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Transforming growth factor-β synthesized by stromal cells and cancer cells participates in bone resorption induced by oral squamous cell carcinoma

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ABSTRACT

Transforming growth factor beta (TGF-β) plays a significant role in the regulation of the tumor microenvironment. To explore the role of TGF-β in oral cancer-induced bone destruction, we investigated the immunohistochemical localization of TGF-β and phosphorylated Smad2 (p-Smad2) in 12 surgical specimens of oral squamous cell carcinoma (OSCC). These studies revealed TGF-β and p-Smad2 expression in cancer cells in all tested cases. Several fibroblasts located between cancer nests and resorbing bone expressed TGF-β in 10 out of 12 cases and p-Smad2 in 11 out of 12 cases. Some osteoclasts also exhibited p ~ Smad2 expression. The OSCC cell line, HSC3, and the bone marrow-derived fibroblastic cell line, ST2, synthesized substantial levels of TGF-β. Culture media derived from HSC3 cells could stimulate *Tgf-β1* mRNA expression in ST2 cells. Recombinant TGF-β1 could stimulate osteoclast formation induced by receptor activator of nuclear factor kappa-B ligand (RANKL) in RAW264 cells. TGF-β1 could upregulate the expression of p-Smad2 in RAW264 cells, and this action was suppressed by the addition of a neutralizing antibody against TGF-β or by SB431542. Transplantation of HSC3 cells onto the calvarial region of athymic mice caused bone destruction, associated with the expression of TGF-β and p-Smad2 in both cancer cells and stromal cells. The bone destruction was substantially inhibited by the administration of SB431542. The present study demonstrated that TGF-β synthesized by both cancer cells and stromal cells participates in the OSCC-induced bone destruction.

1. Introduction

Bone destruction by oral cancer predicts poor prognosis and reduction of quality of life [1–4]; however, the molecular mechanism of bone destruction by oral cancer is poorly understood. Cancer-associated bone destruction is essentially mediated through osteoclastic bone resorption. We demonstrated that fibroblasts interspersed within the cancer and bone played a crucial role in the osteoclastic bone destruction by oral squamous cell carcinoma (OSCC) by producing receptor activator of nuclear factor-κB (RANK) ligand (RANKL) [5]. RANKL, an essential factor for osteoclastogenesis [6], is primarily synthesized by bone marrow stromal cells and osteoblast-lineage cells and it promotes hematopoietic progenitors of monocyte/macrophage lineage cells to mature osteoclasts [7]. We also reported that RANKL synthesized by both, cancer and stromal cells, was involved in the osteoclastic bone resorption in oral cancers [8].

Cancer cells express various factors that stimulate expression of RANKL. Although interleukin (IL)-8 produced by breast cancer directly enhances osteoclastogenesis independent of the RANKL pathway [9], various factors synthesized by cancer cells stimulate osteoclastogenesis via the RANK/RANKL pathway. We demonstrated that IL-6 and parathyroid hormone-related protein synthesized by OSCC induced RANKL expression in stromal cells [10]. We also reported that CXCL2 synthesized by oral cancer cells participates in bone resorption by stimulating RANKL expression and inhibiting osteoprotegerin, which is a decay receptor for RANKL [11].

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Transforming growth factor-β (TGF-β) plays a crucial role in the regulation of the tumor microenvironment, including cell proliferation, angiogenesis, and metastasis of tumors [12,13]. TGF-β binds to TGF-β receptor 2 (TGFBR2) and TGF-β receptor 1 (TGFBR1), and the TGFBR1/TGFBR2 complex in turn activates Smad2 and Smad3 by phosphorylation. Activated Smad2 and Smad3 create a Smad complex with Smad4, and translocate to the nucleus [12]. It has been reported that TGF-β derived from breast cancer [14] and prostate cancer [15] could stimulate osteoclastic bone resorption during bone metastases. Although the molecular mechanisms for TGF-β-mediated osteoclastogenesis is not clearly understood, Yasui et al. showed that TGF-β is essential for RANKL-induced osteoclastogenesis through a molecular interaction between Smad3 and Traf6 [16]. Moreover, Gingery et al. showed TGF-β could promote osteoclast survival through the TAK1/MEK/AKT/NFκB and Smad2/3 pathways [17].

