CXC chemokine-7 inhibits growth and migration of oral tongue squamous cell carcinoma cells, mediated by the epithelial-mesenchymal transition signaling pathway

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Abstract. Oral tongue squamous cell carcinoma (OTSCC) is the most common oral malignancy with different histopathological symptoms and etiology of tumorigenesis. Migration and invasion is the most important characteristics of OTSCC, and limits tumor therapy in clinics. The epithelial-to-mesenchymal transition (EMT) signaling pathway is an important process in the progress of tumor cell migration and invasion. Previous studies have indicated that C-X-C chemokine receptor-7 (CXCR-7) promotes the progression and metastasis of tumor cells, presenting a potential target molecule for cancer therapy. The present study investigated the inhibitory effects of C-X-C chemokine-7 (CXC-7) on human OTSCC cells both *in vitro* and *in vivo*. The results demonstrated that the Tca8113 human OTSCC cell line expressed higher levels of CXC-7 mRNA compared with the hNOE human normal oral epithelial cell line. MTT assays indicated that CXC-7 suppressed Tca8113 cell growth, and the cytotoxicity of CXC-7 was indicated as the cell survival of the negative control group was significantly decreased compared with the blank control and hNOE cells. Migration and invasion assays revealed that CXC-7 inhibited Tca8113 cell local expansion and distant metastasis. In addition, the results demonstrated that the extracellular signal-regulated kinase (ERK)/protein kinase B (AKT) signaling pathway was inhibited after CXC-7 treatment in Tca8113 cells. N-cadherin, E-Cadherin, Snail and Slug expression levels in the ERK/AKT signaling pathway were inhibited in Tca8113 cells after treatment with CXC-7. It was demonstrated that important extracellular matrix proteins involved in cell migration, including Slug, collagen type I and Vimentin, were significantly downregulated by CXC-7 treatment. In conclusion, CXC-7 inhibited growth and migration in OTSCC cells, mediated by the EMT signaling pathway. This suggests that CXC-7 serves an inhibitory role in OTSCC migration, implicating CXCR-7 as a promising biomarker for chemokine receptor-based drug development.

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Introduction

Oral cancer is currently the 11th most common cancer in the world, with the highest incidence and mortality rates in developing countries (1). Oral tongue squamous cell carcinoma (OTSCC) is the most common type of oral tumor, accounting for 94% of all oral malignancies (2,3). OTSCC is characterized by local infiltrating growth in the oral cavity, expanding invasion into the lymph node and uterine metastasis (4,5). Clinically, the significance of primary tumor thickness identified direction and partial glossectomy as primary treatments. These are usually only suitable for OTSCC patients of clinical stage I and II (6). However, OTSCC has been traditionally believed to be associated with easy recurrence, metastasis and poor prognosis due to rapid migration and invasion (6). Local migration to perienchymas, and long distance metastasis to organs, are the main reasons leading to mortality and poor survival rate among patients with OTSCC (7). Previous studies have suggested that patients diagnosed with early-stage OTSCC (T1-2, N0) have improved 5-year survival rates of between 75 and 89% (8,9). In addition, a previous study indicated that prognosis of patients with OTSCC can be improved by early detection and appropriate treatment (10). Therefore, efficient diagnoses and treatments for patients with OTSCC are required to improve the survival rate for patients.

A previous study indicated that CXC chemokine receptor-7 (CXCR-7) promotes progression and metastasis of tumor cells, representing a potential target molecule for cancer therapy (11). CXCR-7 has been characterized as a novel receptor for CXC-7 (12). A previous study demonstrated that

the CXCR-7 gene encodes members of the G protein-coupled receptor family, which was identified as an orphan receptor for vasoactive intestinal peptides (13). CXCR-7 is now classified as a novel receptor for the CXC motif chemokine 12/stromal cell-derived factor 1-alpha, which may serve a role in the progression, metastasis and angiogenesis of otorhinolaryngologic tumors (14). In addition, CXCR-7 has been considered to serve an essential role in the pathogenesis of tumor angiogenesis and metastasis (11). Furthermore, Yates *et al* (11) demonstrated that CXCR-7 forms a functional complex associated with epidermal growth factor (EGF)-receptor, extracellular signal-regulated kinase (ERK), phosphoinositide 3-kinase/protein kinase B (AKT) and proto-oncogene tyrosine-protein kinase Src, which subsequently induced the phosphorylation and upregulation of B-cell lymphoma 2 (Bcl-2) and cyclin-D1. These studies indicated that suppression of CXCR-7 expression by CXC-7 reversed these effects and resulted in suppressed growth, inhibition of migration and induction of apoptosis. Therefore, it was hypothesized that CXC-7 inhibits OTSCC cell migration and invasion via regulation of the ERK/AKT signaling pathway.

