European Review for Medical and Pharmacological Sciences 2018; 22: 1035-1043

Semaphorin-7A contributes to growth, migration and invasion of oral tongue squamous cell carcinoma through TGF-β-mediated EMT signaling pathway

T.-J. LIU[1,2], J.-L. GUO[3], H.-K. WANG[4], X. XU[1]

[1]Department of Implantology, Shandong Provincial Key Laboratory of Oral Biomedicine, School of Stomatology, Shandong University, Jinan, Shandong, P.R. China
[2]Department of Stomatology, The Jinan Central Hospital Affiliated to Shandong University, Jinan, Shandong, P.R. China
[3]Department of Ophthalmology, The Eighth People's Hospital of Jinan, Jinan, Shandong, P.R. China
[4]Department of Oral and Maxillofacial Surgery, Weihai Municipal Hospital, Weihai, Shandong, P.R. China

Abstract. – OBJECTIVE: Oral tongue squamous cell carcinoma (OTSCC) is the most frequently encountered malignant epithelial tumors. Semaphorin-7A is a membrane-associated/secreted protein that plays an essential role in the migration and progression of human malignancies. We aimed to investigate the mechanisms of Semaphorin-7A in the growth and migration of OTSCC.

MATERIALS AND METHODS: The expressions of Semaphorin-7A in cells were tested by RT-PCR, Western blot, and Immunofluorescence, separately. The activities of OTSCC cells (HSC-3 and Tca8113) were analyzed by MTT, following treatment with Semaphorin-7A or PBS. The migration, invasion, and apoptosis of cells were also determined. The protein expressions of epithelial mesenchymal transition (EMT) pathway were analyzed by Western blot, after treated with Semaphorin-7A *in vitro* and *in vivo*. Finally, the mouse model of OTSCC was treated with antibody target for Semaphorin-7A (AntiSema-7A), Semaphorin-7A or PBS, then the tumor size was determined, and histopathological examination and western blot was applied for further confirmation.

RESULTS: In OTSCC cells, Semaphorin-7A was highly expressed, and Semaphorin-7A promoted growth in multiple metastatic OTSCC cell lines. Further study indicated that Semaphorin-7A resulted in up-regulation of Snail, N-cadherin and Vimentin expression, and downregulating of E-cadherin. In addition, The Ets2-repressor factor (ERF) expression was down-regulated, and transforming growth factor (TGF-β)-induced EMT was promoted in OTSCC cells. Then, the proteins of collagen types I (CT-I) and fibronectin (FIB) were also up-regulated after Semaphorin-7A treatment. Furthermore, our results indicated that inhibition of Semaphorin-7A by antibody target for Semaphorin-7A (AntiSema-7A) suppressed OTSCC growth and increased survival in a mouse model of OTSCC. Histopathological examination confirmed the inhibitory effects *in vivo*.

CONCLUSIONS: Semaphorin-7A promoted growth and migration of OTSCC by regulating TGF-β-induced EMT signaling pathway in OTSCC cells, which provided a new interconnection between the Semaphorin-7A and TGF-β-induced EMT signaling pathway.

Key Words:
Oral tongue squamous cell carcinoma, Semaphorin-7A, EMT, TGF-β.

Abbreviations

CT-I = collagen types I; DMEM = Dulbecco's Modified Eagle Medium; DMSO = Dimethyl Sulphoxide; EGF = epidermal growth factor; EMT = epithelial mesenchymal transition; ELISA = enzyme-linked immunosorbent assay; ERF = Ets2-repressor factor; Erk = extracellular-response kinase; FIB = fibronectin; HE = Hematoxylin and eosin; MAPK = mitogen-activated protein kinase; MTT = (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide); NOEC = normal oral epithelial cells; OD = optical density; OTSCC = Oral tongue squamous cell carcinoma; PCR = polymerase chain reaction; PBS = phosphate buffered saline; RT-PCR = reverse transcriptase-polymerase chain reaction SPF = Specific Pathogen Free; TGF = transforming growth factor.

