Involvement of matrix metalloproteinase-3 in CCL5/CCR5 pathway of chondrosarcoma metastasis

INTRODUCTION:
CCL5 (previously called RANTES) was originally recognized as a product of activated T cells, and plays a crucial role in the migration and metastasis of human cancer cells. It has been reported that the effect of CCL5 is mediated via CCR receptors. However, the effect of CCL5 and CCR receptor on migration activity in human chondrosarcoma cells is mostly unknown. We hypothesized that CCL5 might be capable of regulating chondrosarcoma cells migration and matrix metalloproteinase (MMP) expression. We found that human chondrosarcoma tissues had significant expression of the CCL5 and CCR5, which was higher than that in normal cartilage. In addition, MMP-3 small interfering RNA and inhibitor inhibited the CCL5-induced cell migration. Activations of phosphatidylinositol 3-kinase (PI3K), Akt and NF-κB pathways after CCL5 treatment was demonstrated, and CCL5-induced expression of MMP-3 and migration activity was inhibited by the specific inhibitor of PI3K, Akt and NF-κB cascades. Taken together, these results indicate that CCL5 and CCR5 interaction enhanced migration of chondrosarcoma cells through the increase of MMP-3 production.

METHODS:
Cell culture: The human chondrosarcoma cell line (JJ012; Grade II chondrosarcoma cell line) 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, USA; pore size, 8-μm) in 24-well dishes. Before performing the migration assay, cells were pretreated for 30 min with different concentrations of inhibitors, including the Ly294002 (10 μM), Akt inhibitor (10 μM), PDTC (10 μM), TPCK (3 μM) or vehicle control (0.1% DMSO). The concentrations of inhibitors didn’t affect cell death of JJ012 cells as shown by a cell viability assay (data not shown). Approximately 1×10^5 cells in 200 μl of serum-free medium were placed in the upper chamber, and 300 μl of the same medium containing CCL5 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.

Zymography analysis; qPCR; ELISA assay; Western blot analysis; Immunofluorocytometry; Transfection and reporter gene assay.

RESULTS
CCL5 has been reported to stimulate directional migration and invasion of human cancer cells. CCL5-activated migration in chondrosarcoma cells was examined using the Transwell assay with correction of CCL5-induced proliferation effects on human chondrosarcoma cells. CCL5 directed human chondrosarcoma cell (JJ012 cell) migration (Fig. 1A). Interaction of CCL5 with its specific receptor CCR on the surface of cancer cells has been reported to induce cancer invasion. Stimulation of cells with CCL5 increased the mRNA expression of CCR5 (Fig. 1B), suggesting that the amplification loop strengthens the CCL5-CCR5-signaling pathway. Pretreatment of cells with CCR5 mAb or CCR5 receptor inhibitor (Met-RANTES) reduced CCL5-induced cell migration (Fig. 1C). In addition, transfection of cells with CCR5 siRNA reduced CCR5 protein expression (data not shown). Next, we examined human chondrosarcoma patients for the expression of the CCL5 and CCR5 using qPCR. Expression of mRNA levels of CCL5 in chondrosarcoma patients (Fig. 1E; lines 4-6) were significantly higher than those in normal cartilage (Fig. 1E; lines 1-3). Therefore, CCL5 and CCR5 interaction is very important in migration activity in chondrosarcoma cells. Previous studies have shown a significant expression of MMP-1, -2, -3, -9 and -13 in human chondrosarcoma cell. We therefore, hypothesized that any of these human chondrosarcoma cells-associated MMPs may be involved in CCL5/CCR5-directed chondrosarcoma cell migration. qPCR analysis showed that CCL5 significantly increased the expression of MMP-3 mRNA but not MMP-1, -2, -9 and -13 in JJ012 cells (Fig. 2A). Furthermore, CCL5 further increased protein expression of MMP-3 in JJ012 cells in a time-dependent manner using Western blot (Fig. 2B). To further explore whether MMP-3 might play a crucial role in CCL5-mediated migration activity, the MMP-3 siRNA was used. It was reduced CCL5-mediated migration activity (Fig.2C). Therefore, MMP-3 upregulation is involved in CCL5-mediated cell migration. Stimulation of cells with CCL5 increased PI3K and Akt phosphorylation. Treatment of cells with NFκB inhibitor reduced CCL5-mediated cell migration. Therefore, CCL5-directed migration was transcriptional up-regulation of MMP-3 and activation of CCR5, PI3K, Akt and NFκB pathways.

DISCUSSION:
We present here a novel mechanism of CCL5-directed migration of human chondrosarcoma cells by up-regulation of MMP-3 and activation of CCR5, PI3K, Akt and NF-κB pathways. The discovery of CCL5/CCR5-mediated signaling pathway helps us understand the mechanism of human chondrosarcoma metastasis.