The epithelial-to-mesenchymal transition (EMT) signaling pathway is a key process in tumor cell progression and metastasis stimulated by EGF/Ras and transforming growth factor-β (TGF-β) signaling pathways, which lead to a complex biochemical reaction processes in tumor cells (15,16). A previous study indicated that suppressing the progress of EMT may be clinically helpful for cancer therapy (17). EMT was reported as a fundamental process in cancer cell progression, during which epithelial cells disassemble, fibroblastic-mesenchymal phenotype acquire, basement membranes digest, and tosurrounding tissues transmigrate (18). EMT is involved in pathological situations of tumor cells such as the acquisition of an invasive, metastatic phenotype in tumors of epithelial origin (19). A previous study examined the molecular mechanisms of the EMT signaling pathway by Ras-induce signaling, which regulates EMT process in human tumor cells (20). In process of carcinogenesis, EMT promotes epithelial cells to dislodge epithelial polarity, digest the basement membrane and invade adjacent tissues, and even enter the bloodstream and colonize new palingenetic base (21). These references suggested that EMT signaling pathway was a potential tumor-therapy process during progression of neoplasm, and indicated that inhibition of EMT signaling pathway may suppress migration, invasion and metastasis through loss of inhibitory signaling in EMT signaling pathway.

The present study investigated CXCR-7 expression and the function of CXC-7 in the growth and migration of OTSCC cells. The CXCR-7 signaling pathway in relation to migration, invasion and protein expression in OTSCC cells was examined. It was demonstrated that inhibition of CXCR-7 expression suppressed OTSCC cell growth, migration and invasion both *in vitro* and *in vivo*. These results suggested that CXCR-7 is a potential target for OTSCC therapy.

Materials and methods

Cell lines. The Tca8113 and SCC25 oral squamous carcinoma cell lines were purchased from the American Type Culture Collection (Manassas, MA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA). The hNOE human normal oral epithelial cell line was obtained from the School of Stomatology of Shandong University (Jinan, China). All cells were cultured in a humidified incubator with 2 mM penicillin/streptomycin (37˚C, 5% CO_2).

MTT assay. Tca8113 and SCC25 cells ($1x10^6$) were treated with CXC-7 (2 mg/ml) or PBS in 6-well plates for 48 h in triplicate at 37˚C. After culturing in DMEM, 20 μl MTT (5 mg/ml) was added to the cells. Then the cells were further incubated for 4 h at 37˚C. The medium was removed and 100 μl dimethylsulfoxide was added into the wells to solubilize the crystals. The results were measured using an ELISA reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) reader at a wavelength of 450 nm.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated from Tca8113 and SCC25 cells using an RNA Easy Mini Extract kit (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) at 4˚C. The expression levels of CXCR-7 in SCC25 and Tca8113 cells were calculated by RT-qPCR using iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc.) with β-actin expression as an endogenous control. Primer sequences were as follows: Forward, 5'-CAC TGAAGGAGCCTGCAGC-3' and reverse, 5'-CATCTTCTT CCTCGCATGCA-3' for CXCR-7; forward, 5'-GTGGGCGCC CAGGCACCA-3' and reverse 5'-CTCCTTAATGTCACG CACGATTT-3' for β-actin. Cycling conditions were as follows: 45 cycles of denaturation at 95˚C for 2 min, annealing at 66˚C for 30 sec with touchdown to 56˚C for 30 sec and extension at 72˚C for 10 min. All procedures were performed according to the manufacturer's protocol. All primers were synthesized by Invitrogen; Thermo Fisher Scientific, Inc. Relative CXCR-7 expression level was determined by the $2^{-\Delta\Delta Cq}$ method (22). The final results were presented as the n-fold manner compared to β-actin.

Cell migration and invasion assays. Tca8113 cells were incubated with CXC-7 in serum-free DMEM media. For the migration assay, CXC-7-treated cells were suspended at a density of $5x10^5$ in serum-free DMEM and then transferred to the tops of BD BioCoat Matrigel Migration Chambers (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's protocol. For the invasion assay, Tca8113 and SCC25 cells were treated with CXC-7 or PBS for 24 h using a control insert (BD Biosciences) instead of a Matrigel Migration Chamber. The tumor cells invasion and migration were observed in at least three random fields under a photomicroscope (Olympus Corporation, Tokyo, Japan).

