BDNF increases human chondrosarcoma cells migration and β5 integrin expression via TrkB, PI3K/Akt and NF-κB dependent pathways

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Objectives

Brain derived neurotrophic factor (BDNF) is a Small-molecule protein from the "neurotrophin" family of growth factors. Neurotrophic factors are found in the brain and the periphery. TrkB is the receptor for the BDNF, it has been shown to play a key role in metastasis of tumor cells. However, the effect of BDNF on migration activity in human chondrosarcoma cells is mostly unknown. Here, we found that BDNF Increased the migration and expression of β5 integrin in human chondrosarcoma cells (JJ012 cells). TrkB inhibitors (K252a), Phosphatidylinositol 3-kinase (PI3K) inhibitors (LY294002 and Wortmannin), Akt inhibitors (Akti) and NF-κB inhibitors (TPCK and PDTC) inhibited the BDNF-induced the migration and β5 integrin upregulation of chondrosarcoma cells. BDNF stimulation increased the PI3K and Akt signaling pathway. BDNF-mediated NF-κB-luciferase activity was inhibited by p85 and Akt mutant. Taken together, our results indicated that BDNF enhances the migration of chondrosarcoma cells by increasing integrin β5 expression via the PI3K/Akt and NF-κB signal transduction pathways.

Methods

Cell culture: The human chondrosarcoma cell line (JJ012) was kindly provided by the laboratory of Dr. Sean P Scully (University of Miami School of Medicine, Miami, FL, USA). The cells were cultured in Dulbecco's modified Eagle's medium/a-minimum essential medium supplemented with 10% fetal bovine serum and maintained at 37°C in a humidified atmosphere of 5% CO2.

Western blot analysis; Wound-healing migration assay; Immunohistochemistry; Transfection and reporter gene assay; Quantitative real-time PCR

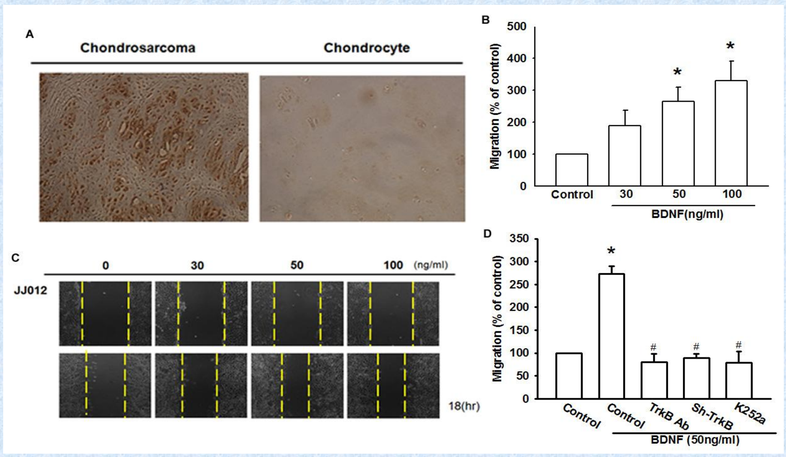

Figure 1. BDNF increases migration activity of human chondrosarcoma cells .

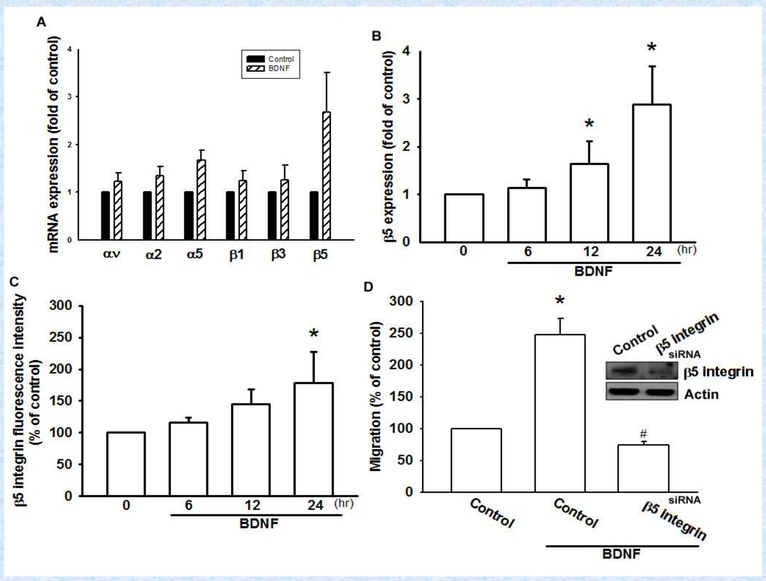

Figure 2. BDNF induced β5 integrin expression .

