin E.shRNA clones (five clones) with BD Matrigel Matrix (BD Biosciences) in 5 mg/ml of culture medium containing 15% FCS at a volume of 100 μl. Tumor formation was assessed every day. Seven weeks after injection, the tumors were dissected for western blotting experiments. Tumor volumes were calculated as (L × W × W)/2, where W represents the width and L represents the length. Statistical analysis was performed on mean tumor volumes at the end of the study using Dunnett's test. To detect the expression of cyclin E and β-actin, whole-cell lysates,

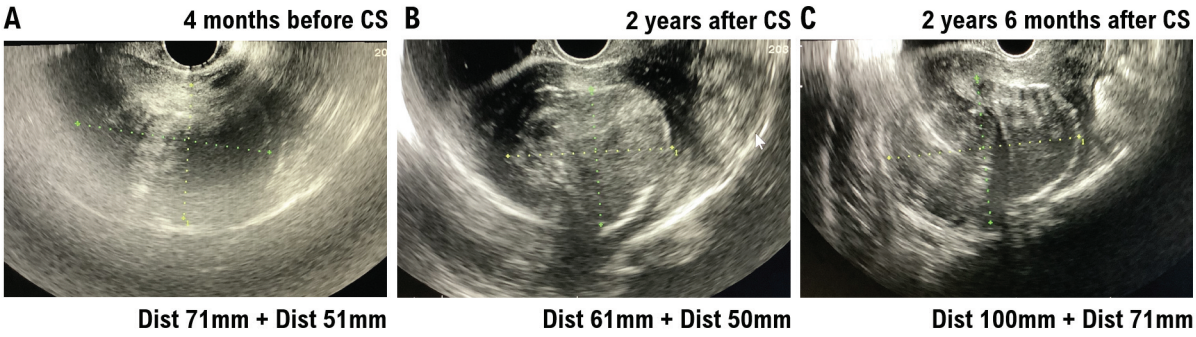

Figure 1. *Diagnosis of myoma uteri in a 47-year-old female. (A) Transabdominal ultrasonography imaging showing a myoma uterus (51×74 mm) at 4 months before cesarean section (CS). (B) Transabdominal ultrasonography imaging confirms no apparent changes in the myoma uteri (61×50 mm) at 2 years after CS. (C) Transvaginal ultrasonography imaging showing a thin endometrium at 2 years and 6 months after CS. Growth of the myoma uteri (100×71 mm) is observed compared with the previous examination.*

nuclear extracts, or cytosolic extracts were resolved by 10% SDS-PAGE and immunoblotting was performed using appropriate antibodies and standard procedures. The experiments with BALB/c nu/nu mice were conducted at the Shinshu University and the National Hospital Organization Kyoto Medical Center following institutional guidelines (approval no. M192).

Ethical approval and consent to participate. This study was reviewed and approved by the Central Ethics Review Board of the National Hospital Organization Headquarters in Japan (Meguro, Tokyo, Japan) and Shinshu University (Matsumoto, Nagano, Japan). The authors attended a 2020 and 2021 educational lecture on medical ethics supervised by the Japanese government. The completion numbers for the authors are AP0000151756, AP0000151757, AP0000151769, and AP000351128. This research was not a clinical study. Therefore, consent to participate was not required. The authors attended a seminar on the ethics of experimental research using small animals and became familiar with the importance and ethics of animal experiments (National Hospital Organization Kyoto Medical Center and Shinshu University School of Medicine).

Results

Case report. A 47-year-old patient visited an obstetrics and gynecology clinic with the primary complaint of genital bleeding. A uterine mass (84×66 mm) was found upon pelvic examination and ultrasonography imaging, and leiomyoma was suspected. The patient was advised to undergo surgical treatment and was referred by the doctor in charge of our Institution. During the outpatient visit, she reported a 6-week history of amenorrhea and her pregnancy test was positive. Ultrasonography imaging revealed an intrauterine fetus (biparietal diameter 37 mm, abdominal circumference 121 mm, femur length 24 mm) with fetal heart movement. Transabdominal ultrasonography revealed a 10-cm-sized myoma uteri near the posterior wall of the median with the gestational sac pushed to the right ventral side. We considered the risks to fetal growth restriction; however, because she was pregnant, no MRI was performed. At 19 weeks of gestation, transabdominal ultrasonographic imaging confirmed that the placenta was attached directly above the myoma uteri. At diagnosis, the myoma uterus was 10 cm, but