Flow cytometric analysis of apoptosis. Tca8113 and SCC25 cells were cultured in DMEM supplemented with 10% fetal bovine serum for 48 h. Tca8113 and SCC25 cells were treated with CXC-7, CXCR-7 or PBS as a control for 48 h. All cells were subsequently treated with cisplatin for 12 h. The apoptosis of suspended cells were analyzed by flow cytometry (BD Biosciences) using FACSDiva software version 10.0.7 (Flowjo LLC, BD Biosciences, Franklin Lakes, NJ, USA) as described previously (23).

Animal study. Specific pathogen-free male BALB/c mice (age, 6-8 weeks; n=40, body weight, 32-35 g) were purchased from Slack Experimental Animals Co., Ltd. (Shanghai, China). All animals had free access to food and water and were housed in a temperature-controlled facility at 23±1˚C and relative humidity of 50±5% with a 12 h light/dark cycle. Tca8113 cells at a density of $1x10^7$ suspended in 100 μl PBS were subcutaneously injected into BALB/c mice. When tumor diameters reached 5-6 mm on day 7 after tumor inoculation, Tca8113-bearing mice were randomly divided into 2 groups (n=20/group), which received CXC-7 (80 mg/kg) or the same volume of PBS. The total treatments were 6 times at 2-day intervals. The mice were observed for 25 days and were sacrificed using sodium pentobarbital anesthesia (50 mg/kg, Invitrogen; Thermo Fisher Scientific, Inc.) when the tumor diameter reached 12 mm. Tumor diameters were recorded every 2 days and tumor volume was calculated by using the formula: 0.52 x smallest diameter^2 x largest diameter, as described previously (24). Animal experiments were approved by the Animal Care and Welcome Committee of Shandong University (Jinan, China).

Western blotting. Tca8113 cells were cultured to 90% monolayer cell formation. The cells were lysed with radioimmunoprecipitation assay lysate buffer containing protease-inhibitor (Applied Biosystems; Thermo Fisher Scientific, Inc.) and were centrifuged at 6,500 x g at 4˚C for 10 min. The supernatant of mixture was analyzed using western blotting for ERK, TGF-β, Vimentin (Vim), collagen type I (CT-I) and Slug protein expression, as described previously (25). For western blotting, the following rabbit anti-human primary antibodies were used: CXC-7 (1:1,000; catalog no. ab89256; Abcam, Cambridge, UK), EGF (1:500; catalog no. ab9695; Abcam) and TGFβ (1:500; catalog no. ab92486; Abcam) and (1:500; catalog no. ab8226; Abcam). The horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (Bio-Rad Laboratories, Inc.) was used at a 1:5,000 dilution and detected using an Enhanced Chemiluminescence substrate solution (GE Healthcare, Chicago, IL, USA) according to the manufacturer's protocol.

Histological analysis. Tumors from experimental mice were fixed in 10% formaldehyde and were then embedded in paraffin. Tumor samples were sectioned (4 μm) using a microtome and subjected to deparrafinization in a series of alcohols and antigen retrieval. Tumor sections were incubated with the following primary antibodies: CXC-7 (1:1,000; catalog no. ab89256; Abcam), EGF (1:500; catalog no. ab9695; Abcam) and TGFβ (1:500; catalog no. ab92486; Abcam) and (1:500; catalog no. ab8226; Abcam) for 12 h at 4˚C. Horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (Bio-Rad Laboratories, Inc.) was used at a 1:5,000 dilution for 1 h at 37˚C. Tumor sections were captured with an inverted fluorescence microscope (Olympus Corporation) at x400 magnification and Leica Application Suite X software (version 3.0.2).

Immunofluorescence. Tca8113 and SCC25 cells were cultured until 90% monolayer cells were formatted. The cells subsequently incubated with a mouse anti-human CXCR-7 primary antibody (1:1,000; catalog no. ab89256; Abcam). Following this, a goat anti-mouse CXCR-7 secondary antibody (1:1,000; catalog no. ab89251; Abcam) was applied for immobilized cells. Finally, the cells were washed with PBS to completely remove the residual antibody. The Ventana Benchmark automated staining system was used for observation of CXCR-7 expression by confocal microscopy.