Results

BDNF has been reported to stimulate directional migration and invasion of human cancer cells. However, the effect of BDNF in migration of chondrosarcoma is mostly unknown. We examined human chondrosarcoma tissues for the expression of the BDNF using immunohistochemistry analysis. Expression of protein levels of BDNF in human chondrosarcoma tissues was significantly higher than that detected in normal cartilage (Fig. 1A). The BDNF for chondrosarcoma cells migration was examined using the Transwell assay and Wound-healing assay with correction of BDNF-induced proliferation effects on human chondrosarcoma cells. Treatment of BDNF (30–100 ng/ml) with chondrosarcoma cells (JJ012) directed cells migration, and (Fig. 1B&C). BDNF-induced migration of JJ012 cells were greatly reduced by treatment with TrkB inhibitor (K252a), TrkB mAb and Sh-RNA (Fig. 1D). We hypothesized that β5 integrin may be involved in BDNF-directed migration of human chondrosarcoma. Treatment of cells with BDNF increased β5 integrin mRNA expression in a time-dependent manner (Fig. 2B). Transfection of cells with β5 integrin siRNA inhibited β5 integrin expression (Fig. 2D). In addition, transfection of cells with p85 and Akt inhibiter reduced the BDNF-mediated cell migration and β5 integrin expression (Fig. 3A&4A). Treatment of cells with BDNF increased phosphorylation of p85, Akt and p65 (Fig. 3D&4D). BDNF treatment of JJ012 cells for 24 hr resulted in increased NF-κB-luciferase activity. In addition, K252a, Ly294002, Wortmannin, Akti, PDTC and TPCK antagonized the BDNF-induced NF-κB-luciferase activity (Fig. 5A&B). The p65 phosphorylation and the binding of p65 to the NF-κB element by BDNF were attenuated by the Ly294002, Wortmannin and Akti (Fig. 4E). Stimulation of cells with BDNF increased p65 translocation into the nucleus as measured by immunofluorescence staining. Ly294002, Wortmannin and Akti, all reduced the BDNF-mediated translocation of p65 (Fig. 5C). Finally, these data suggest that activation of PI3K/Akt and p65 are required for BDNF-induced NF-κB activation in human chondrosarcoma cells.

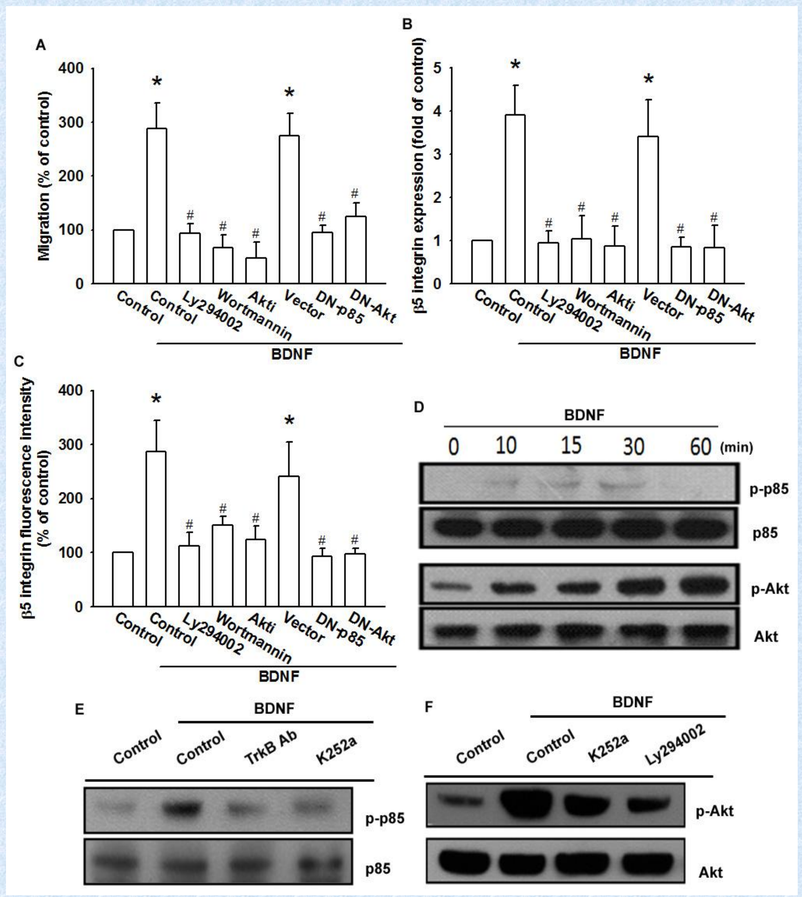

Figure 3. PI3K/Akt is involved in the potentiation of β5 integrin expression by BDNF .