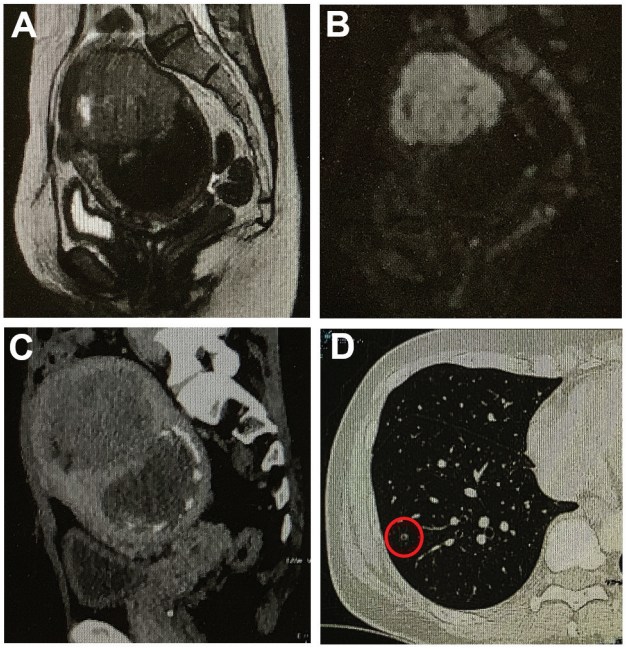

Figure 2. *MRI imaging reveals a new tumor adjacent to the previously diagnosed myoma uteri. (A) MRI T1 and T2 imaging shows a marked low signal at T2W1 and an iso-signal at T1W1 on the caudal side of the tumor. (B) On the cranial side of the tumor, T2W1 shows mild hyperintensity and marked diffusion limitation. (C) Enhanced CT imaging reveals two tumors touching the top and bottom and occupying the left wall of the uterus. The caudal tumor appears associated with marginal calcification. (D) Enhanced CT imaging reveals a nodule suspected to be inflammatory in the lower lobe of the right lung, located inside the red circle.*

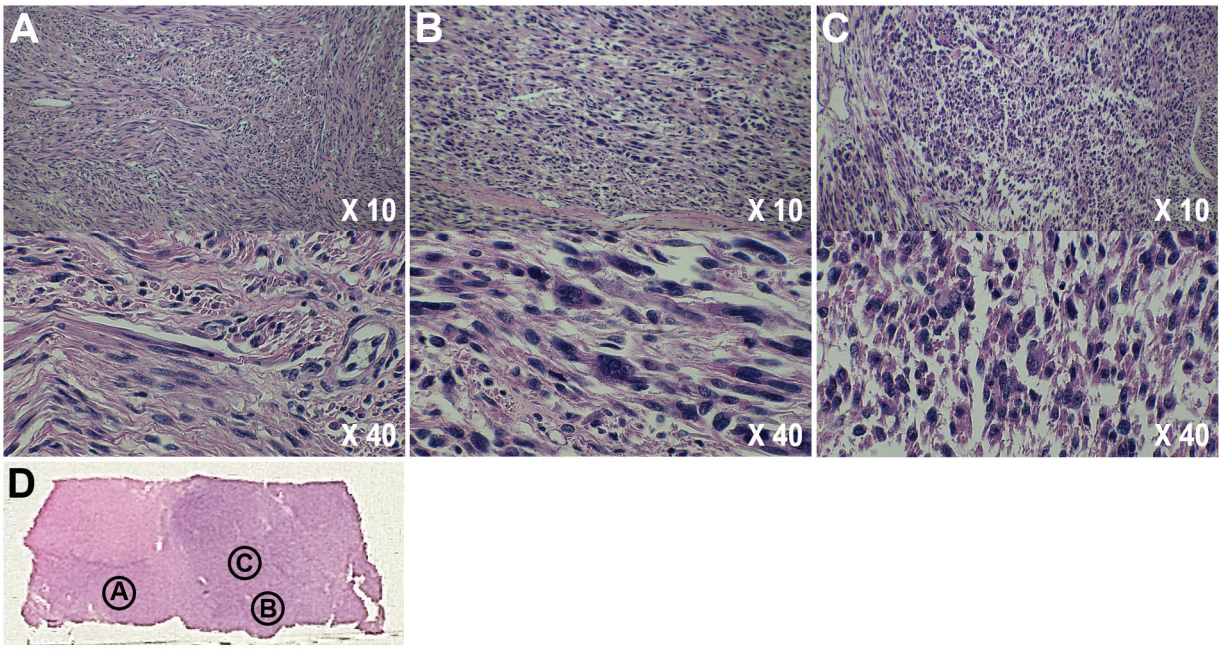

Figure 3. *Histopathological studies of a new tumor adjacent to the previously diagnosed tumor. Pathological images show uterine smooth muscle tumors with severe cell atypia and lobular proliferative morphology. The margin of the tumor was pushing and the boundary was clear. Coagulative necrosis was evident and pathological images showed a mixture of (A) uterine leiomyoma, (B) uterine leiomyoma cells with bizarre-like nuclear deformities, and (C) uterine leiomyosarcoma in uterine tumor #1 section.*

no increase was observed. At 19 weeks of gestation, to prevent imminent miscarriage and premature birth, treatment with an oral tocolytic agent was started.