Statistical analysis. All data in this study are presented as the mean ± standard error of triplicate experiments. Data was analyzed using SPSS Statistics version 19.0 software (IBM Corp., Armonk, NY, USA), Graphpad Prism version 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Data was analyzed by one-way analysis of variance followed by Fisher's exact test, or a Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of CXCR-7 in cultured OTSCC lines. To elucidate the role of CXCR-7 in the migration and progress of Tca8113, SCC25 and hNOE cells were cultured and harvested for RT-qPCR analysis. CXCR-7 mRNA expression levels were upregulated in Tca8113 and SCC25 cells compared with hNOE cells (Fig. 1A). Western blotting revealed that the protein expression levels were higher in Tca8113 and SCC25 cells compared with hNOE cells (Fig. 1B). Immunofluorescence staining demonstrated that CXCR-7 expression was lower in hNOE cells compared with the other two cell lines (Fig. 1C). Furthermore, EGF and TGF-β expression levels were increased in Tca8113 and SCC25 cells compared with hNOE cells (Fig. 1D). These data indicated that CXCR-7 expression was upregulated in Tca8113 and SCC25 cells compared with hNOE cells, suggesting that these factors are upregulated in OTSCC.

In vitro effects of CXC-7 on OTSCC cells. The effects of CXC-7 on growth induction of OTSCC cells were examined *in vitro*. The results in Fig. 2A showed that CXC-7 significantly decreased CXCR-7 expression in cultured Tca8113 and SCC25 cells. Tca8113 and SCC25 cell growth was inhibited by CXC-7 via suppression of CXCR-7 expression (Fig. 2B). The results in Fig. 2C demonstrated the inhibitory effect on cell growth in a dose-dependent manner in Tca8113 and SCC25 cells. The inhibition arrived at maximum degree for tumor cell growth when the concentration reached 800 mg/ml. Therefore, 800 mg/ml CXCR-7 was chosen for further analysis. As shown in Fig. 2D, the apoptosis rate was significantly promoted in Tca8113 and SCC25 cells after CXCR-7 treatment for 48 h. Collectively, these results suggested that inhibition of CXCR-7 expression by CXC-7 significantly suppressed growth and promoted apoptosis in Tca8113 and SCC25 cell lines.

CXC-7 inhibits migration and invasion via regulating EMT pathways in OTSCC cells. To investigate whether EMT may be associated with OTSCC cell migration and invasion, the effect of CXC-7 on EMT-associated proteins and aggressive behavior in Tca8113 cells was investigated *in vitro*. Migration and invasion abilities of Tca8113 cells after treatment with CXC-7 were analyzed. As shown in Fig. 3A and 3B, CXC-7

