

HGF increases migration and MMP-2 expression via PI3K, AKT and NF-kappaB dependent pathway in human chondrosarcoma cells

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INTRODUCTION:

Chondrosarcoma is characterized by a high malignant and metastatic potential. It has been reported that hepatocyte growth factor/scatter factor (HGF/SF) induced proliferation, motility and promote invasion of tumor cells. However, the effect of HGF on migration activity in human chondrosarcoma cells is mostly unknown. Here, we found that HGF increased the migration and expression of matrix metalloproteinase (MMP)-2 in human chondrosarcoma cells (JJ012 cells). Pretreatment of chondrosarcoma cells with LY294002 and Wortmannin, which are PI3K inhibitors, inhibited the HGF mediated migration and MMP-2 expression. Stimulation of cells with HGF increased the phosphorylation of PI3K and AKT. In addition, NF-kappaB inhibitor (PDTC) or IkappaB protease inhibitor (TPCK) also inhibited HGF mediated cell migration and MMP-2 up-regulation. Stimulation of cells with HGF induced IkappaB kinase (IKKalpha/beta) phosphorylation, IkappaB phosphorylation and p65 Ser(536) phosphorylation. Furthermore, the HGF mediated increasing MMP-2 expression was inhibited by LY294002, Wortmannin, Akt inhibitor, PDTC and TPCK. Taken together, these results suggest that the HGF acts through activate PI3K and Akt, which in turn activates IKKalpha/beta and NF-kappaB, resulting in the activations of MMP-2 and contributing the migration of human chondrosarcoma cells.

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 lines (SW1353) were obtained from the American Type Culture Collection. The cells were cultured in Dulbecco's modified Eagle's medium/ α -minimum essential medium supplemented with 10% fetal bovine serum and maintained at 37°C in a humidified atmosphere of 5% CO₂.

Migration assay, Real Time-PCR, Western blot analysis

RESULTS

HGF has been reported to stimulate directional migration and invasion of human cancer cells. To examine the effects of HGF on chondrosarcoma cell migration, the Transwell assay was used. SW1353 were treated with various concentrations of HGF and significantly directed cells migration (Fig1). To investigate that MMP-2 is important for chondrosarcoma cell migration, We found that MMP-2 expression is involved in the regulation of growth and metastasis of human cancer cells. Then, treatment of cells with HGF increased protein and mRNA expression of MMP-2 in a time-dependent and dose-dependent by western blot and qPCR analysis (Fig. 2). In addition, MMP2 siRNA also reversed HGF mediated MMP-2 expression. Pretreatment of chondrosarcoma cells with LY294002 and Wortmannin and DN-PI3K, DN-AKT inhibited the HGF mediated migration and MMP-2 expression. Stimulation of cells with HGF increased the phosphorylation of PI3K and AKT by western blot. Transfection of cells with p85 and Akt mutant also reversed HGF mediated MMP-2 expression. (Fig. 3) As previously mentioned, NF- κ B activation is necessary for the migration and invasion of human chondrosarcoma cells. To examine whether NF- κ B activation is involved in the signal transduction pathway caused by HGF that leads to cell migration and MMP-2 expression, the NF-kappaB inhibitor (PDTC) or IkappaB protease inhibitor (TPCK) also inhibited HGF mediated cell migration and MMP-2 up-regulation of cell migration. In addition, the HGF induced increase in MMP-2 activity was also inhibited by treatment with Co-transfection with IKK α and IKK β mutants. Taken together, these data suggest that activation of HGF induced MMP-2 activation in human chondrosarcoma cells (Fig. 4).

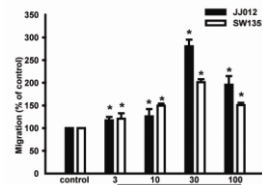


Figure 1. HGF increased cell migration in human chondrosarcoma cells.

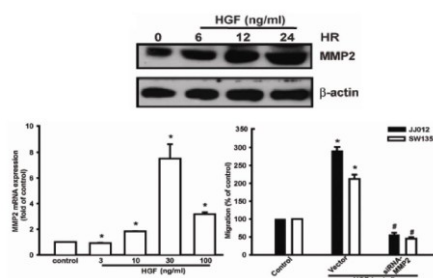


Figure 2. HGF increased MMP-2 expression in human chondrosarcoma cells.

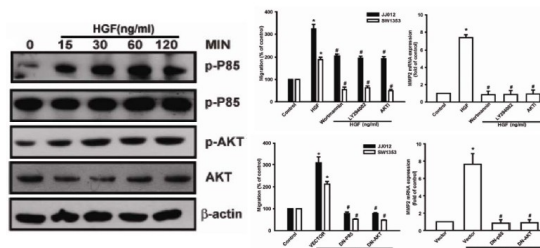


Figure 3. PI3K/AKT involved in HGF-mediated cell migration and MMP-2 expression.

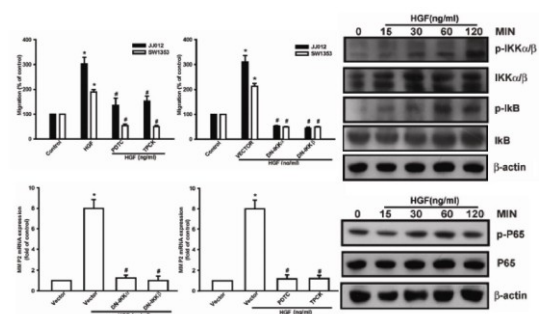


Figure 3. NF- κ B is involved in HGF-mediated cell migration and MMP-2 expression.

DISCUSSION:

In the present study, we explored the intracellular signaling pathway involved in HGF mediated increasing MMP-2 expression in human Chondrosarcoma cells. We explored whether HGF increased the migration of and MMP-2 expression in human chondrosarcoma cells. In addition, phosphatidylinositol 3-kinase (PI3K), Akt and NF- κ B signaling pathways may be involved in the increase of MMP-2 expression and cell migration by HGF may help us.