Targeting the TGF-β signaling pathways in cancer-associated bone resorption may contribute to more effective treatments [18,19]. Although TGF-β is synthesized by tumor cells in various cancers, it has been reported that TGF-β produced by stromal cells is a critical factor that affects the prognosis of esophageal cancers. This led us to investigate the precise expression profile of TGF-β and its signaling molecules in OSCC; we demonstrate that TGF-β synthesized by both, cancer cells and fibroblasts, participates in osteoclastic bone resorption.

2. Materials and methods

2.1. Reagents

Recombinant human TGF-β1 and anti-TGF-β pan-specific rabbit polyclonal neutralizing antibody (AB-100-NA) were purchased from R&D Systems (Minneapolis, MN). SB431542, which is a specific and selective inhibitor of TGF-βR1, activin type-1 receptor (ACVR)-1B, and ACVR-1C, was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Anti-Smad2 and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) rabbit monoclonal antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA). Anti-phospho-Smad2 (Ser465/467) rabbit polyclonal antibody (AB3849) for immunohistochemical staining and anti-phospho-Smad2 (Ser465/467) rabbit monoclonal antibody (04–953) for western blot analysis were purchased from Merck Millipore (Billerica, MA). Anti-TGF-β1/2/3 rabbit polyclonal antibody (NBP1-72193) for immunohistochemical staining was purchased from Novus Biologicals (Littleton, CO).

2.2. Cell culture

The human OSCC cell line, HSC3, was purchased from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). The mouse bone marrow-derived stromal cell line, ST2, and the mouse macrophage cell line, RAW264, were purchased from the RIKEN Bioresource Center (Tsukuba, Japan). HSC3 cells were maintained in Dulbecco's modified Eagle medium: nutrient mixture F-12 (DMEM/F12). The ST2 cells were cultured in RPMI 1640 medium. The RAW264 cells were cultured in α-minimum essential medium (α-MEM). All media contained 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO), 100 U/ml penicillin G, and 100 mg/ml streptomycin. To harvest conditioned medium from HSC3 cells, the cells were grown to confluence in 100 mm dishes, and cultured for an additional 48 h in 4 ml serum-free α-MEM. The collected culture media were centrifuged at 1500 rpm for 5 min and filtered through a 0.22-μm filter unit and kept at −80 °C until use.

2.3. Enzyme-linked immunosorbent assay (ELISA)

The concentrations of TGF-β1 were quantified by using a human/mouse TGF-β1 ELISA Ready-SET-Go! (eBioscience, San Diego, CA) according to the manufacturer's instructions.

2.4. Reverse transcriptase-polymerase chain reaction analyses

For reverse transcriptase-polymerase chain reaction analyses, total RNA was extracted using NucleoSpin (Macherey–Nagel, Duren, Germany). RNA samples were reverse transcribed to cDNA using oligo (dT) primers (Roche Applied Science, Basel, Switzerland), M-MuLV reverse transcriptase (Thermo Fisher Scientific, Waltham, MA), and dNTP mix (Promega, Madison, WI). The mRNA expression was examined using the Light Cycler System and FastStart Essential DNA Green Master (Roche Applied Science). The relative expression level of each mRNA was normalized using the 18S rRNA expression level. The primer sequences used for mouse *Tgf-β1* were as follows: 5′-CTCCCGTGGCTTCTAGTGC-3′ (forward) and 5′-GCCTTAGTTTGGACAGGATCTG-3′ (reverse).

2.5. Osteoclast formation

RAW264 cells were cultured in 96-well plates for 24 h, the medium was subsequently changed to α-MEM containing 20 μmol/L MEK1 inhibitor (PD98059; Cell Signaling Technology) and RANKL (100 ng/ml). The cells were treated with various concentrations of TGF-β1 and SB431542. After fixing in 10% formalin, osteoclast formation was identified by tartrate-resistant phosphatase (TRAP) staining; briefly, cells were incubated with 0.1 mol/L sodium acetate buffer (pH 5.0) containing fast red violet LB salt (Sigma-Aldrich), AS-MX phosphatase (Sigma-Aldrich), and 50 mmol/L sodium tartrate (Sigma-Aldrich) as previously described [10]. TRAP-positive cells containing more than 3 nuclei were counted as osteoclasts.