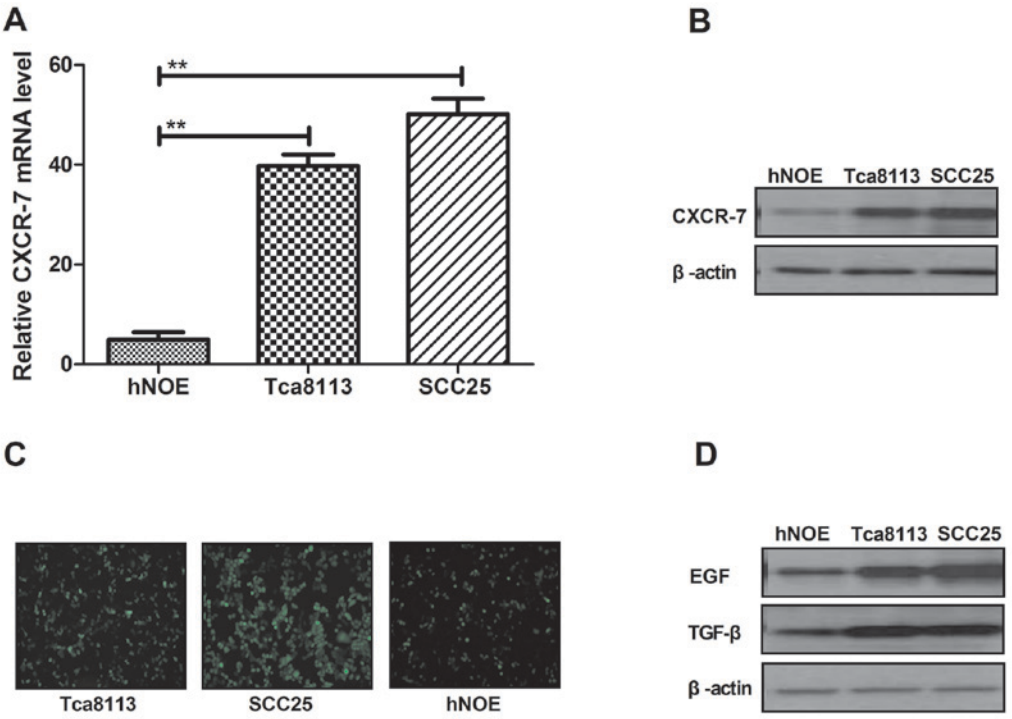

Figure 1. Upregulation of CXCR-7 expression in OTSCC cell lines. Tca8113, SCC25 and hNOE cells were treated with CXC-7. (A) CXCR-7 mRNA and (B) CXCR-7 protein expression levels. (C) Fluorescence microscope images of the expression of CXCR-7 in Tca8113, SCC25 and hNOE cells (x400 magnification). (D) mRNA expression levels of EGF and TGF-β. Data are presented as the mean ± standard error. **P<0.01. TGF-β, transforming growth factor-β; OTSCC, oral tongue squamous cell carcinoma; CXCR-7, C-X-C chemokine receptor-7; CXC-7, C-X-C chemokine-7; EGF, epidermal growth factor.

suppressed migration and invasion of Tca8113 cells. Next, migration-associated protein expression in Tca8113 cells prior and post treatment of CXC-7 was investigated. As demonstrated in in Fig. 3C, CXC-7 downregulated key functional proteins including TGF-β, ERK and AKT in Tca8113 cells compared to control group. In addition, migration-associated proteins, Vim, CT-I and Slug were analyzed after CXC-7 treatment. Results showed that CXC-7 downregulated the expression levels of these migration-promoting proteins in OTSCC cells compared to control group (Fig. 3D). These data suggested that CXC-7 inhibited migration and invasion via the TGF-β-mediated EMT signaling pathway.

Inhibition of CXCR-7 expression exhibits beneficial effects on OTSCC-bearing mice. The *in vivo* effects of CXC-7 was analyzed in Tca8113-bearing mice *in vivo*. Tca8113 ($1x10^6$) cells were injected subcutaneously into BALB/c nude mice. The mice received CXC-7 or the same volume of PBS as a control when tumor diameter reached 5-6 mm. CXC-7 significantly suppressed tumor growth compared with PBS-treated mice in a 25 day observation (Fig. 4A). After 25 days CXC-7 treatment, CXCR-7 expression in tumors was detected in each experimental mouse. The results in Fig. 4B demonstrated that apoptosis was increased after CXC-7 treatment compared to the PBS-treated group. In addition, TGF-β, ERK and AKT expression was detected in tumors by histological staining in the different treatment groups. The results in Fig. 4C demonstrated that CXC-7 downregulated TGF-β, ERK and AKT expression compared with the PBS group. Furthermore, CXC-7 treatment significantly downregulated E-cadherin, Snail, N-cadherin and Vim compared with the control group, as determined by western blotting (Fig. 4D). These results suggested that CXC-7 may be inhibit tumor growth, angiogenesis and apoptosis in OTSCC.

Discussion

The current treatments for the majority of patients with OTSCC are partial glossectomy performed by surgery, with generally promising outcomes (24,25). However, the rapid growth of OTSCC cells, local migration toward to adjacent tissue, and fast metastasis to cervical, lymphatic and other organs shortens the 5-year survival period (26). Therefore, cell migration and invasion is the most important characteristic of OTSCC, and limits drug tumor therapy in clinics, which has contributed to adjacent migration and long distance metastasis to other organs or tissues (27,28). A previous study has suggested that early diagnosis is beneficial for patients with OTSCC in eradication of tumor cells (29). The results of the present study indicated that inhibition of OTSCC adjacent migration and long distance metastasis may be efficient to improve the efficacy and clinical outcomes of the drug.

Previous studies have reported that patients with OTSCC overexpress levels of CXCR-7, leading to a poorer prognosis. Overexpression of CXCR-7 significantly increased cell growth, migration and invasion, while inhibition of CXCR-7 expression markedly suppresses the invasive properties of OTSCC cell lines (2,30,31). Therefore, the mechanism of CXCR-7-associated signaling pathways on the invasive properties of tumor cells requires further study. Previous studies