Transabdominal ultrasonography imaging at 19 weeks of gestation revealed good fetal movement and amniotic fluid level. Transabdominal ultrasound confirmed that the myoma uteri had not increased in size (51×74 mm) (Figure 1A). We confirmed that the placenta was attached above the myoma uteri and that it was not placenta previa. Umbilical cord ptosis was not observed and a fetal morphology screen revealed no major malformations.

At 38 weeks and 1 day of gestation, an elective CS was performed as scheduled. The newborn weighed 2,406 g [Apgar score 8/9, Umbilical cord arterial blood gas test value (UmA)-pH 7.385, base excess (BE) −1]. The newborn was admitted to pediatrics as a low-birth-weight infant. The patient was informed that she would be followed for ULs if there were no overt symptoms. Eighteen months after CS, transvaginal sonographic imaging showed a stable myoma uteri (59×52 mm) present on the posterior wall of the uterus. Two years after CS, transvaginal sonographic imaging showed no significant increase in the size of the myoma uteri (61×50 mm) present on the posterior wall of the uterus.

Two years and 6 months after CS, the patient presented with symptoms of tumor growth. At this time, her menstrual cycle was irregular. Transvaginal ultrasonography imaging revealed a thin endometrium and the myoma uteri (100×71 mm) had increased in size compared to the previous examination (Figure 1B and C). MRI revealed a new tumor adjacent to the previously diagnosed tumor with myoma uteri. MRI T1 and T2 imaging showed a marked low signal at T2W1 and an iso-signal at T1W1 on the caudal side of the tumor (Figure 2A). On the cranial side of the tumor, T2W1 showed a mild hyperintensity and marked diffusion limitation (Figure 2A and B). An enhanced computed tomography (CT) indicated that the cranial side of the tumor appeared as a lobulated region with a non-uniform enhancing effect, including a dorsal defective area. MRI T1 and T2 imaging showed an area of suspected necrosis, suggestive of uterine LMS, a malignant tumor associated with UL (Figure 2A and B). There were no findings indicating clear cancer infiltration into the surrounding organs. Lymph node swelling was not observed in the imaging range. CT imaging revealed two tumors touching the top and bottom on the left wall of the uterus (Figure 2C). The caudal tumor was associated with marginal calcification (Figure 2C). CT imaging revealed a nodule suspected to be inflammatory in the right lower lobe (Figure 2D).

Two years, 7 months after the CS, a simple hysterectomy and bilateral salpingo-oophorectomy were performed. Pathological images showed a uterine smooth muscle tumor with severe cell

