BMP-2 increases migration of human chondrosarcoma cell via PI3K/Akt pathway

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INTRODUCTION:
Chondrosarcoma is a malignant primary bone tumor with a poor response to current chemotherapy and radiation treatment. Clinically, surgical resection remains the primary mode of therapy for chondrosarcoma. It has been reported that the chondrosarcoma has a potent capacity to invade locally and distant metastasis. Patients who develop metastatic chondrosarcomas have poor prognosis. Bone morphogenetic protein, a member of the transforming growth factor-β (TGF-β) superfamily, plays a crucial role in migration and metastasis of human cancer cells. Integrins are the major adhesive molecules in mammalian cells. But the relationship between BMPs and integrins in human chondrosarcoma is still unclear. The aim of this study was to investigate whether BMPs and integrins are associated with chondrosarcoma cells migration.

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 human chondrosarcoma cell line SW1353 was obtained from American Type Culture Collection. JJ012 cells were cultured in DMEM/α-MEM supplemented with 10% Fetal Bovine Serum and SW1353 cells were cultured in Leiovitz’s L-15 medium supplemented with 10% Fetal Bovine Serum. All of these cells were maintained at 37°C with 5% CO2.

The migration assay was performed using Transwell (Costar, NY; pore size, 8-μm) in 24-well dishes. The cell surface β1 integrin was measured by flow cytometry using FACS Calibur and CellQuest software (BD Biosciences, CA). β1 integrin mRNA levels were measured using RT-PCR analysis. PI3K, AKT, p65, IKKa/β phosphorylation were examined by using Western blot method. The xB-luciferase plasmid was transfected to JJ012 cells by Lipofectamine 2000 (LF2000; Invitrogen, CA). The luminescence was then measured in a microplate luminometer.

RESULTS
We hypothesized that BMP-2 might direct the chondrosarcoma cells migration. Indeed, we found that BMP-2 enhanced human chondrosarcoma cancer cells (JJ012 and SW1353) migration dose-dedentently (Fig 1A and 1B). Flow cytometry analysis showed that BMP-2 induced the cell surface expression of β1 integrin dose-dependently (Fig 1C). In addition, BMP-2 also increased the mRNA expression of β1 integrin (Fig 1D) (The quantitative RT-PCR data are shown in the Fig. 1D median panel). Thus, β1 integrin may be involved in BMP-2-mediated migration of JJ012 cells.

To study the cell signaling between BMP-2 stimulation and β1 integrin expression in JJ012 cells, western blot method was then performed. Here we found that BMP-2 increased the phosphorylation of p85 subunit of PI3K and serine 473 of Akt. Stimulation of JJ012 cells with BMP-2 also induced IκB kinase (IKKα/β) phosphorylation, IκBα phosphorylation, and p65 Ser536 phosphorylation (Fig 2A). The time kinetics of p85 (~30 min) and Akt (~30 min) phosphorylation and phosphorylation of IKK (~60 min), IκBα (~60 min) and p65 (~60 min) after BMP-2 stimulation. These data indicated that p85 and Akt may act upstream molecules signaling of IKK, IκBα and p65 in the BMP-2 signaling. In addition, the BMP-2-induced increase in xB-luciferase activity was also inhibited by treatment with PI3K inhibitor (Ly294002), Akt inhibitor, NF-κB inhibitor (PDTC) or IκBα protease inhibitor (TPCK) (Fig 2B). Co-transfection with p85, Akt, IKKα and IKKβ mutants also reduced the BMP-2-induced xB-luciferase activity (Fig 2C). Taken together, these results suggested that the BMP-2 may act through PI3K/Akt, which in turn activate IKKα/β and NF-κB, resulting in the activation of β1 integrin and contributing the migration of human chondrosarcoma cells (Fig 3).

DISCUSSION:
In this study, we presented that BMP-2 increased the activity of β1 integrin via PI3K, Akt, IKKα/β, and NF-κB-dependent pathway and increased migration of human chondrosarcoma cells. The discovery of BMP-2-mediated integrin/NF-κB-dependent pathway may help us to understand the mechanism of human chondrosarcoma metastasis more clearly.