2.6. Immunohistochemical staining

Surgical specimens obtained from 12 cases of surgical excisions for mandibular gingival squamous cell carcinoma were retrieved from the archives of the Department of Oral Pathology, Tokyo Medical and Dental University. They were fixed in 10% neutral buffered formalin, then decalcified in 20% ethylenediaminetetraacetic acid (EDTA) and embedded in paraffin. For immunohistochemical staining, the sections were pretreated in 0.01 mol/L citric acid for 30 min in the microwave. Endogenous peroxidase activity was quenched by incubation with 3% hydrogen peroxide solution for 30 min; the sections were then incubated with primary antibodies against TGF-β and phosphorylated Smad-2 (p-Smad2) overnight at 4 °C. The sections were washed with phosphate buffered saline and treated with peroxidase-conjugated secondary antibody (Envision + Dual Link System-HRP; Dako, Glostrup, Denmark) for 1 h. Diaminobenzidine was used as the chromogen. This experimental procedure was reviewed and approved by the Ethics Review Committee of the Tokyo Medical and Dental University.

2.7. Western blot analysiss

Proteins were extracted from RAW264 cells with RIPA buffer (20 mM Tris–HCl (pH 7.5), 1 mM EDTA, 50 mM β-glycerophosphate, 150 mM NaCl, 1 mM Na_3VO_4, 1% NP-40, 25 mM NaF) containing a protease and phosphatase inhibitors cocktail (Sigma-Aldrich). The harvested proteins were resolved using sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto

polyvinylidenefluoride membranes (ATTO, Tokyo, Japan). The proteins were identified by western blotting using primary antibodies against p-Smad2, Smad2, and GAPDH, followed by secondary antibodies conjugated with horseradish peroxidase. Immunoreactive bands were detected using enhanced chemiluminescence reagents (GE healthcare; Pittsburgh, PA).

2.8. Xenograft experiments of human oral cancer cells in athymic mice

HSC3 cells (5×10^5 cells/mouse) were injected onto the periosteal region of the parietal bone in 6 athymic mice as previously described [10]; 3 of the athymic mice received subcutaneous injections of SB431542 (10 mg/kg) at tumor-injected sites, whereas the remaining 3 received the dimethyl sulfoxide (DMSO) as vehicle immediately after the transplantation of HSC3 cells. Two weeks after the transplantation, the samples were dissected and fixed with 4% paraformaldehyde. A micro-computed tomography (micro-CT) imaging system (SMX-100CT; Shimadzu, Kyoto, Japan) was used for micro-CT analysis at a resolution of 8 μm. The area of bone destruction was calculated from these data using the ImageJ version 1.46 software package (NIH, Bethesda, MD). The experimental procedures were reviewed and approved by the Animal Care and Use Committees at Tokyo Medical and Dental University.

2.9. Statistical analyses

The results are presented as Mean ± SD. Statistical analyses were performed using the Student's *t*-test; *p*-values <0.05 were considered to be statistically significant.

3. Results

3.1. Expression of TGF-βand p-Smad2 in the bone invading area in human OSCC

Immunohistochemical staining for TGF-β in 12 human OSCC cases revealed that cancer cells were positive for TGF-β in all the tested cases; in addition, the fibroblasts interspersed between the cancer cells and bone were positive for TGF-β in 10 cases (Fig. 1A). Some osteoclasts were positive for TGF-β (Fig. 1A). Immunohistochemical staining for p-Smad2 that is involved in the TGF-β signaling pathway revealed the nuclear localization of p-Smad2 in almost all cancer cells, and in some fibroblasts in 11 cases (Fig. 1B). Some osteoclasts showed also nuclear localization of p-Smad2 (Fig. 1B). These results suggest that both cancer cells and fibroblasts express TGF-β, indicating that TGF-β activates osteoclasts, fibroblasts, and cancer cells through the Smad signaling pathway.

