IL-8 upregulated by osteosarcoma-macrophage interactions promotes osteosarcoma growth and metastasis via the FAK pathway

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INTRODUCTION: Osteosarcoma is a common, highly malignant bone tumor affecting children. Although the efficacies of treatments for osteosarcoma have gradually improved with advances in chemotherapy and surgery, survival has not improved over the past 20 years. Tumor-associated macrophages (TAMs), a major component of the tumor microenvironment, were recently reported to be involved in tumor growth, metastasis, and chemoresistance. One of the mechanisms underlying the tumor-promoting effects of TAMs involves chemokines, such as IL-8, produced by TAMs (1). The significance of IL-8 in carcinoma has been reported, e.g., the association between elevated serum IL-8 and metastasis in breast cancer (2), and the correlations of high IL-8 expression with tumor progression and chemoresistance in colorectal cancer (3). On the other hand, the pathological significance and mechanism of IL-8 actions in osteosarcoma are not clear. This study aimed to elucidate the effects of IL-8, produced by the interactions between osteosarcoma and TAMs, on osteosarcoma.

METHODS: Cell culture and reagents: An osteosarcoma cell line, 143B (ATCC), a monocyte cell line, and THP-1 (ATCC) were maintained under standard culture conditions. THP-1 monocytes were treated using 100 ng/ml phorbol-12-myristate-13-acetate (Sigma Aldrich) for 48 h to induce their attachment and differentiation into macrophages. Osteosarcoma and macrophages were co-cultured for 24 hours, and supernatants (co-culture medium (CM)) were collected and used in assays.
The reagents used were recombinant IL-8 (R&D Systems), anti-IL-8 antibody (R&D Systems), phospho-FAK antibody (cell signaling), Focal Adhesion Kinase (FAK) antibody (cell signaling), and PND-1186 (Selleck Chemicals).
IL-8 expression in CM was quantified by ELISA (BioLegend ELISA MAX Deluxe Set Human IL-8). Western blot was performed with specific antibodies against phospho-FAK and FAK antibodies, and images were captured using a ChemiDoc touch (Bio-Rad).
Osteosarcoma proliferation and invasion with or without CM were examined by WST assay (CCK-8 Dojindo) and matrigel invasion assay. We also examined whether suppression of IL-8 in CM with anti-IL-8 antibody inhibited osteosarcoma proliferation and invasion. We also examined whether inhibition of the IL-8-FAK axis with PND-1186 (FAK inhibitor) suppressed osteosarcoma proliferation and invasion.
Statistical analysis was performed using Student's t-test with significance of $p<0.05$.

RESULTS: Although osteosarcoma and macrophages alone produced a small amount of IL-8, the production of IL-8 was markedly increased by co-culture (Fig. 1). Next, we examined the effects of TAM-derived IL-8 on osteosarcoma in terms of proliferation and invasion, and found that the addition of CM increased the proliferation and invasion of osteosarcoma. On the other hand, the addition of anti-IL-8 antibody to the CM inhibited the effect (Fig. 2). Finally, intracellular signaling was examined. The addition of recombinant IL-8 resulted in phosphorylation of FAK, and the addition of CM also resulted in phosphorylation of FAK, which was suppressed by anti-IL-8 antibody. Finally, the effects of FAK inhibitors on proliferation and invasion of osteosarcoma were examined, and the addition of PND-1186 inhibited the proliferation and invasion of osteosarcoma, which were elevated by CM (Fig. 3).

DISCUSSION: In this study, we found that the interaction between osteosarcoma and macrophages increases IL-8 production and enhances the proliferation and invasion of osteosarcoma via the FAK pathway. Reports of associations between osteosarcoma and macrophages have suggested the involvement of other chemokines, such as CCL18 produced by TAMs, which in turn promotes osteosarcoma growth and metastasis (4), while TAM-derived COX-2 and MMP9 promote osteosarcoma migration and invasion (5). This is the first report showing increased IL-8 and its effects on osteosarcoma-macrophage interactions. Furthermore, we found that proliferation and invasion induced by CM was inhibited by anti-IL-8 antibody. Research on therapies targeting IL-8 in some carcinomas is underway, and phase I clinical trials are being conducted for prostate and ovarian cancers to demonstrate the tumor suppressive effects of IL-8 neutralizing antibodies (6). IL-8 inhibition may also be an effective therapeutic strategy for osteosarcoma. FAK regulates tumor growth, invasion, and self-renewal of cancer stem cells (7), and the IL-8-FAK axis reportedly promotes the growth and invasion of gastric cancer (8). It was suggested that the IL-8-FAK axis is also involved in the growth and metastasis of osteosarcoma.

SIGNIFICANCE: IL-8, which is increased by the interactions between osteosarcoma and macrophages, promotes the proliferation and invasion of osteosarcoma via the FAK pathway, suggesting that IL-8 may be a target for osteosarcoma therapy.

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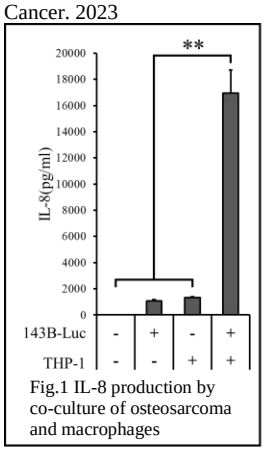

Fig.1 IL-8 production by co-culture of osteosarcoma and macrophages

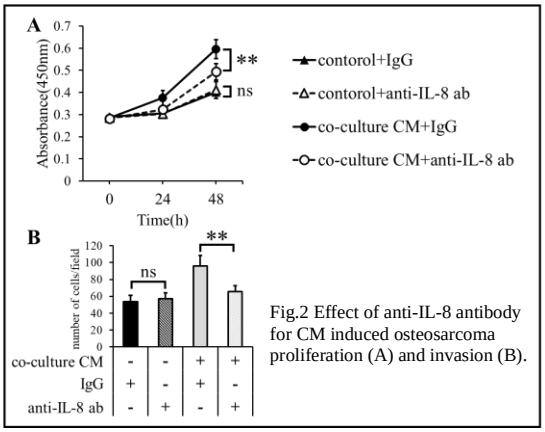

Fig.2 Effect of anti-IL-8 antibody for CM induced osteosarcoma proliferation (A) and invasion (B).

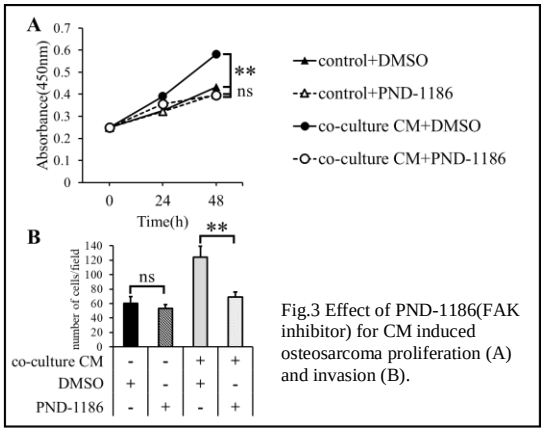

Fig.3 Effect of PND-1186(FAK inhibitor) for CM induced osteosarcoma proliferation (A) and invasion (B).