Corresponding Author: Xin Xu, MD; e-mail: xinxumedicine@163.com

Introduction

Oral tongue squamous cell carcinoma (OTSCC) is one of the most common types of oral tumors[1]. OTSCC was reported frequently occurring more common in male than in female, and it was characterized with local infiltrating growth in oral cavity and expanding invasion into lymph node metastasis[2,3]. In recent years, the incidence of OTSCC in the profile of young patients with squamous cell carcinoma of tongue ranged from 0.4% to 3.9%, and presented an increasing trend[4]. Surgery was the primary treatment used in the patients with OTSCC, of whom median follow-up period was 36 months and 24.1% patients relapsed carcinoma[5,6]. OTSCC has been traditionally believed to be associated with easy recurrence, metastasis and a poor prognosis due to rapid migration and invasion[7]. Metastasis is the main reason leading to mortality of OTSCC, and positive surgical margins are associated with poor prognosis among patients with OTSCC[8]. Previous study[9] indicated that prognosis of patients with OTSCC could be improved by early detection and appropriate medical regimens. Therefore, efficient diagnosis and treatments for patients with OTSCC are required to improve a five-year survival.

Semaphorin-7A was a member of Semaphorins, which induced termination of protein synthesis and activation of ribosomal RNA gene transcription[10]. Semaphorin-7A is a membrane-anchored protein expressed in multiple cell types, which promotes attachment of cells. Semaphorin-7A as a novel substrate for epithelial mesenchymal transition (EMT) can be used to monitor transforming growth factor (TGF-β) activity in mammary epithelial cells. And inhibition of Semaphorin-7A activation could suppress angiogenesis[11,12]. Previous studies[13,14] have indicated that Semaphorin-7A played a vital role in neovascularization during the course of ocular angiogenesis analysis. Semaphorin-7A has been identified as a potent driver of ductal carcinoma in situ progression, and overexpression of Semaphorin-7A occurred in a large percentage of breast cancer cells and promoted tumor cells invasion and lymph angiogenesis via activation of β1-integrin receptor[11]. In addition, Scott et al[15] has indicated that Semaphorin-7A involved in axon guidance and stimulated melanocyte adhesion through opposing actions of β1-integrin and Plexin C1 receptors. Therefore, Semaphorin-7A activity may affect mitogen-activated protein kinase and inactivate cofilin in tumor cells migration and metastasis.

In this work, we investigated the expression and mechanisms of Semaphorin-7A in the growth and migration of OTSCC. We examined the signaling pathway of Semaphorin-7A and migration and invasion-related protein expression in OTSCC cells.

Materials and Methods

Cell Lines

Oral squamous carcinoma cell lines, HSC-3 and Tca8113, were purchased from American Type Culture Collection and cultured in Dulbecco's Modified Eagle Medium (DMEM) (HyClone, South Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and 2 mM penicillin/streptomycin (Gibco, Rockville, MD, USA) in a humidified incubator (37°C, 5% CO_2) (Forma, USA). Cells treated with Semaphorin-7A (Bio-Techne China Co.Ltd., Shanghai, China) or PBS were incubated in 96-well plates for 72 hours in triplicate for each condition.

MTT Assay

After culture, 20 μL of MTT (5 mg/mL, Sigma-Aldrich, St Louis, MO, USA) in PBS solution was added to each well, the plate was further incubated for 4 hours. Most of the medium was removed and 100 μL of dimethyl sulfoxide (DMSO) was added into the wells to solubilize the crystals. The OD was measured by a Bio-Rad (Hercules, CA, USA) (ELISA) reader at wavelength of 450 nm.

Real-time Quantitative PCR (RT-qPCR)

Total RNA was extracted from HSC-3 and Tca8113 cells by using RNA easy Mini Kit (QIAGEN, Gaithersburg, MD, USA). Expressions of Semaphorin-7A in HSC-3 and Tca8113 cells were measured using RT-qPCR kit (QIAGEN, Gaithersburg, MD, USA) with β-actin expression as an endogenous control according to the manufacturer's instructions. All the primers were synthesized by Invitrogen (Invitrogen, Shanghai, China). Relative Semaphorin-7A expression level was calculated by $2^{-\Delta\Delta Ct}$. The results were presented as the n-fold way compared to β-actin.