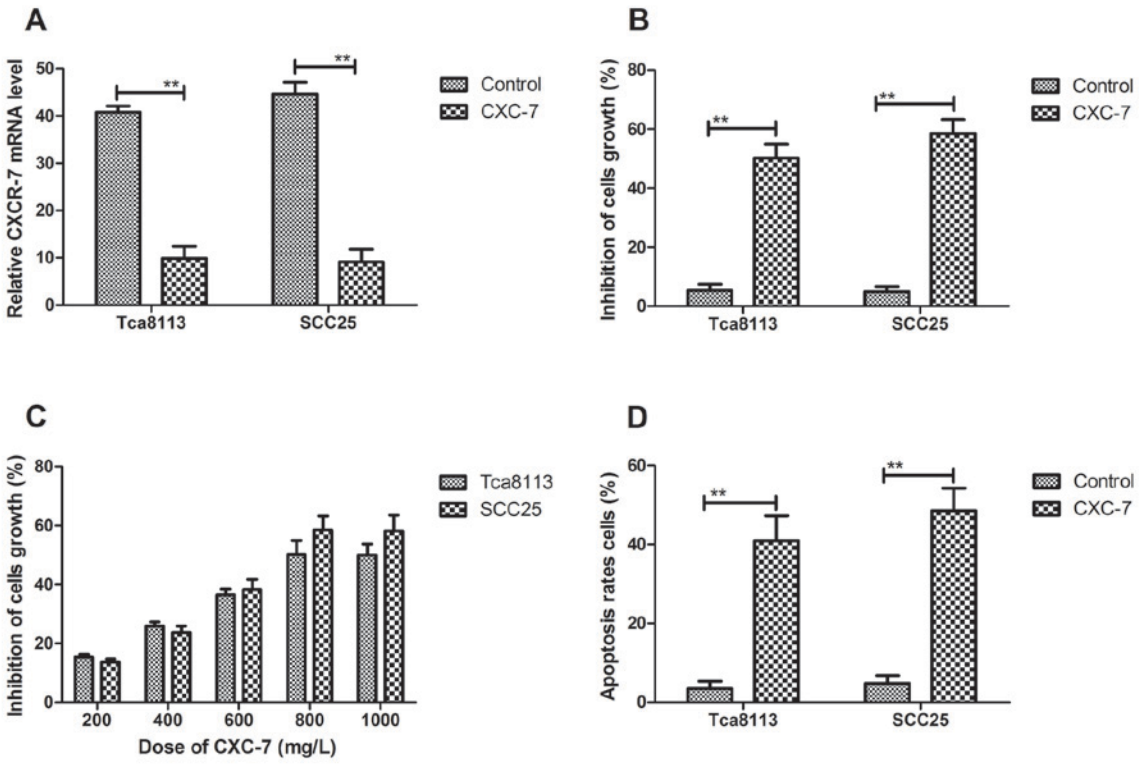

Figure 2. Inhibitory effects of CXC-7 on OTSCC cell lines. (A) Relative CXCR-7 mRNA expression changes in Tca8113 and SCC25 cells after CXC-7 treatment for 48 h. (B) Inhibition of Tca8113 and SCC25 cells growth in treatment of CXC-7 for 48 h. (C) CXC-7 treatment suppressed Tca8113 and SCC25 cell growth in a dose-dependent manner. (D) CXC-7 promoted apoptosis of Tca8113 and SCC25 cells. Data are presented as the mean ± standard error. **P<0.01. OTSCC, oral tongue squamous cell carcinoma; CXC-7, C-X-C chemokine-7.

have indicated that CXCR-7 associates with EGF-receptor to form a complex that recruits downstream signaling molecules and activates phosphorylation of ERK/AKT, as determined by co-localization and co-immunoprecipitation experiments (32,33). In addition, CXCR-7 enhances apoptotic resistance and strengthens cyclin D1 expression levels through modulating AKT and ERK expression, and regulation of B-cell lymphoma 2 expression levels (34). The present study suggested that CXCR-7 expression regulates apoptosis, proliferation, migration, invasion, survival and motility in tumor cells via signaling events such as EGF-receptor activation and the AKT/ERK signaling pathway.

OTSCC is characterized by rapid growth in tumors, local migration toward to adjacent tissue, and rapid metastasis to organs (35,36). In addition, the incidence of OTSCC in young patients is increasing in the world (37). Therefore, more efficient anti-tumor agents for the treatment of OTSCC are urgently required. The present study aimed to investigate the expression of CXCR-7 in OTSCC cells and tissue samples, and to determine the role of CXC-7 in the development of OTSCC. It was demonstrated that CXCR-7 served an essential role in the progression, metastasis and tumor angiogenesis of OTSCC via regulation of the EMT signaling pathway. Immunofluorescence analysis demonstrated that CXCR-7 and migration-associated protein expression levels were significantly upregulated in OTSCC cells. Therefore, CXCR-7 expression may associate with the clinical features, degree of tumor spread, poor overall survival of OTSCC patients and the prognosis of patients receiving oncotherapy. The results of the present study demonstrated that treatment with CXC-7 significantly inhibited TGF-β, ERK and AKT expression and phosphorylation levels both in OTSCC tumors and cell lines, which augmented the anchorage-independent growth, migration and invasive abilities of OTSCC cells. In addition, the results indicated that CXC-7 downregulated Vim, CT-I and Slug expression levels and provoked the ability of OTSCC carcinoma to suppress neovascularization of tumor angiogenesis that enhanced OTSCC carcinoma cell apoptosis, induced by the chemotherapeutic agent cisplatin. These findings provided novel insights into the potential roles of CXCR-7 deregulation in promoting tumor cell metastasis, angiogenesis and progression, which may be a molecular target in the treatment of OTSCC.