3.2. Stimulation of TGF-β1 expression in stromal cells by OSCC cells

Next, we measured the levels of TGF-β1 protein secreted into culture media by the HSC3 human OSCC cell line, and the mouse bone marrow-derived cell line, ST2, using ELISA kits, which recognize both human and mouse TGF-β1. Whereas both cell lines synthesized substantial levels of TGF-β1, ST2 cells synthesized higher levels of TGF-β1 than HSC3 cells (Fig. 1C). Next, we investigated whether culture media derived from HSC3 cells could stimulate *Tgf-β1* expression in ST2; we found that HSC3 cell-derived culture media significantly increased the *Tgf-β1* mRNA expression in ST2 cells (Fig. 1D). These results suggest that OSCC cells stimulate the expression of TGF-β1 in stromal cells.

3.3. Regulation of osteoclastogenesis by TGF-β1

As it has been reported that TGF-β1 is involved in osteoclastogenesis [20,21], we investigated the roles of TGF-β1 and Smad2 in osteoclastogenesis by using RAW264 cells. These cells produced no TRAP-positive multinuclear cells (MNCs) when the cells were cultured in the absence of RANKL (Fig. 2A and B). RAW264 cells cultured with RANKL (100 ng/ml) generated TRAP-positive MNCs, and this stimulatory effect was diminished by addition of SB431542 (Fig. 2A and B). The simultaneous treatment with RANKL (100 ng/ml) and TGF-β1 increased the number of TRAP-positive MNCs in a dose-dependent manner of TGF-β1 (Fig. 2A and B).

Western blot analysis could not reveal detectable levels of p-Smad2 in RAW264 cells cultured with RANKL (100 ng/ml) in the absence of TGF-β1; however, RAW264 cells treated with RANKL and TGF-β1 (0.5 ng/ml) expressed substantial levels of p-Smad2 (Fig. 2C). Addition of anti-TGF-β1 antibody 30 min before protein harvest decreased the expression levels of p-Smad2; treatment with a high concentration of the antibody (100 mg/ml) greatly inhibited the expression of p-Smad2 (Fig. 2C). Addition of SB431542 also inhibited the expression of p-Smad2 produced by addition of TGF-β1 (0.5 ng/ml) (Fig. 2D). These results confirmed that TGF-β1 signaling involving Smad2 is implicated in osteoclastogenesis.

3.4. In vivo assessment of TGF-β mediated bone destruction in the OSCC xenograft model

We investigated the role of TGF-β signaling in OSCC-induced bone destruction by using a xenograft transplantation model of the HSC3 human OSCC cells into the calvarial region of athymic mice. Two weeks after the transplantation, areas of bone destruction were confirmed by micro-CT analyses (Fig. 3A). Immunohistochemical staining showed TGF-β expression in human-derived cancer cells, mouse-derived fibroblasts and osteoclasts adjacent to the bone resorption region (Fig. 3E). The nuclei of almost all cancer cells, and several fibroblasts and osteoclasts were positive for p-Smad2 (Fig. 3G). These results emphasize the similarities in TGF-β signaling between human OSCC cases (Fig. 1A and B) and the xenograft transplantation model of bone destruction.

We used this xenograft model to investigate the effects of SB431542 on bone destruction by HSC3 cells; micro-CT analyses revealed that the subcutaneous injection of SB431542 (10 mg/kg) in tumor-injection sites dramatically reduced the area of bone resorption compared with that in the vehicle-injected group (Fig. 3A and B). The injection of SB431542 also reduced the tumor volume at the transplanted sites (Fig. 3I). In these cases, fewer cancer cells were found around the bone resorption area (Fig. 3D–F). These data suggest that the SB431542-induced growth arrest of the transplanted tumor resulted in the inhibition of tumor-associated bone destruction.

3.5. Distribution of p-Smad2 in cancer cells, stromal cells, and osteoclasts

Immunohistochemical analysis revealed p-Smad2 expression in the nuclei of some osteoclasts localized in the OSCC-associated bone invasion front (Fig. 4B and D), though some osteoclasts exhibited no signal for p-Smad2 (Fig. 4A and C). Approximately 70% of osteoclasts in the xenograft model contained p-Smad2-positive nuclei, and ~30% of osteoclasts showed p-Smad2-positive nuclei in human OSCC cases (Fig. 4E). We hypothesized that if cancer cell-derived TGF-β could directly stimulate osteoclastogenesis upon OSCC-associated bone destruction, the proximity of cancer nests with the bone resorption surface would facilitate TGF-β signaling. To test this hypothesis, we measured the distance between the