Cells Invasion and Migration Assays

For invasion assay, Semaphorin-7A-treated cells were suspended as a density of 1×10^6 in serum-free DMEM and then subjected to the tops

of BD Bio Coat Matrigel Invasion Chambers (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. For migration assay, HSC-3 and Tca8113 cells were treated with Semaphorin-7A or PBS for 24 hours by using a control insert (BD Biosciences, Franklin Lakes, NJ, USA) instead of a Matrigel Invasion Chamber. The tumor cells invasion and migration were counted in at least three randomly stain-field microscope (Nikon, Chiyoda, Tokyo, Japan) every membrane.

Flow Cytometry Analysis

The cells were subsequently treated with cisplatin for 12 hours. The apoptosis of suspended cells were analyzed by flow cytometry described in the previous study[16].

Animal Study

Six-eight weeks old male BALB/c (SPF) nude mice were purchased from Slack Experimental Animals co., LTD (Slack, Shanghai, China). A total volume of 200Ml HSC-3 cells (1×10^6) were injected into subcutaneous back site in BALB/c nude mice. Tumor-bearing mice were randomly divided into 3 groups (n=20). HSC-3-bearing mice were treated with Semaphorin-7A (50 mg), AntiSema-7A (50 mg) or PBS when tumor diameters reached 5 to 6 millimeters at the seventh day after tumor inoculation. The mice were treated every 2 days, and the total treatments were 10 times. Tumor diameters were recorded every two days and tumor volumes were calculated by using the formula: 0.52 × smallest diameter^2 × largest diameter.

Western Blot and Histological Analysis

HSC-3 and Tca8113 cells were cultured and lysed. The expressions of Ras, extracellular-response kinase (Erk), TGF-β, Vimentin (Vim), collagen types I (CT-I) and Slug were determined according to previous study[17]. Tumors from experimental mice were fixed by using 10% formaldehyde followed with embed in paraffin. Tumor samples were fabricated to tumor sections and antigen retrieval was also performed in tumor sections. Tumor sections were incubated with primary antibodies. Then, appropriate secondary antibodies were applied for specimens and specimens were visualized. A Ventana Benchmark automated staining system was used for observation of the purpose protein Ras, Erk, TGF-β, Vim, CT-I and Slug expression. Also, tumor vessel morphology was identified by HE staining.

Immunofluorescence

HSC-3 cells were cultured until the formation of about 95% monolayer cells. The cells subsequently incubated with primary antibodies. Then, appropriate secondary antibodies target for primary antibodies were applied for immobilized cells. Rabbit polyclonal antibody S17S against Ets2-repressor factor(ERF), actin (Sigma-Aldrich, St. Louis, MO, USA), fibronectin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit polyclonal antibodies against p42/p44 mitogen activated protein kinase (MAPK) (Cell Signaling Technology, Beverly, MA, USA), horseradish peroxidase (HRP) anti-rabbit and anti-mouse (Jackson ImmunoResearch Laboratories, West Grove, PA, USA), mouse monoclonal antibody against E-cadherin (BD Transduction Laboratories, Lexington, KY, USA) and S47 conjugated anti-rabbit and anti-mouse (Pierce, Rockford, IL, USA) goat antibodies were used in this experiment. A Ventana Benchmark automated staining system was used for observation of E-Cadherin/DNA and Fibronectin/DNA by confocal microscopy.

Statistical Analysis

SPSS version 19.0 statistical software (IBM Corp., Armonk, NY, USA) was used. All the data are presented as mean ± standard deviation, *t*-test was used for data analysis between the two groups, One-way ANOVA followed by LSD post-test was used. $p < 0.05$, $p < 0.01$ were considered statistically significant.