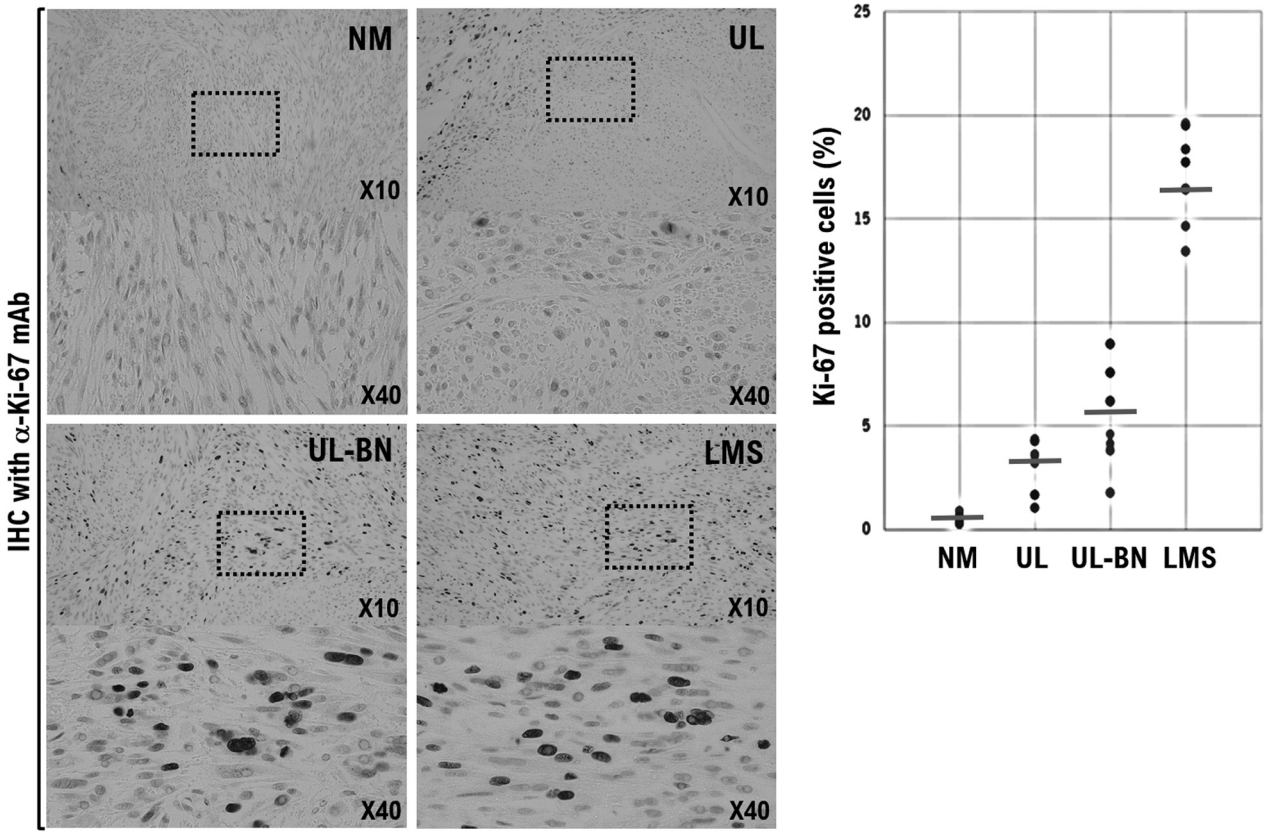

Figure 4. *Percentage of Ki-67 positive cells in morphologically different mesenchymal tumors. Photographs of immunohistochemical staining with anti-human Ki-67 antibody in normal myometrium and different uterine mesenchymal tumor types, normal myometrium (NM) section, uterine leiomyomas (UL) section, uterine leiomyoma with bizarre nuclei (UL-BN) section, and uterine leiomyosarcoma (LMS) section. The upper photographs were taken at 10× magnification and the lower photographs were taken at 40× magnification. Percentage of Ki-67 positive cells in different NM tissues (five different sites), UL (five different sites), leiomyoma with bizarre nuclei (UL-BN) (five different sites), and uterine leiomyosarcoma (LMS) (five different sites).*

atypia and lobular proliferative morphology (Figure 3). The margin of the tumor was pushing, and the boundary was clear. The bizarre cells may have been mononucleated or multinucleated and may have exhibited eosinophilic or globular cytoplasm, smudged chromatin, and nuclear pseudoinclusions (11, 12). Concerning the pathological features of uterine LMS, three main subtypes are recognized. Spindle cell (conventional) tumors are typically cellular (rarely hypocellular) and composed of fusocellular cells with eosinophilic cytoplasm (sometimes scant) and are arranged in long, interlacing, often compact but relatively disorganized fascicles (11, 12). Nuclear pleomorphism is often striking, but a subset of tumors exhibits uniform cytological features and may appear deceptively benign at low magnification. There are varying proportions of spindle and pleomorphic cells. Multinucleated tumor cells and asteoclast-like cells may be present (11, 12). Coagulative necrosis was found in the tumor. Pathological images appeared to show a mixture of (A) UL, (B) UL cells with bizarre-like nuclear deformities, and (C) uterine LMS in the uterine tumor section (Figure 3). We informed the patient that she would be followed every 3 months after surgery.

A recent report demonstrated that cyclin E-deficient cells actively proliferate in conditions of continuous cell cycling but are unable to re-enter the cell cycle from G0 and are resistant to oncogenic transformation (13-15). Cyclin E, a regulator of the cell cycle, and Ki-67, which is broadly used as a diagnostic marker in proliferating cancer cells, affect the behavior of breast cancer cells and uterine LMS (15-17). We investigated whether the levels of cyclin E and Ki-67 in the tumors correlated with mesenchymal malignancy. The average proportion of Ki-67-positive cells in each morphologically classified tissue was 0.47% for normal myometrium (NM), 3.09% for UL cells, 6.12% for UL with bizarre nuclei (UL-BN), and 17.02% for uterine LMS (Figure 4). From this result, the malignancy of uterine mesenchymal tumor depends on a high level of Ki-67 positive cells.