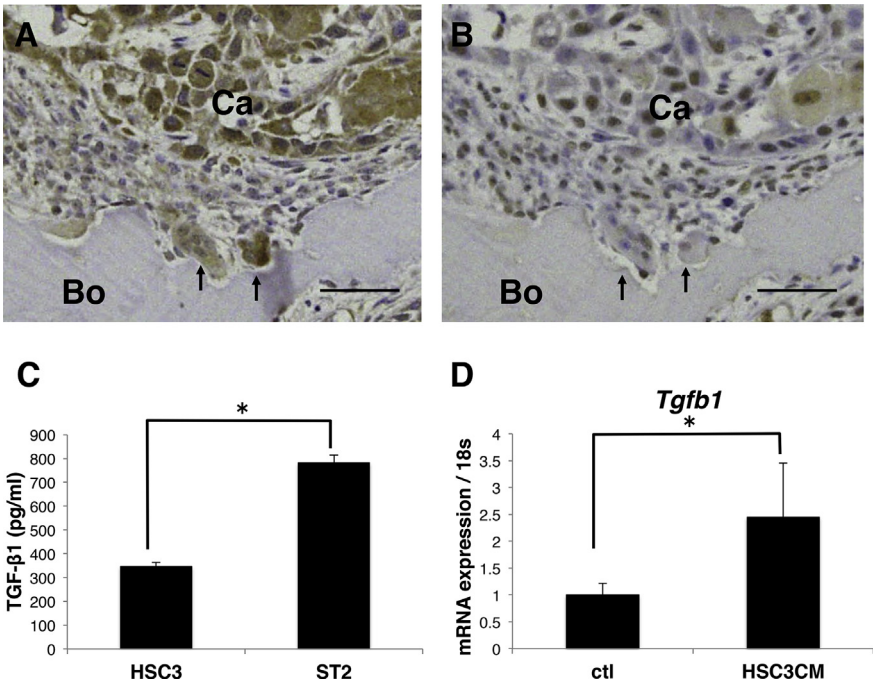

Fig. 1. Expression of TGF-β and p-Smad2 in OSCC cells and stromal fibroblastic cells. Expression of TGF-β (A) and p-Smad2 (B) in human OSCC cases. Arrows: osteoclasts. Bar: 50 μm. Protein level of TGF-β1 in the culture media of HSC3 and ST2 cells, as measured using an enzyme-linked immunosorbent assay (C). $^{*}p < 0.05$ indicates significant difference compared to the culture medium of HSC3. (D) The expression levels of *Tgf-β1* mRNA from ST2 cells with or without treatment of HSC3 cell-derived culture medium, as measured using reverse-transcriptase polymerase chain reaction. $^{*}p < 0.05$ indicates significant difference compared to control (Ctl).

cancer nests and osteoclast-covered bone surfaces. In both, the mouse xenograft model and human surgical specimens, no significant differences were observed between osteoclasts with (Fig. 4B and D) or without (Fig. 4A and B) p-Smad2-positive nuclei, in the distance of the cancer nests from bone resorption surface (Fig. 4F). Of note, fibroblastic cells with p-Smad2 nuclei were often interspersed between cancer nests and osteoclasts with p-Smad2-positive nuclei; however, such fibroblasts were less frequently observed in cancer nests and osteoclasts with p-Smad2-negative nuclei (Fig. 4A–D).