Results

Expression of Semaphorin-7A in Cultured OTSCC Lines

OTSCC cells (HSC-3 and Tca8113) and normal oral epithelial cells (NOEC) were cultured and harvested for RT-qPCR analysis. The results in Figure 1A demonstrated that mRNA level of Semaphorin-7A was up-regulated in HSC-3 and Tca8113 cells compared with NOEC. We found that Semaphorin-7A protein level was higher in HSC-3 and Tca8113 cells compared with NOEC ($p < 0.01$, Figure 1B). In addition, in order to determine whether Semaphorin-7A is expressed in OTSCC, the cultured HSC-3 and Tca8113 cells were immune-stained for Semaphorin-7A. As shown in Figure 1C, we found that Semaphorin-7A expression level was lower in NOEC. Furthermore, an increase in Semaphorin-7A expression was confirmed by immunofluorescence

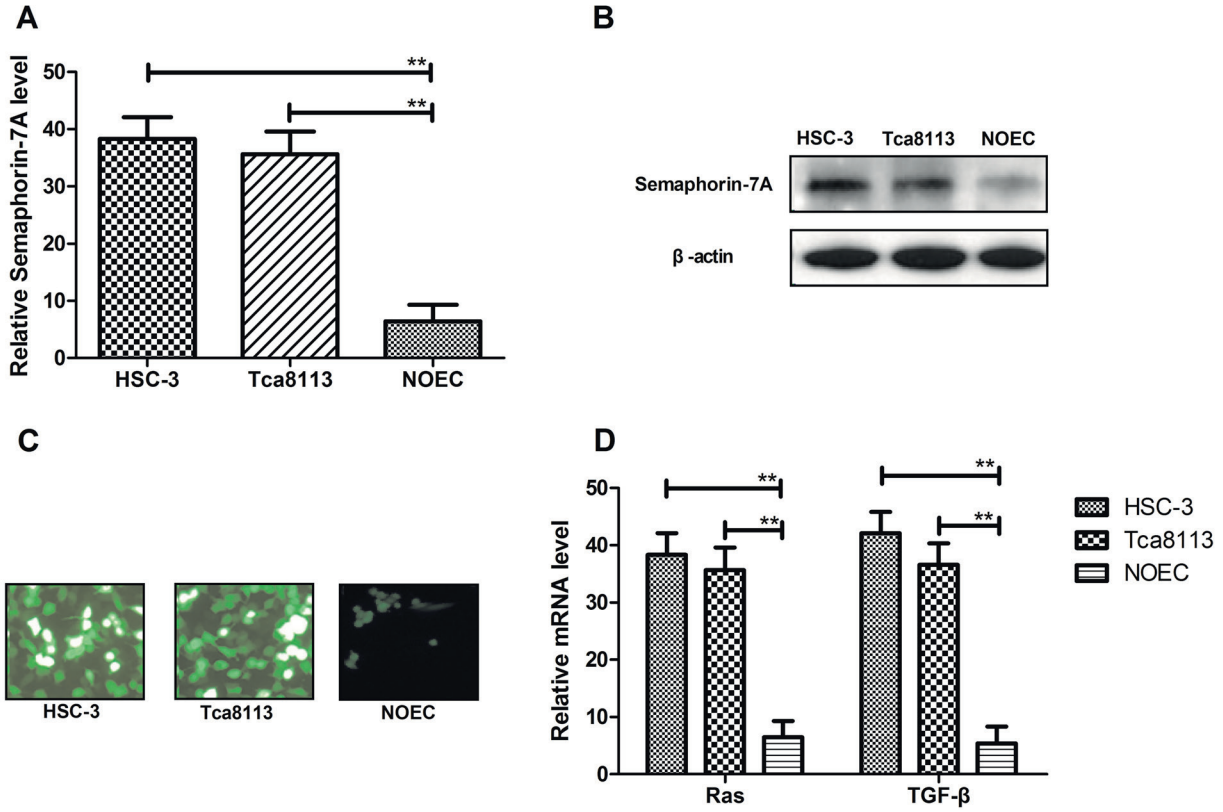

Figure 1. Expression of Semaphorin-7A in cultured HSC-3 and Tca8113 cells. ***A,*** Relative Semaphorin-7A mRNA expression in OTSCC cell lines, HSC-3 and Tca8113. ***B,*** Semaphorin-7A protein expression level in OTSCC cells. ***C,*** Immunofluorescence analysis of Semaphorin-7A protein expression level in OTSCC cells or oral epithelial cells as control. ***D,*** Relative Ras and TGF-β mRNA expression in OTSCC cell lines, HSC-3 and Tca8113 cell lines. Student *t*-test revealed a significant effect. *$p < 0.05$, **$p < 0.01$, *vs.* control.

in the cultured HSC-3 and Tca8113 cells. Then, the results in Figure 1D showed that Ras and TGF-β expression were increased in HSC-3 and Tca8113 cells compared with NOEC. These data indicated that Semaphorin-7A expression was up-regulated in HSC-3 and Tca8113 cells compared with NOEC.