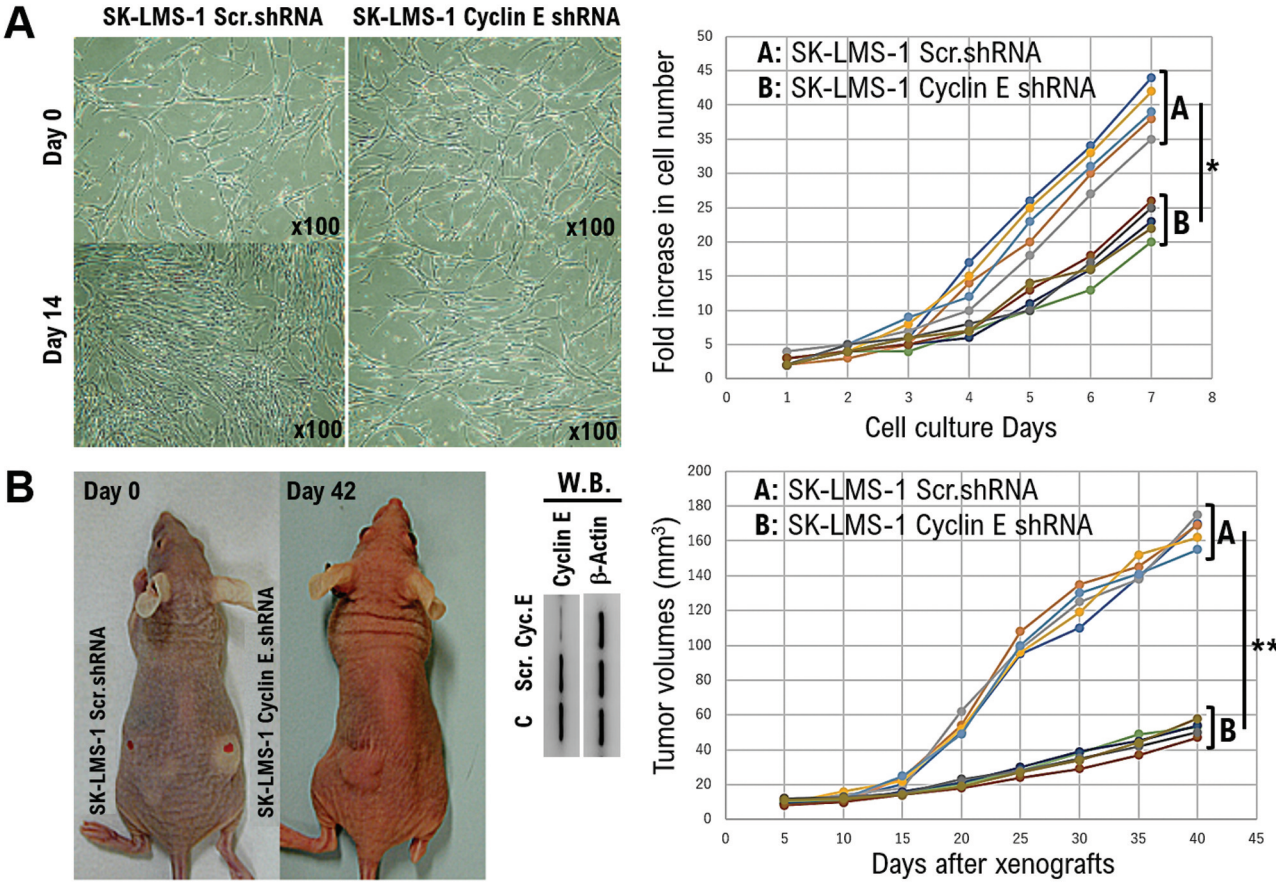

Figure 5. *Biological activity of cyclin E in uterine leiomyosarcoma (LMS). (A) Phase-contrast micrographs of the parental transformed SK-LMS-1 Scr.shRNA clone and the SK-LMS-1 cyclin E.shRNA clone (100× magnification) (upper left panel). Fold increase in cell number of the parental transformed SK-LMS-1 Scr.shRNA clones (different five clones) and the SK-LMS-1 cyclin E.shRNA clones (different five clones) (upper right panel). The number of cells was measured by the Giemsa staining method. Statistical analysis was performed on mean tumor volumes at the end of the study using Dunnett's test; *p<0.01 (B) Changes in the human uterine LMS cell line, SK-LMS-1 transfectant, the parental transformed SK-LMS-1 Scr.shRNA clone (different five clones), and the SK-LMS-1 cyclin E.shRNA clone (different five clones) xenografts in mice (n= 20). Representative photographs of the xenografts in mice (lower left panel). Tumor growth of the SK-LMS-1 cyclin E.shRNA clones (different five clones) was markedly reduced in comparison with that of the parental transformed SK-LMS-1 Scr.shRNA clones (different five clones). Tumor growth kinetics after subcutaneous injection of the parental transformed SK-LMS-1 Scr.shRNA clones (different five clones) and the SK-LMS-1 cyclin E.shRNA clones (different five clones) (lower right panel). Western blot experiments reveal cyclin E expression in tumors (lower center panel). The experiments were performed four times with similar results. Statistical analysis was performed on mean tumor volumes at the end of the study using Dunnett's test; **p<0.001.*