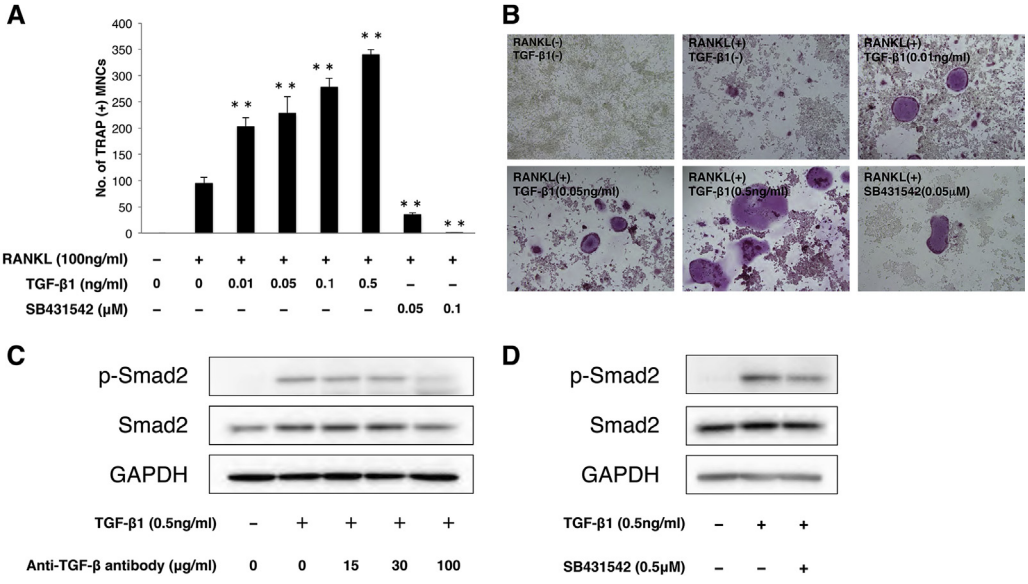

Fig. 2. The effects of recombinant human TGF-β1 on osteoclastogenesis. Raw264 cells were cultured for 24 h, and the culture media were changed to α-MEM containing of RANKL (100 ng/ml) and various concentration of TGF-β1 for 3 days. (A) Number of TRAP-positive multinuclear cells. $^{*}p < 0.01$ indicates significant difference compared to the cells treated with only RANKL. (B) Representative image of the cells with TRAP immunostaining. (C) Raw264 cells were cultured for 24 h, and the medium was changed to α-MEM containing RANKL (100 ng/ml) and TGF-β1 (0.5 ng/ml) and treated with various concentrations of anti-TGF-β antibody. Expression of p-Smad2, total Smad2, and GAPDH was examined with western blot analyses. (D) Raw264 cells were cultured for 24 h, and culture media were changed to α-MEM containing RANKL (100 ng/ml), TGF-β1 (0.5 ng/ml), and SB431542 (0.5 μM). Expression of p-Smad2, total Smad2, and GAPDH were examined with western blot analyses.