The clinical value of identifying OTSCC target molecules not only helps determine the degree of aggressive tumor behaviors, but also provides target therapeutic agents for patients with OTSCC, which allows for the early diagnosis and efficient treatment of patients in earlier stages (29,38,39). The present study demonstrated that CXCR-7 regulated progression, metastasis and tumor angiogenesis of OTSCC cells via regulation of the EMT signaling pathway. The EMT signaling pathway has been indicated as a developmental process with a function in tumor progression and metastasis, and has been extensively studied previously (20,40,41). TGF-β and Ras are essential for the EMT signaling pathway; however, their expression levels often depend on tumor cell type, which hinders comprehensive analysis of

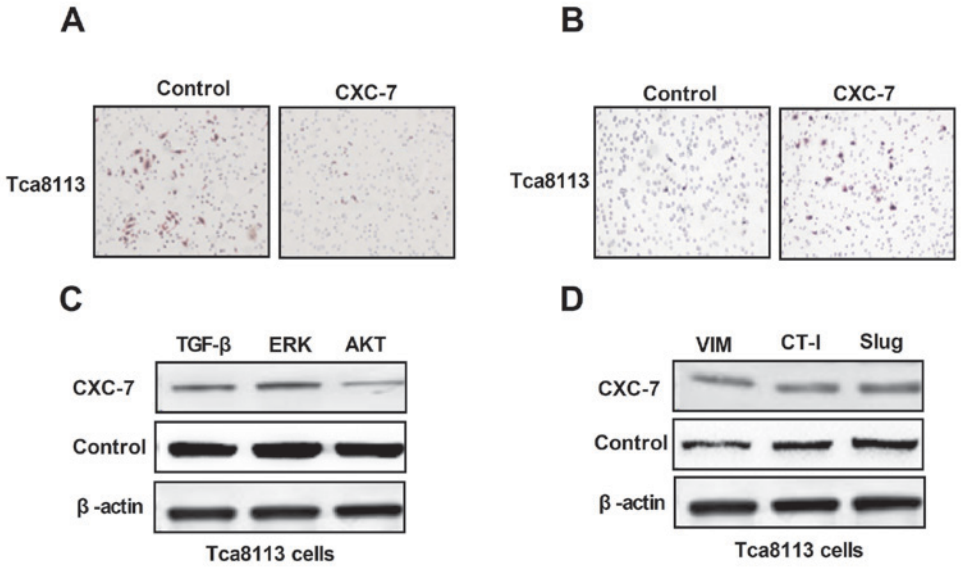

Figure 3. CXC-7 inhibits metastasis of Tca8113 cells by regulating epithelial-to-mesenchymal transition pathways. (A) Migration of Tca8113 cells after 48 h CXC-7 trreatment. (B) Inhibition of invasive effects of CXC-7 on Tca8113 cells after incubation 48 h. Representative western blot images of (C) TGF-β, ERK and AKT expression in Tca8113 cells after treatment with CXC-7 for 48 h, and (D) migration-associated proteins, Vim, CT-I and Slug in Tca8113 cells after 48 h treatment with CXC-7. TGF-β, transforming growth factor-β; CXC-7, C-X-C chemokine-7; Vim, vimentin; CT-I, collagen type I; ERK, extracellular signal-regulated kinase.