We transfected Scr.shRNA or cyclin E.shRNA into SK-LMS-1, a human LMS cell line established from human LMS, to establish SK-LMS-1 Scr. shRNA clones (five clones) and SK-LMS-1 cyclin E. shRNA clones (five clones). The proliferation rate of the SK-LMS-1 Scr.shRNA clones (five clones) and SK-LMS-1 cyclin E.shRNA clones (five clones) was compared. The cell proliferation ability of SK-LMS-1 cyclin E.shRNA clones, in which expression of cyclin E was suppressed by cyclin E.shRNA, was reduced compared with the cell proliferation of the SK-LMS-1 Scr.shRNA clones (Figure 5A).

Cyclin E1 was highly expressed in LMS but was very poorly expressed or negatively expressed in NM and UL cells. To examine the biological connection between cyclin E and tumorigenesis, we analyzed tumorigenesis of LMS and the expression pattern of cyclin E in the SK-LMS-1 Scr.shRNA clones (five clones) and the SK-LMS-1 cyclin E.shRNA clones (five clones) (Figure 5). Tumor growth was clearly observed in control mice inoculated with the SK-LMS-1 Scr.shRNA clones (five clones), whereas a reduction in tumor growth was observed in mice inoculated with the SK-LMS-1 cyclin E.shRNA clones (five clones) (Figure 5B). Since suppression of cyclin E

