Involvement of integrin up-regulation in RANKL/RANK pathway of chondrosarcomas migration

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INTRODUCTION:
Chondrosarcoma is a malignant primary bone tumor with a poor response to currently-used chemotherapy or radiation treatment, making the management of chondrosarcomas a complicated challenge. Clinically, surgical resection remains the primary mode of therapy for chondrosarcoma. Due to the absence of an effective adjuvant therapy, this mesenchymal malignancy has a poor prognosis and therefore, it is important to explore a novel and adequate remedy. Receptor activator of nuclear factor-xB ligand (RANKL) and its receptor, RANK, play a key role in osteoclastogenesis and tumor metastasis. We found that human chondrosarcoma tissues had significant expression of the RANKL and RANK, which was higher than that in normal cartilage. We also found that RANKL directed the migration and increased cell surface expression of β1 integrin in human chondrosarcoma cells (JJ012 cells). Here we found a phenomenon whereby RANKL and RANK interaction increased the migration and expression of integrin in human chondrosarcoma cells. In addition, MAPK kinase (MEK), ERK, IKKα/β and NF-xB signaling pathways may be involved in the increase of integrin expression and cells migration by RANKL.

METHODS:
Cell culture: The human chondrosarcoma cell line (JJ012) was kindly provided from the laboratory of Dr. Sean P Scully (University of Miami School of Medicine, Miami, FL, USA). The JJ012 cells were cultured in DMEM/α-MEM supplemented with 10% Fetal Bovine Serum and maintained at 37°C in a humidified atmosphere of 5% CO2.

Migration assay: The migration assay was performed using Transwell (Costar, NY; pore size, 8-μm) in 24-well dishes. Before performing the migration assay, cells were pretreated for 30 min with different concentrations of inhibitors or vehicle control (0.1% DMSO). Approximately 1×106 cells in 100 μl of serum-free medium were placed in the upper chamber, and 300 μl of the same medium containing RANKL was placed in the lower chamber. The plates were incubated for 24 h at 37°C in 5% CO2, then cells were fixed in methanol for 15 min and stained with 0.05% crystal violet in PBS for 15 min. Cells on the upper side of the filters were removed with cotton-tipped swabs, and the filters were washed with PBS. Cells on the underside of the filters were examined and counted under a microscope. Each clone was plated in triplicate in each experiment, and each experiment was repeated at least three times.

qPCR: Flow cytometric analysis; Western blot analysis; Transfection and reporter gene assay

RESULTS
RANKL has been reported to stimulate directional migration and invasion of human cancer cells [Armstrong et al., 2008; Heymann et al., 2008]. We first examined whether RANKL express in human chondrosarcoma cells. Expression of mRNA levels of RANKL in chondrosarcoma patients (Fig. 1A, lines 4-6) were significantly higher than those in normal cartilage (Fig. 1A, lines 1-3). RANKL-triggered migration in chondrosarcoma cells was examined using the Transwell assay with correction of RANKL-induced proliferation effects on human chondrosarcoma cells. RANKL directed human chondrosarcoma cell (JJ012 cell) migration (Fig. 1B). Interaction of RANKL with its specific receptor RANKL on the surface of cancer cells has been reported to induce cancer invasion. Next, we examined human chondrosarcoma patients for the expression of the RANK using qPCR. Expression of mRNA levels of RANK in chondrosarcoma patients (Fig. 1C, lines 4-6) were significantly higher than those in normal cartilage (Fig. 1C, lines 1-3). Therefore, RANKL and RANK interaction is mediated cell migration of chondrosarcoma. Previous studies have shown significant expression of integrins in human chondrosarcoma cells. We therefore, hypothesized that integrins may be involved in RANKL-directed chondrosarcoma cells migration. Flow cytometry analysis showed that RANKL-induced the cell surface expression of β1 but not α2, α5, β3 and αvβ3 integrin (Fig. 2A). In addition, RANKL also increased the mRNA expression of β1 integrin (Fig. 2B). Pretreatment of cells for 30 min with anti-β1 monoclonal antibody (mAb) (3 μg/ml) markedly inhibited the RANKL-induced cancer migration (Fig. 2C). Therefore, involvement of β1 integrin up-regulation in RANKL-induced cell migration of human chondrosarcoma. Pretreated of JJ012 cells with MAPK kinase (MEK) inhibitors PD98059 or U0126 inhibited the RANKL-mediated migration and integrin expression. Stimulation of cells with RANKL increased the phosphorylation of MEK and extracellular signal-regulating kinase (ERK). In addition, NF-xB inhibitor (PDTC) or hB protease inhibitor (TPCK) also inhibited RANKL-mediated cells migration and integrin up-regulation.

DISCUSSION:
The prognosis of patients with chondrosarcoma distant metastasis is generally considered very poor; hence, preventing human chondrosarcoma metastasis is an important issue nowadays. Our study observes that RANKL increases the activity of β1 integrin via the RANK, MEK, ERK, IKKα/β and NF-xB-dependent pathway and enhances migration of human chondrosarcoma cells. Furthermore, the discovery of RANKL/RANK-mediated signaling pathway helps us understand the mechanism of human chondrosarcoma metastasis and may lead us to develop effective therapy in the future.