TGF-β1 Stimulates IL-8 Release via AP-1 and NF-κB in Human Cancer Cell

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INTRODUCTION:
The bone marrow microenvironment is further enriched by growth factors released during osteoclastic bone resorption. It has been reported that the chemokine IL-8 is a potent and direct activator of osteoclastic differentiation and bone resorption. However, the effect of bone-derived growth factors on the IL-8 production in human cancer cells and the promotion of osteoclastogenesis are largely unknown. The aim of this study was to investigate whether osteoblast-derived TGF-β1 is associated with osteolytic bone diseases

METHODS:
Cell culture: The human prostate cancer cell line (PC-3, DU-145), human breast cancer cell line (MDA-231, MDA-435), lung adenocarcinoma cell line (A549) and human osteosarcoma cell line (MG-63) were obtained from the American Type Culture Collection. The cells were maintained in RPMI-1640 medium which was supplemented with 20 mM HEPES and 10% heat-inactivated FCS, 2 mM-glutamine, penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37°C with 5% CO₂.

IL-8 mRNA levels were measured using RT-PCR analysis. MAPKs phosphorylation was examined by using Western blot method. siRNA was used to inhibit the expression of TGF-β1, BMP-2 and IGF-1. DAPA and CHIP assays were used to study in vitro and in vivo binding of c-Fos, c-Jun, p65 and p50 to the IL-8 promoter.

RESULTS:
We hypothesized that osteoblasts might be capable of regulating cancer cells IL-8 levels. Indeed, osteoblast conditioned medium (OBMC; 75%) significantly increased IL-8 mRNA expression in prostate (PC-3 and DU-145), breast (MDA-231 and MDA-435) and lung (A549) cancer cells (Fig. 1A). The quantitative RT-PCR data are shown in the Fig. 1A bottom panel. To study the effect of OBMC on IL-8 promoter in these cells, the luciferase construct that contains human IL-8 promoter (-162/+44) was used to measure the promoter activity. As demonstrated in Figure 1B, OBMC (75%) activated IL-8 promoter in all the cells examined. OBMC also induced IL-8 promoter activity concentration-dependently. In this study, we used OBMC (75%) to measure the IL-8 expression in human cancer cells. We found that OBMC also increased the transcriptional activity of promoter constructs with AP-1 and NF-κB sites (Fig. 1C). Because both the AP-1 and NF-κB sites are present in the IL-8 promoter, we conducted the same experiments in cells transfected with DNA constructs containing the IL-8 wild-type (wt; -162/+44), AP-1 site (-126/-120) mutant (IL-8ΔAP-1), and κB site (-82/-70) mutant (IL-8 Δ κB) to pinpoint the one responsible for OBMC-induced IL-8 expression. As exhibited in Figure 1D&E, the lack of either AP-1 or NF-κB site significantly suppressed OBMC-induced IL-8 promoter activity. In addition, mutation of both AP-1 and NF-κB sites (IL-8ΔAP-1 & κB) reduced the OBMC-mediated IL-8 promoter activity completely (Fig. 1E). These results indicated their indispensability in OBMC-promoted IL-8 transcription.

It is well established that osteoblasts can synthesize and secrete TGF-β1, BMP-2 and IGF-1, which play important roles in bone formation and bone cell differentiation. In an attempt to pinpoint the one in OBMC responsible for IL-8 expression in cancer cells, we transfected osteoblasts with control or TGF-β1, BMP-2 or IGF-1 siRNA, then their OBMC was collected and applied to cancer cells. As demonstrated in Figure 2A, only TGF-β1 siRNA could block OBMC-induced IL-8 release in PC-3 cells. OBMC from osteoblasts with TGF-β1 siRNA also exerted similar effect on both MDA-231 and A549 cells (Fig. 2B). (The quantitative RT-PCR data are shown in the Fig. 2A bottom panel). In addition, TGF-β1, BMP-2 and IGF-1 siRNA specifically inhibited the expression of mRNA levels of TGF-β1, BMP-2 and IGF-1 in human osteoblasts, respectively (Fig. 2C). The inhibition of TGF-β1 expression in response to TGF-β1 siRNA was also observed using quantitative RT-PCR analysis (Fig. 2D). These results suggested that TGF-β1 was crucial in OBMC-mediated IL-8 induction in cancer cells. To corroborate this hypothesis, we utilized TGF-β1 to stimulate cancer cells and found that TGF-β1 (10 ng/ml) could significantly increase both IL-8 mRNA levels and IL-8 promoter activity. Besides, TGF-β1 could effectively elevate the transcriptional activity of luciferase construct-bearing AP-1 and NF-κB sites in PC-3 cells. Mutations in either AP-1 or NF-κB sites will block the full activation of IL-8 promoter elicited by TGF-β1. These findings indicated that TGF-β1 could induce IL-8 expression by activation of AP-1 and NF-κB.

DISCUSSION:
In this study we provide evidence to demonstrate that the osteoblasts release growth factors, including TGF-β1, BMP-2 and IGF-1. TGF-β1 is the major contributor to the activation of ERK, p38 and JNK, leading to the activation of AP-1 and NF-κB on the IL-8 promoter, and initiation of IL-8 mRNA and protein release, thereby promoting osteoclastogenesis.