Semaphorin-7A Promoted Growth, Migration and Invasion of OTSCC

In order to investigate the biological role of Semaphorin-7A in OTSCC progression, HSC-3 and Tca8113 cells were treated by Semaphorin-7A. The results in Figure 2A showed that Semaphorin-7A treatment promoted the growth of HSC-3 and Tca8113 cells compared with control ($p < 0.01$). Our results in Figure 2B indicated that Semaphorin-7A treatment significantly promoted the growth rate and augmented the anchorage-independent growth ability in HSC-3 and Tca8113 cells ($p < 0.01$). Moreover, the invasive ability of OTSCC cells was significantly enhanced in the OTSCC cells treated with Semaphorin-7A (Figure 2C). Furthermore, overexpression of Semaphorin-7A also enhanced the apoptotic resistance of HSC-3 and Tca8113 cells induced with the chemotherapeutic agent cisplatin (Figure 2D). We also found that Semaphorin-7A treatment promoted HSC-3 and Tca8113 cells growth as a dose-depend manner. Collectively, these results suggested that up-regulation of Semaphorin-7A promoted the growth and aggressiveness of HSC-3 and Tca8113 cells *in vitro*.

Semaphorin-7A Promoted Growth, Migration and Invasion Through TGF-β-mediate EMT Signaling Pathway

In order to investigate the possible mechanism of Semaphorin-7A-induced OTSCC cells migration, ERF as a Ras/Erk mediator during TGF-β-induced EMT signaling pathway was analyzed in cells. The results in Figure 3A indicated that Ras, Erk and TGF-β expression levels were up-regulated after Semaphorin-7A treatment. In

