Involvement of ERK, Akt and NF-κB-dependent pathway in osteoblasts-derived factors increase motility and integrin up-regulation in human prostate cancer cells

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Introduction: Metastasis, the major cause of mortality for cancer patients, is a complex and multi-stage process in which secondary tumors are formed in distant sites [1]. Bone is one of common sites for cancer metastasis, particularly including breast, prostate and lung cancers that have predilection for metastasis to bone [2]. Integrins are a family of transmembrane adhesion receptors comprising 19 α and 8 β subunits that interact noncovalently to form up to 24 different heterodimeric receptors. The combination of different integrin subunits on the cell surface allow cells to recognize and respond to a variety of different ECM proteins including fibronectin, laminin, collagen and vitronectin. Bone-derived growth factor and chemokines also play central roles as trophic factors that attract breast, prostate cancer cells to bone tissue. However, the effect of osteoblast-derived factors on integrins expression and migration activity in human prostate cancer cells is mostly unknown. Here we found that osteoblast conditioned medium (OBCM) increased the migration and the expression of integrins of human prostate cancer cells.

Materials and Methods: Cell culture: The human prostate cancer cell lines (PC-3) was obtained from the American Type Culture Collection. A murine osteoblastic cell line MC3T3-E1 was obtained from Riken Cell Bank (Tsukuba, Japan).
Preparation of conditioned medium (CM): Murine osteoblastic cell line MC3T3-E1 were grown to confluence on reaching confluence, culture media were changed with α-MEM without FCS. CMs were collected 2 days after the media changed and stored at -70°C until use.

Results: Bone-derived growth factor and chemokines play central roles as trophic factors that attract breast, prostate cancer cells to bone tissue. The OBCM for prostate cancer cells migration was examined using the Transwell assay with correction of OBCM induced proliferation effects on human prostate cancer cells. OBCM concentration-dependently directed prostate cancer cells migration(Fig. 1). Previous studies have shown significant expression of β1 and β3 integrin in human cancer cells. We therefore, hypothesized that integrins may be involved in OBCM-directed prostate cancer cells migration. Flow cytometry analysis showed that OBCM-induced the cell surface expression of β1 and β3 integrin(Fig. 2). Pretreatment of cells for 30 min with anti-β1 and β3 monoclonal antibody (mAb) (10 μg/ml) or transfected with siRNA against β1 and β3 integrin for 24 hr markedly inhibited the OBCM-induced cancer migration(Fig. 3). These data suggest that OBCM-induced cancer migration may occur via activation of β1 and β3 integrin receptor.

Discussion: Prostate cancer cells have a striking tendency to metastasize to bone. The analysis of trophic signals that control bone metastasis of prostate cancer is crucial for the identification of new molecular targets for anti-metastasis therapy. We hypothesized that osteoblast-derived factors would help to direct the migration of prostate cancer cells. We found that osteoblasts-derived factors from osteoblast induced the migration of human prostate cancer cells. One of the mechanisms underlying OBCM directed migration was transcriptional upregulation of β1 and β3 integrins and activation of Akt, ERK, IKKα/β and NF-κB pathways.

References:

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