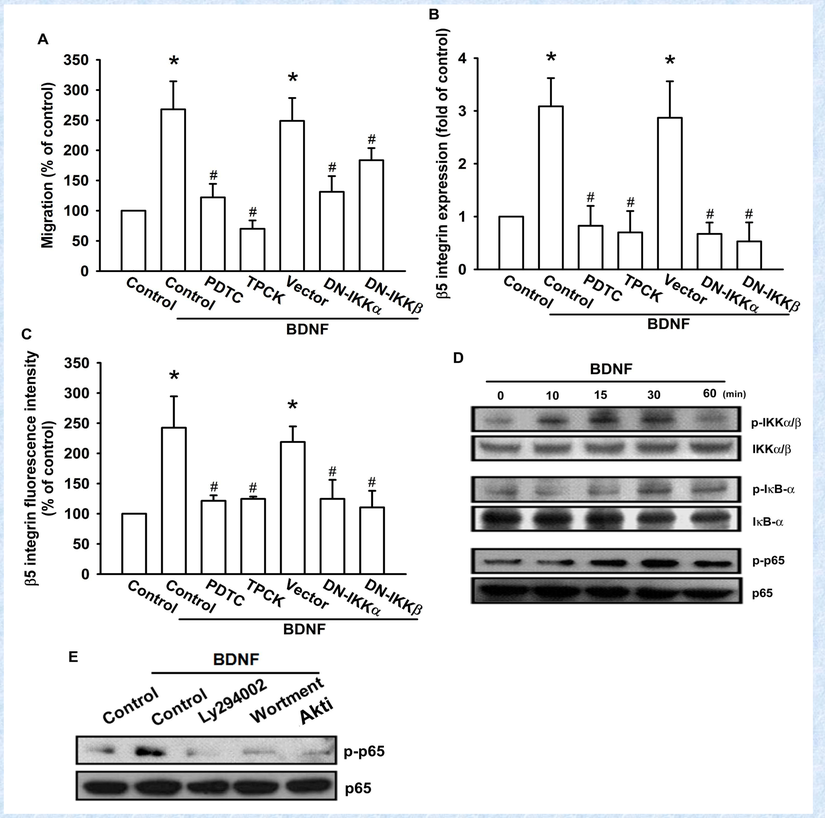

Figure 4. NF-κB is involved in the potentiation of β5 integrin expression by BDNF .

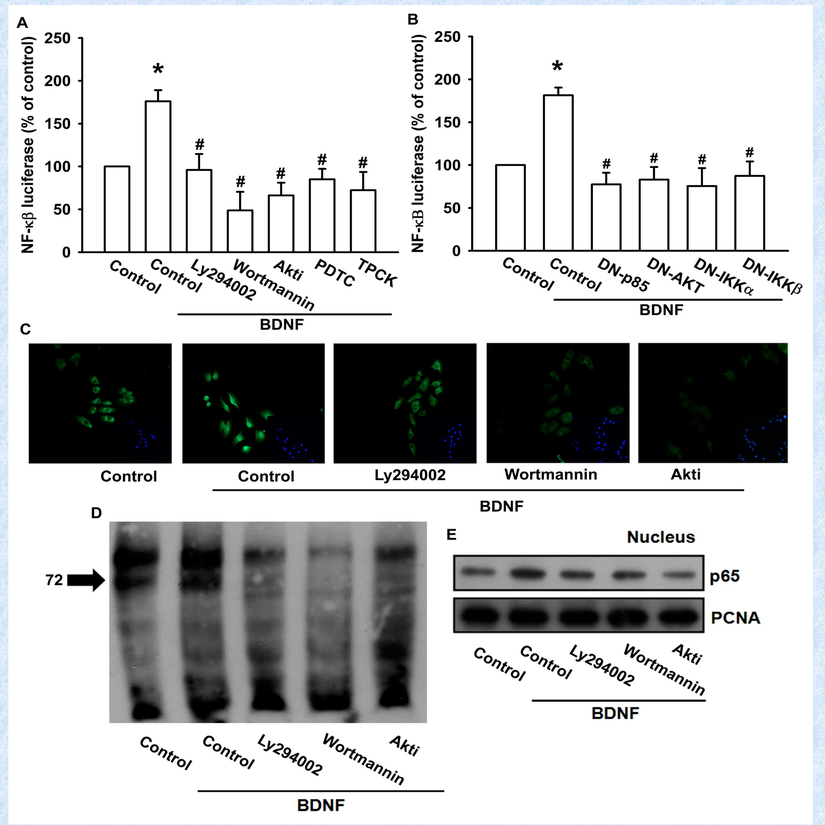

Figure 5. PI3K/Akt is involved in BDNF induced NF-κB promoter activity.

Conclusions

We present a novel mechanism of BDNF-directed migration of chondrosarcoma cells via up-regulation of β5 integrin production. BDNF increases cell migration and β5 integrin expression by activation of TrkB receptor, p85, Akt, p65 and NF-κB-dependent pathway and contribute to tumor metastasis.