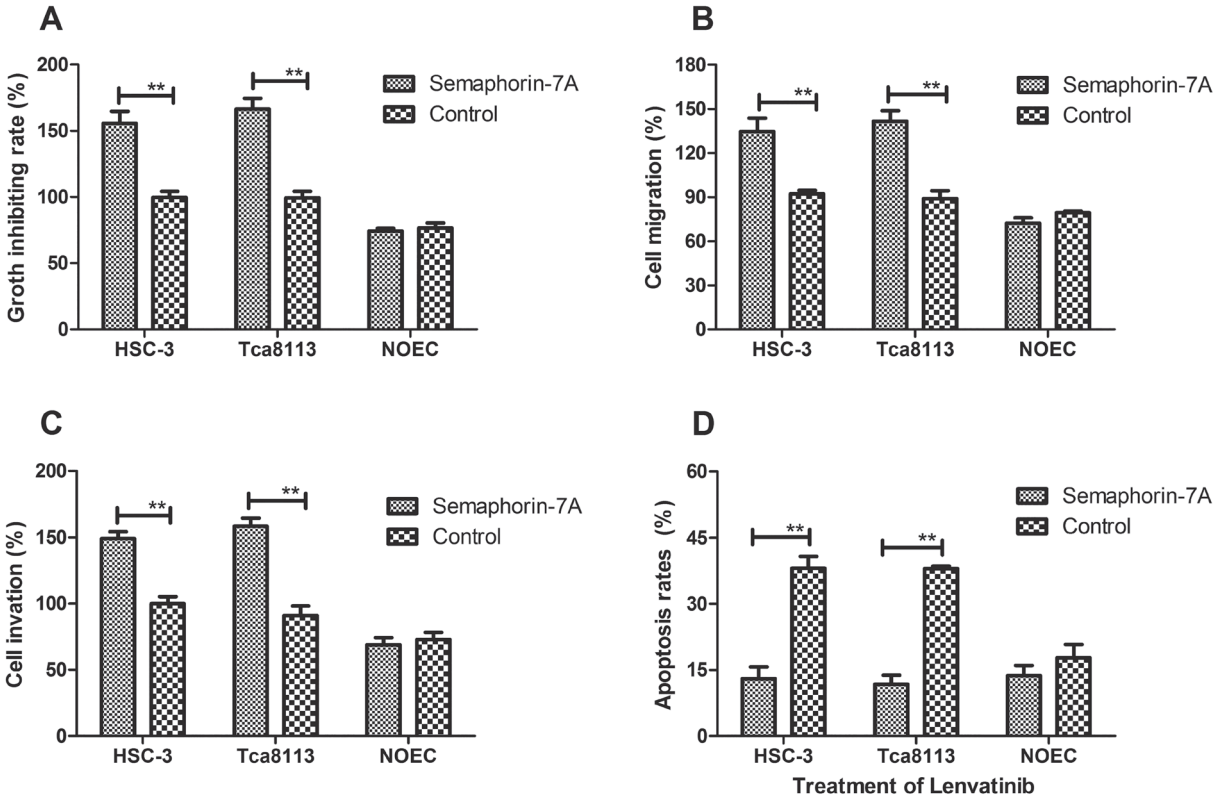

Figure 2. The promotive effects of Semaphorin-7A on OTSCC cells *in vitro*. ***A,*** Semaphorin-7A promoted OTSCC cells growth after inoculation 72 hours. ***B,*** OTSCC cells migration was promoted by Semaphorin-7A treatment. ***C,*** Semaphorin-7A treatment enhanced OTSCC cells invasion compared to normal oral epithelial cells. ***D,*** Semaphorin-7A treatment enhanced apoptotic resistance of OTSCC cells induced by cisplatin. Student *t* test revealed a significant effect. $*p < 0.05$, $**p < 0.01$, *vs*. control.

addition, migration and invasion-related proteins of Vim, CT-I and Slug were analyzed after Semaphorin-7A treatment. Our results showed that Semaphorin-7A up-regulated these invasion-promoted protein expression levels in OTSCC cells (Figure 3B). In order to analyze EMT signaling pathway, immunofluorescence was used to analyze Semaphorin-7A promoted TGF-β–induced EMT signaling pathway in HSC-3 and Tca8113 cells. The results in Figure 3C and D showed that E-Cadherin/DNA levels were downregulated (Figure 3C) and Fibronectin/DNA (Figure 3D) expression levels were up-regulated in HSC-3 and Tca8113 cells after Semaphorin-7A treatment. These indicated that Semaphorin-7A promoted growth, migration and invasion through TGF-β-mediated EMT signaling pathway.

Inhibition of Semaphorin-7A Expression Showed Beneficial Effects on OTSCC-Bearing Mice

For confirming the Semaphorin-7A efficacy, we analyzed the effects of Semaphorin-7A in HSC-3-bearing mice *in vivo*. HSC-3 (1×10^6) cells were injected subcutaneously into BALB/c nude mice. The mice were divided into three groups and received Semaphorin-7A, Anti Sema-7a with the same volume of PBS as control respectively, when tumor diameter reached 5-6 mm. We found that Semaphorin-7A significantly contributed to tumor growth (Figure 4A), while AntiSema-7A-treated mice greatly inhibited tumor growth compared with PBS-treated mice. At the 25th day after tumor cells inoculation, we analyzed Semaphorin-7A expression in tumors of each experimental mouse (Figure 4B). In addition, we detected TGF-β, Ras, ERK and ERF expression in tumors by Western blot in different treatment groups. The results in Figure 4C indicated that Semaphorin-7A up-regulated TGF-β, Ras, ERK and ERF expression *in vivo*, while AntiSema-7A inhibited TGF-β, Ras, ERK and ERF expression compared to PBS group. Furthermore, the Snail, N-cadherin and Vimentin were down-regulated after Anti-Sema-7A treatment compared with Semaphorin-7A and PBS groups

