Bradykinin enhances migration in human chondrosarcoma cells through BK receptor signaling pathways

INTRODUCTION:

Bradykinin (BK) is an inflammatory mediator, and shows elevated levels in regions of severe injury and inflammatory diseases. BK has recently been shown to be involved in carcinogenesis and cancer progression. In this study, we found that BK increased the migration and the expression of α2β1 integrin in human chondrosarcoma cells. We also found that human chondrosarcoma tissues had significantly higher expression of the B1 and B2 receptors compared to normal cartilage. BK-mediated migration and integrin up-regulation was attenuated by B1 and B2 BK receptor siRNA. In addition, phospholipase C (PLC), protein kinase Cα (PKCα), and NF-κB signaling pathways may be involved in the increase of α2β1 integrin and cells migration by BK.

METHODS:

Cell culture: The human chondrosarcoma cell line (JJ012) was kindly provided by Dr. Sean P Scully (University of Miami School of Medicine, Miami, FL, USA). The cells were cultured in DMEM/α-MEM supplemented with 10% Fetal Bovine Serum (FBS) 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, including the U73122, GF109203X, Rottlerin, PDTC, TPCK or vehicle control (0.1% DMSO). 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 BK were placed in the lower chamber. The plates were incubated for 24 hr at 37°C in 5% CO2, and then cells were 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. The number of invading cells in each experiment was adjusted by the cell viability assay to correct for proliferation effects of the BK treatment (corrected number of invading cells = counted number of invading cells/percentage of viable cells).

Flow Cytometric Analysis; Western blot analysis; PKC kinase activity assay; Quantitative Real-Time PCR; Reporter assay

RESULTS

BK has been reported to stimulate migration and invasion of human cancer cells directionally. The role of BK in chondrosarcoma cell migration was examined using the Transwell assay with correction of BK-induced proliferation effects on human chondrosarcoma cells. Figure 1A shows that BK enhanced the migration of human chondrosarcoma cells (JJ012 cells) in a dose-dependent manner. Previous studies have shown significant expression of integrins in human chondrosarcoma cells. We therefore, hypothesized that integrins may be involved in BK-directed chondrosarcoma cell migration. Flow cytometry analysis showed that BK induced the cell surface expression of α2β1 and α2 but not α3β1, αvβ3, β3 and α5 integrin in JJ012 cells (Fig.1B). To confirm this finding, expression of mRNAs for the integrins in response to BK was analyzed by qPCR. Treatment of JJ012 cells with BK induced the mRNA expression of α2 and β1 integrins (Fig. 1C). Pretreatment of cells for 30 min with anti-α2β1 monodonal antibody (mAb) (3 μg/ml) markedly inhibited the BK-induced cell migration (Fig. 1D). Therefore, the α2β1 integrin is involved in BK-induced migration of human chondrosarcoma cells.

BK exerts their effects through interaction with specific BK receptors (B1 and B2). However, little is known about the expression of B1 and B2 receptor in human chondrosarcoma cells. We examined human chondrosarcoma patients for the expression of the BR receptors using qPCR. Expression of mRNA levels of B1 and B2 in chondrosarcoma patients were significantly higher than those in normal cartilage.

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 BK increases the activity of α2β1 integrin via the BK receptors, PLC, PKCα, IKKα/β, and NF-κB-dependent pathway and enhances migration of human chondrosarcoma cells.