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Original contribution

Uterine leiomyosarcoma with osteoclast-like giant cells associated with high expression of receptor activator of nuclear factor κB ligand☆

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Summary The occurrence of osteoclast-like giant cells (OLGCs) in uterine leiomyosarcomas (LMSs) is a rare phenomenon. The nature of OLGCs and the significance of their accumulation in these tumors are poorly understood. Recent studies revealed that the formation of osteoclasts requires a specific cytokine, receptor activator of nuclear factor κB ligand (RANKL), in bone. In this study, we investigated the expression of RANKL in 2 cases of uterine LMS with OLGCs by means of immunohistochemistry and compared the extent of RANKL expression with that in conventional uterine LMSs and leiomyomas by using real-time reverse-transcription quantitative polymerase chain reaction. Our cases of uterine LMS with OLGCs showed markedly high expression of RANKL messenger RNA with clear RANKL immunoreactivity compared with messenger RNA expression and immunoreactivity of conventional uterine LMSs and leiomyomas. These findings suggest that the tumors producing RANKL may account for accumulation of OLGCs in tumor tissue because of RANKL-related osteoclastogenesis.

1. Introduction

Uterine leiomyosarcomas (LMSs) comprise approximately 1% to 2% of uterine malignancies [1]. Osteoclast-like giant cells (OLGCs) are rarely seen in uterine LMSs [1], and this type of LMS is named uterine LMS with OLGCs [2-13], which has a poor prognosis [7]. This phenomenon of infiltrating OLGCs in tumor tissues was reported not only for uterine LMS but also for several other malignant tumors from various organs including carcinomas and sarcomas [14-21].

Certain studies revealed that the regulation of osteoclast formation requires a specific cytokine, receptor activator of nuclear factor κB ligand (RANKL), in bone [22,23]. RANKL expressed by osteoblasts or osteocytes interacts with its receptor, receptor activator of nuclear factor κB (RANK), expressed by the monocyte/macrophage lineage precursor cells of osteoclasts, and this RANK-RANKL

Abbreviations LMS, leiomyosarcoma; OLGC, osteoclast-like giant cell; RANKL, receptor activator of nuclear factor κB; RT-qPCR, reverse transcription–quantitative polymerase chain reaction; SMA, α-smooth muscle actin

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interaction induces precursor cells that transform into osteoclasts [22,23].

This evidence led previous studies to report finding expression of RANKL in certain bone tumor tissues with OLGCs [24-27]. However, few studies reporting RANKL expression in tumors with OLGCs arising from organ or tissue sites other than bone exist [18,28], and no report of uterine LMS with OLGCs has appeared. The nature of these OLGCs and the significance of their accumulation in uterine LMSs are still unknown.

We therefore studied the expression of RANKL in uterine LMS with OLGCs and compared it with RANKL expression in conventional uterine LMS and leiomyomas using immunohistochemistry and real-time reverse-transcription quantitative polymerase chain reaction (RT-qPCR).

2. Materials and methods

2.1. Case 1

The patient was an 81-year-old Japanese woman who presented with a 30-year history of leiomyoma. She reported abdominal bloating. Pelvic examinations revealed an enlarged uterus, and magnetic resonance imaging showed an irregularly shaped mass, with a diameter of approximately 12 cm, in the uterus. A chest radiograph and a computed tomographic image suggested multiple metastases to the lungs. A total hysterectomy and bilateral oophorectomy were performed. No significant peripheral lymphadenopathy was observed. The patient was treated with the chemotherapeutic agents gemcitabine and docetaxel. However, the metastatic lung lesions grew larger, and new metastatic bone lesions appeared. She died 11 months after the operation.

2.2. Case 2

The patient was a 60-year-old Japanese woman who presented with an 8-year history of leiomyoma. She complained of abdominal bloating and dysuria. Magnetic resonance imaging revealed an irregularly shaped mass with a diameter of approximately 13 cm on the right side of the uterine wall. Several enlarged peripheral lymph nodes were noted. A total hysterectomy, bilateral oophorectomy, omentectomy, and lymph node biopsy were performed. Despite chemotherapy with gemcitabine and docetaxel, the patient had recurrence and died 21 months after the operation.

2.3. Comparative cases

To compare RANKL expression in other cases by means of immunohistochemistry (IHC) and RT-qPCR, we collected and evaluated 3 cases of uterine leiomyomas and 3 cases of conventional uterine LMSs. The diagnosis of uterine LMS was based on the presence of nuclear atypia, a high mitotic index, coagulative tumor cell necrosis, and immunoreactivity for smooth muscle markers of tumor cells [29].

2.4. Histopathology and IHC

Surgical specimens of uterine tumors were obtained during routine clinical investigations. The study protocol was approved by the Human Ethics Review Committee of Nippon Medical School; a signed consent form was obtained from each patient.

Formalin-fixed tissues from uterine tumors were embedded in paraffin blocks according to standard histopathologic laboratory methods. Sections were cut 4 μm thick and stained with hematoxylin and eosin. Immunohistochemical studies were performed by using autoclave heating and polymer immunocomplex (N-Histofine Simple Stain MAX PO; Nichirei Biosciences, Tokyo, Japan) methods with appropriately reacting positive and negative controls. We used the following primary antibodies for diagnosis: SMA (Dako, Carpinteria CA), *h*-caldesmon (Dako), desmin (Dako), Ki-67 (Dako), CD68 (Dako), and cytokeratin AE1/AE3 (Dako). To detect RANKL expression, we used anti-RANKL antibody (rabbit polyclonal antibody; Abcam plc, Cambridge, UK).

2.5. Real-time RT-qPCR amplification

We analyzed RANKL messenger RNA (mRNA) expression by performing real-time RT-qPCR. We collected 6 serial 10-μm paraffin sections from each case and extracted total RNA with TRIzol reagent (Qiagen, Hilden, Germany) according to the manufacturer's instructions. To remove contaminating DNA, poly A+ RNA was treated with DNase I (RNase-Free DNase Set; Qiagen). All samples had a ratio of the optic density at 260 and 280 nm (OD260/OD280) of greater than 1.8. To generate first-strand complementary DNA, we reverse transcribed total RNA (100 ng) with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) in a total volume of 20 μL. For each PCR, we used 5 μL of cDNA (total volume 20 μL). To detect RANKL mRNA, we used ready-to-use primer and probe sets from Applied Biosystems (Assays-on-Demand Gene Expression Catalog nos. Hs00243522_m1 for RANKL and Hs03929097_g1 for GAPDH). We optimized primer and probe concentrations for each target gene according to the manufacturer's procedure. PCR (2 minutes at 50°C, 10 minutes at 95°C, and 45 cycles of 15 seconds of denaturation at 95°C and 60 seconds of annealing at 60°C) was performed with the ABI PRISM 7000 Sequence Detection System (Applied Biosystems) and the fluorescent TaqMan methodology. For all experiments, we quantified RANKL mRNA and GAPDH mRNA in duplicate and normalized RANKL mRNA against GAPDH mRNA and calculated relative gene expression data using the $2^{-\Delta\Delta}$Ct method [30].

3. Results

3.1. Pathologic findings: histopathology and IHC

The gross examination of case 1 showed that a tumor measuring 12 × 10 cm occupied the whole uterine corpus,