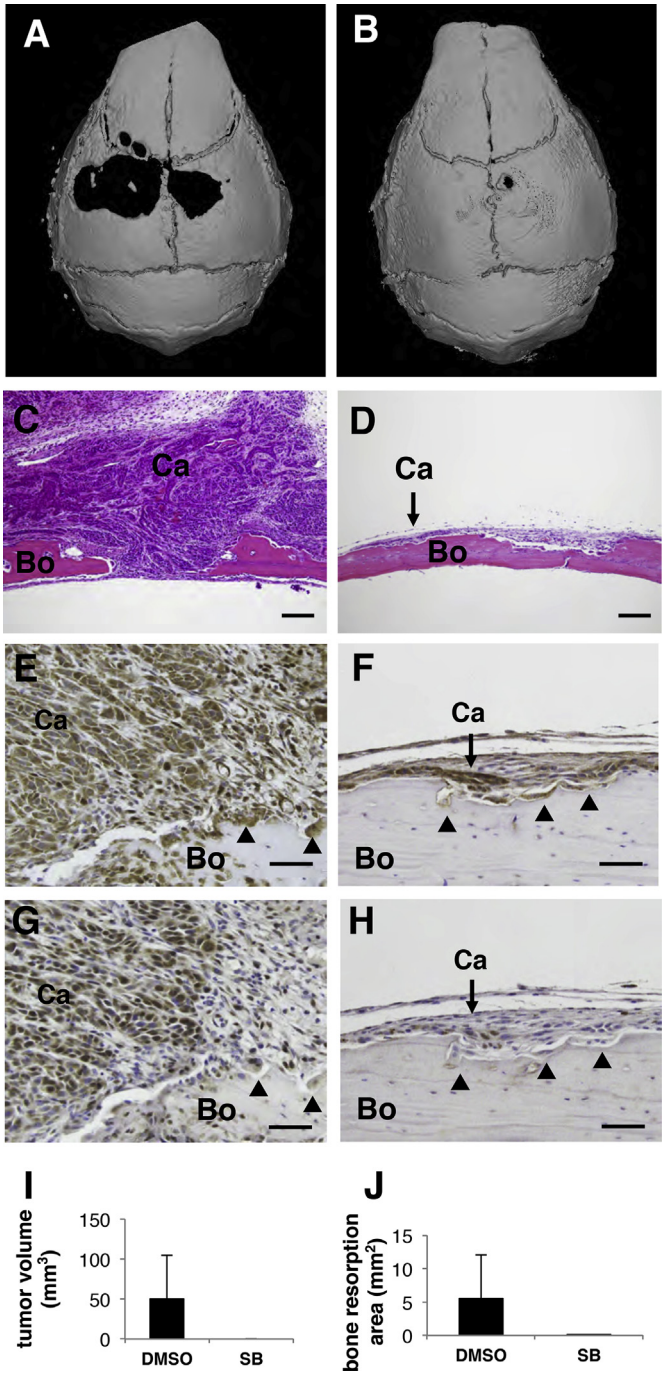

Fig. 3. Bone destruction in the oral squamous cell carcinoma xenograft model. HSC3 cells were injected onto the periosteal region of the parietal bone in athymic mice, and SB431542 (10 mg/kg) or DMSO was injected subcutaneously into tumor-injection sites. Typical micro-CT images of mice with HSC3 cell xenografts with (A) DMSO and (B) SB431542 treatment. Typical histology (hematoxylin and eosin staining) of the xenograft treated with (C) DMSO or (D) SB431542. Immunohistochemical expression of TGF-β in xenografts treated with (E) DMSO or (F) SB431542, and phosphorylated Smad2 in xenografts treated with (G) DMSO or (H) SB431542. Ca, cancer nest; Bo, bone; arrowheads, osteoclasts. Scale bars: 100 μm (C, D); 25 μm (E–H). (I) Tumor volume calculated by a formula $L \times W^2 \times \pi/6$ (L, tumor length; W, tumor width). (J) Bone resorption area in micro-CT images measured using ImageJ software.

4. Discussion

To explore the role of TGF-β in OSCC-associated bone destruction, we conducted an immunohistochemical analysis to determine the localization of TGF-β and its signaling molecule, p-Smad2, in the bone resorption area in human histological specimens. This study revealed TGF-β and p-Smad2 expression in all cases of OSCC; moreover, TGF-β (10 out of 12 cases) and p-Smad2 (11 out of 12 cases) expression was observed in some fibroblasts interspersed between cancer nests and the bone surface. In the present study, similar expression profiles for TGF-β and p-Smad2 were observed in the cancer cells and fibroblasts interspersed within cancer nests and the bone surface in the mouse xenograft model. These results indicate that both, the cancer cells and the fibroblasts at the cancer–bone interface synthesized TGF-β.

Immunohistochemical analyses revealed that ~30% of osteoclasts in the human surgical specimens, and ~70% of osteoclasts in the xenograft model exhibited phosphorylation of Smad2 in their nuclei, indicating that TGF-β signaling was activated in such osteoclasts; there are three possible sources of TGF-β that stimulated the phosphorylation of Smad2 in osteoclasts. First, TGF-β synthesized by cancer cells might directly stimulate the phosphorylation of Smad2 in osteoclasts; however, the layer of fibroblasts located between the cancer nests and bone surface may interfere with the localization of TGF-β to bone surface. As the distance between the cancer and osteoclast-containing bone surface might be a critical factor in the localization of TGF-β to osteoclasts, we compared the relative distances between cancer nests and the bone surface in two groups of osteoclasts, those with or without p-Smad2 in their nuclei. This study revealed that the distances between the two groups were almost identical, in both the human surgical specimens and mouse xenograft model, suggesting that the cancer cell-synthesized TGF-β was an unfavorable source for osteoclast-stimulating TGF-β. The second possible source includes TGF-β derived from fibroblasts located between the cancer and bone surface, which might directly stimulate the osteoclasts. We showed that the HSC3-derived culture media could upregulate the expression of *Tgf-β1* mRNA in ST2 stromal cells. This raises the possibility that cancer cells may stimulate TGF-β1 synthesis in the fibroblasts located between the cancer and bone surface, which may, in turn, stimulate the osteoclasts. Further experiments are necessary to prove this hypothesis. The third possible source may include bone matrix-derived TGF-β, which is released during osteoclastic bone resorption. Although this is a favorable source of TGF-β, further studies are necessary, as we could not observe any phosphorylation of Smad2 in osteoclasts located in Howship's lacunae, which represents active bone resorption (Fig. 4A and C).