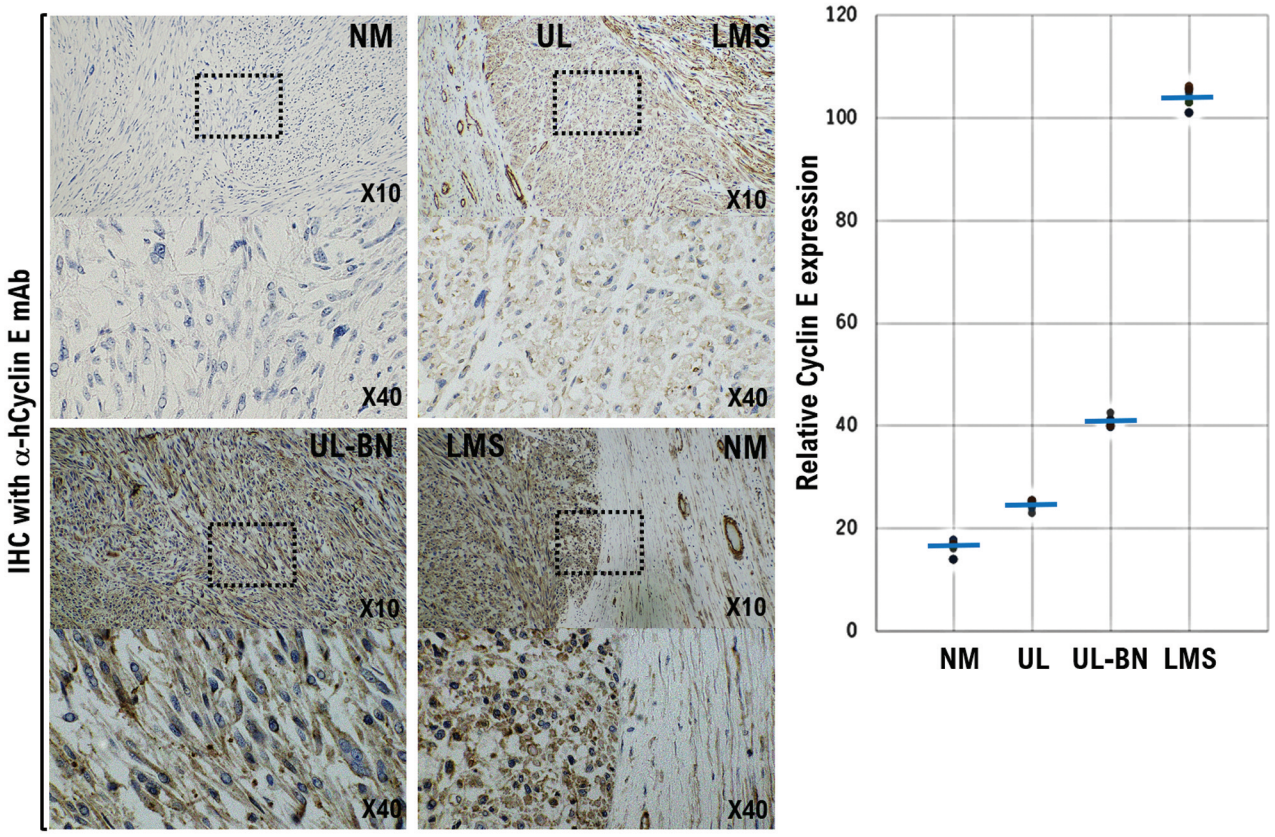

Figure 6. *Percentage of cyclin E positive cells in morphologically different mesenchymal tumors. Photographs of immunohistochemical staining with anti-human cyclin E antibody in a different normal myometrium (NM) section, uterine leiomyomas (UL) section, uterine leiomyoma with bizarre nuclei (UL-BN) section, and uterine leiomyosarcoma (LMS) section. The upper photographs were taken at 10× magnification and the lower photographs were taken at 40× magnification. Percentage of Ki-67 positive cells in different NM tissue (five different sites), UL (five different sites), leiomyoma with bizarre nuclei (UL-BN) (five different sites), and uterine leiomyosarcoma (LMS) (five different sites).*

expression by cyclin E.shRNA blocked tumorigenesis, it became necessary to rule out a toxic effect of reducing cyclin E expression in the control cancer cell line. Additional experiments demonstrated no toxic effects of either in reducing cyclin E expression in HeLa cervical cancer cells (data not shown). The results of western blot analysis supported the suppression of cyclin E expression by cyclin E.shRNA (Figure 5B).

The average relative expression of cyclin E in each morphologically classified tissue was 16.83 for normal UL cells (NM), 24.57 for UL cells (UL), 40.81 for UL-BN, and 103.97 for uterine LMS (Figure 6). From these results, the content of Ki-67 positive cells and the relative expression of cyclin E may represent useful diagnostic biomarkers for malignancy of uterine mesenchymal tumors (Figure 7).

Discussion

The development of medical technology has been remarkable; however, since uterine mesenchymal tumors have various morphologies, the full scope of their biological characteristics remains unclear (18, 19). Furthermore, there is no medical or biological evidence that a benign tumor or UL transforms into a malignant tumor (*i.e.*, uterine LMS) (10). However, several uterine LMSs appear concurrently with ULs. Mitotically active leiomyomas are usually found in women of reproductive age and associate with secretory endometrium, pregnancy, and drugs (progestogens and tamoxifen) (12). Although cyclin E has been reported as a biomarker in some malignancies (20-24), no targeted therapeutic data have been curated. Recent research findings showed that the expression of miR-1 was strongly suppressed in uterine LMS tumor tissue compared to adjacent healthy tissue (25). Tumor suppressive mechanisms of miR-1, seem to be inhibited in uterine LMS SK-UT-1 cells, maybe as part of the malignant transformation process (25). miR-1 suppresses the growth of esophageal squamous cell carcinoma *in vivo* and *in vitro* through the downregulation of MET, cyclin D1 and CDK4 expression (26),