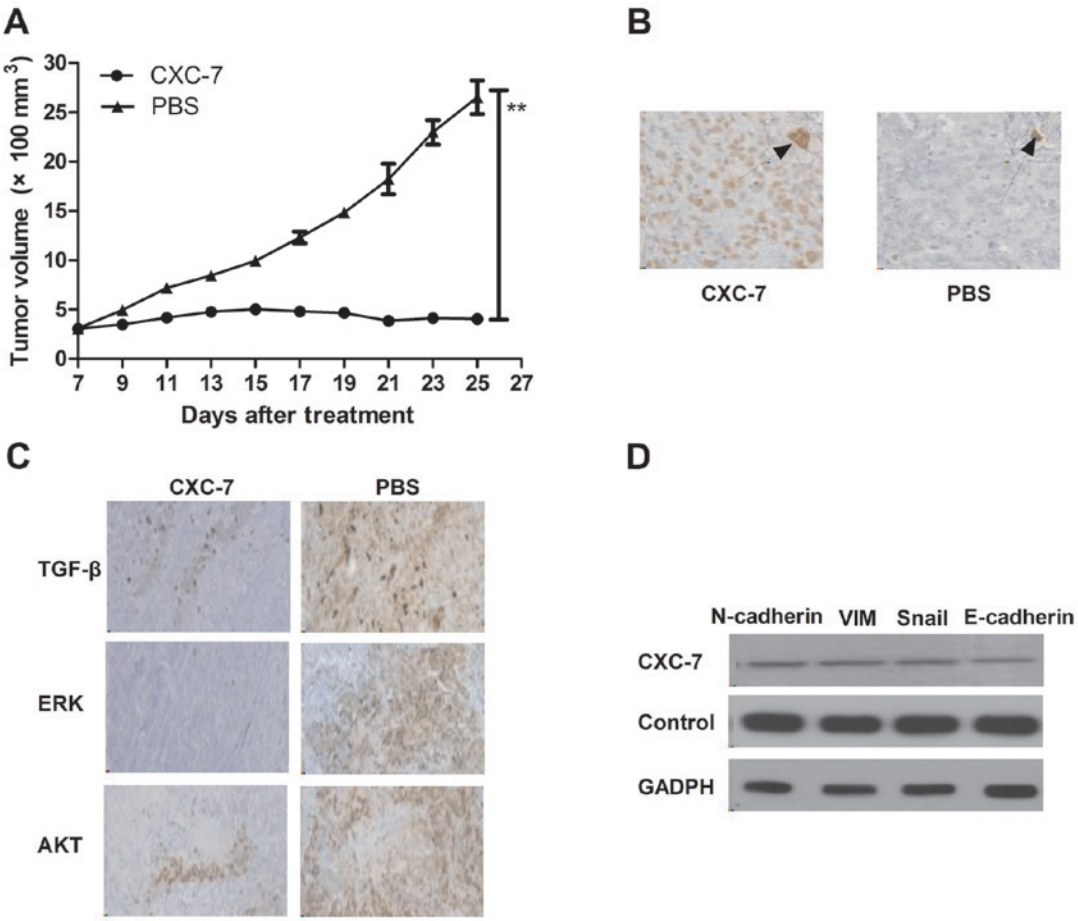

Figure 4. CXC-7 suppressed Tca8113-bearing mice. (A) Tumor growth was analyzed after CXC-7 or PBS treatment in a 25-day short term observation. Data are presented as the mean ± standard error. **P<0.01. (B) CXCR-7 expression was analyzed in tumors from experimental mice on day 25. Arrows indicate CXCR-7 expression. Analysis of protein expression levels of (C) TGF-β, ERK and AKT and (D) E-cadherin, N-cadherin, Snail and Vim. TGF-β, transforming growth factor-β; CXC-7, C-X-C chemokine-7; ERK, extracellular signal-regulated kinase.

key underlying mechanisms in EMT (20). The present study suggested that CXC-7 significantly inhibits TGF-β-induced EMT signaling in OTSCC cell lines. In addition, levels of the epithelial marker E-cadherin and the mesenchymal marker fibronectin were downregulated in OTSCC cells. Furthermore, Vim, CT-I and Slug expression levels were also downregulated after CXC-7 treatment. Therefore, the identification of CXCR-7 can define the stage of OTSCC, and achieve more efficient target therapy of CXC-7 for patients with OTSCC.

In conclusion, the present study identified that the CXCR-7 gene is upregulated in OTSCC cells and tumors, and the ability of CXC-7 to repress CXCR-7 may inhibit OTSCC cell growth, migration and invasion through TGF-β-induced EMT signaling. Additionally, CXC-7 significantly inhibited OTSCC cell growth, migration and invasion, and the expression of epithelial markers was retained and the blood vessel density was suppressed in OTSCC. These results suggested that CXC-7 regulates CXCR-7 gene expression via TGF-β-induced EMT signaling, leading to decreased apoptosis resistance, tumor progression and metastasis. These results provide novel therapeutic agents for patients with OTSCC.

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