Several reports have demonstrated that various cancer cells including breast cancer [14] and prostate cancer [15] cells produce TGF-β, and that it is involved in osteoclastic bone destruction during bone metastases. We observed that OSCC cells produced TGF-β and participated in osteoclastic bone resorption. Of note, we demonstrated that fibroblasts located between the cancer and bone could also synthesize TGF-β. Another important observation was that the culture media from HSC3 cells could stimulate the expression of *Tgf-β* mRNA in ST2 cells, indicating that OSCC cells regulated the expression of TGF-β in stromal cells. Although the cancer cell-derived TGF-β is regarded as an important factor in cancer-associated bone resorption, our results indicate that more extensive investigation is necessary to understand the role of TGF-β in OSCC-associated bone destruction. Furthermore, Ozawa et al. recently reported the importance of TGF-β1 expression in stromal cells in the prognosis of esophageal squamous cell carcinomas [22].

We investigated the effects of TGF-β on bone destruction, using a xenograft model. Our studies revealed that SB431542 injections could dramatically reduce bone destruction; although this is closely related to the inactivation of TGF-β signaling during bone resorption, the retardation of tumor growth might be an important factor in reducing cancer-associated bone resorption.

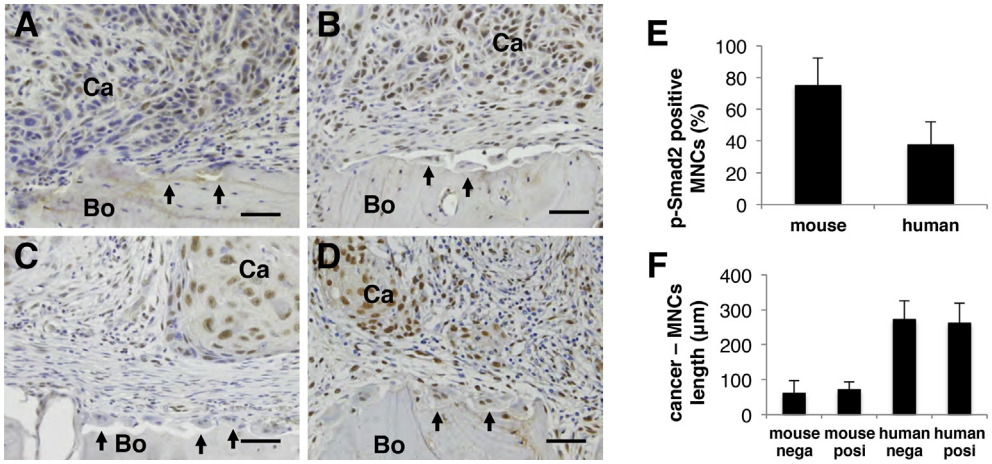

Fig. 4. Distribution of p-Smad2-positive cells in OSCC-associated bone resorption areas. Distribution of p-Smad2 in mouse xenograft model (A, B) and human OSCC cases (C, D). Some osteoclasts localizing in the OSCC-associated bone resorption front are positive for p-Smad2 (B, D), others are negative for p-Smad2 (A, C). Ca, cancer nest; Bo, bone; arrows, osteoclasts. Scale bar: 25 μm. Osteoclasts positive for p-Smad2 localizing bone invasion front were counted (E). Percentage of p-Smad2-positive osteoclasts in mouse xenograft model and human OSCC cases. The distance between cancer nests and osteoclast-covered bone surfaces (F). Mouse nega: p-Smad2-negative osteoclasts in mouse xenograft model, mouse posi: p-Smad2-positive osteoclasts in mouse xenograft model, human nega: p-Smad2-negative osteoclasts in human OSCC cases, human posi: p-Smad2-positive osteoclasts in human OSCC cases.

In summary, we show that both cancer cells and stromal cells produce TGF-β in the OSCC-induced bone destruction, and propose the role of TGF-β produced by stromal fibroblasts in such bone destruction.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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