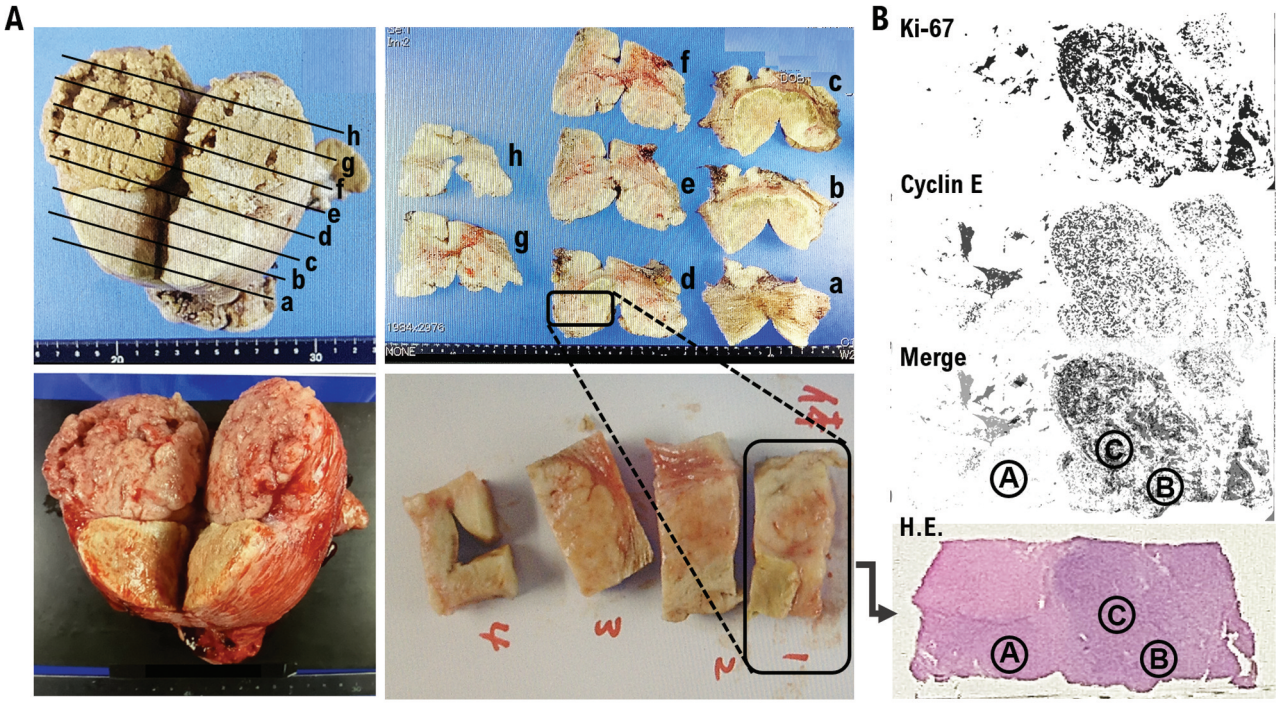

Figure 7. *Gross and histopathological morphology of the uterine mesenchymal tumors. (A) Cut surface of the resected uterus: Whitish masses in the uterine corpus are typical leiomyomas on gross and microscopic examination (lower masses). At the uterine body, gray-white masses are observed (upper masses) which included necrosis and bleeding tissues. (B) Slide scanning view of the whole tissue sections of hematoxylin and eosin (H&E) staining (lower panel), immunohistochemical staining with anti-Ki-67 antibody, immunohistochemical staining with anti-cyclin E antibody, image of overlaying image stained with anti-cyclin E antibody, and image stained with anti-Ki-67 antibody (Merge). Only anti-Ki-67 antibody-positive cells were converted to black dots by the software of the digital microscope (BZ-X800, Keyence, Osaka, Japan). Only anti-cyclin E antibody-positive cells were converted into black dots by the software of the digital microscope. The tissue in which the black dots of the anti-Ki-67 antibody-positive cells and the black dots of the anti-cyclin E antibody-positive cells overlapped was considered to be a highly malignant mesenchymal tumor tissue. Tissue region of uterine leiomyoma is indicated (A), tissue region of a uterine leiomyoma with bizarre nuclei is indicated (B), and tissue region of uterine leiomyosarcoma is indicated (C) in merged panel.*

furthermore cyclin E1 is reportedly down-regulated by mRNAs in lung cancer (27). In the future, the expression status of miR-1 in other types of mesenchymal tumor or benign mesenchymal tumors, *i.e.*, uterine leiomyoma, must be investigated. Regardless of the microRNA's cellular functionality, miR-1 combined with differential expression of Ki-67, cyclin E and other candidates may represent a promising biomarker of diagnosis in uterine LMS therapy.

In this case study, we showed that the expression status of the combination of cyclin E and Ki-67 may be an indicator of the malignancy of uterine mesenchymal tumors. Immunohistochemical biomarkers such as cyclin E and Ki-67 may be useful as an adjunct diagnosis of malignancy in uterine mesenchymal tumors that are extremely difficult to diagnose surgically.

In cases involving pregnancy with uterine fibroids, physicians should consider actively performing uterine enucleation. Previous cohort studies have shown (28, 29) that uterine fibroid resection can be safely performed during CS without increasing maternal morbidity during the perinatal period. Regular follow-up after delivery enabled us to detect a rapid increase in uterine mesenchymal tumors. We diagnosed the patient with a uterine LMS, and treatment was started early.

Conclusion

There is no medical evidence that ULs transform into uterine LMSs. This case elucidates the natural history of uterine LMSs. Since regular follow-ups for uterine myomas were performed after childbirth and during pregnancy, we reported a case of early detection of uterine LMS.

Conflicts of Interest

The Authors declare no potential conflicts of interest.

Authors' Contributions

KW and MK performed most of the clinical medicine and coordinated the project. KM conducted the diagnostic pathological studies. TH created the study and wrote the manuscript. IK and KA carefully reviewed this manuscript and commented on the aspects of medical science. IK shared information on clinical medicine and oversaw the entirety of the study.

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