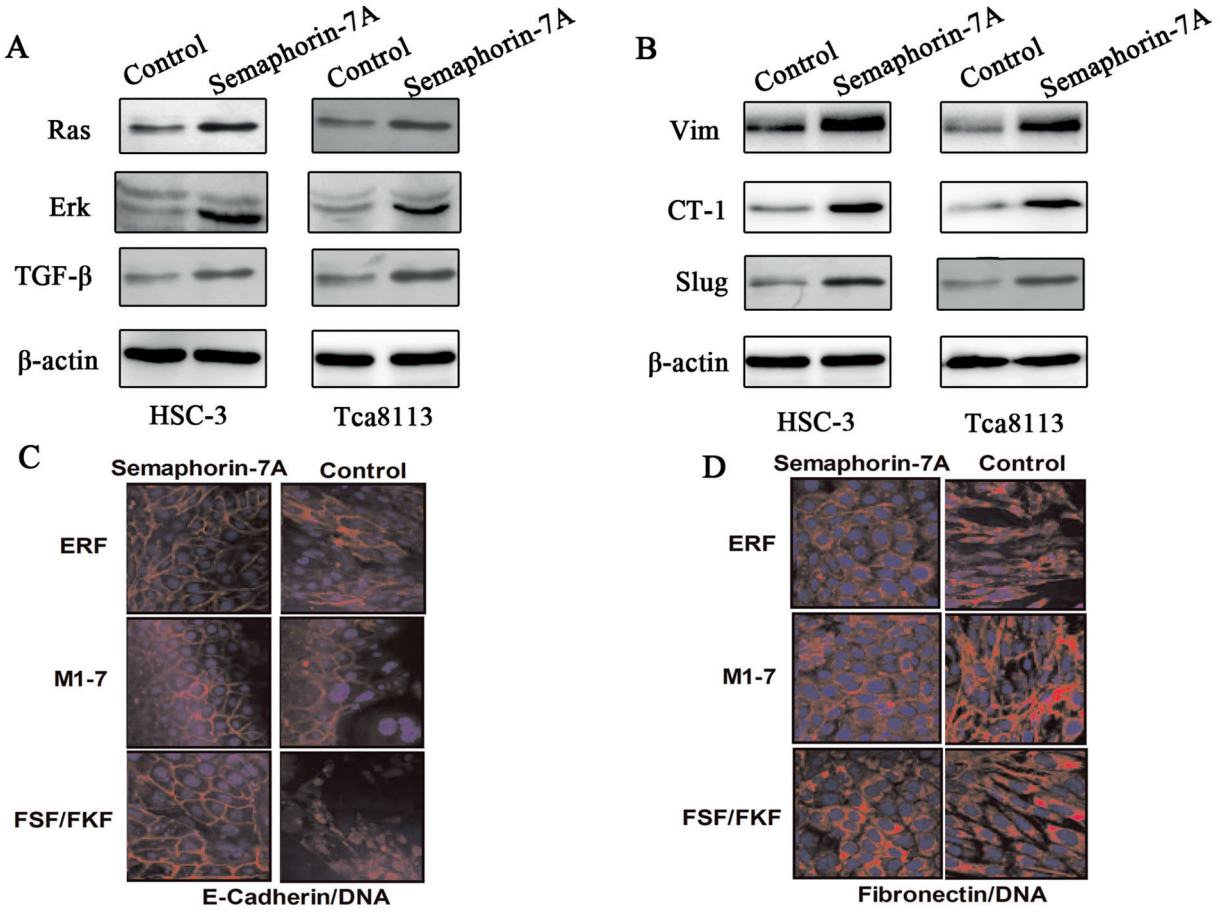

Figure 3. Molecules changes in TGF-β-mediate EMT signaling pathway after Semaphorin-7A treatment. ***A,*** Analysis of Ras, Erk and TGF-β protein expression changes in HSC-3 and Tca8113 cells after treatment with Semaphorin-7A. ***B,*** Analysis of Vim, CT-I and Slug protein expression changes in HSC-3 and Tca8113 cells after treatment with Semaphorin-7A. ***C,*** E-cadherin expression (ERF, M1-7 and FSF/FKF) in HSC-3 cells by staining for E-cadherin with the respective specific antibody (*red*) and TOPRO-3 for DNA (*blue*) and analyzed by confocal microscopy. ***D,*** Fibronectin expression (ERF, M1-7 and FSF/FKF) in HSC-3 cells by staining for E-cadherin with the respective specific antibody (*red*) and TOPRO-3 for DNA (*blue*) and analyzed by confocal microscopy.

(Figure 4D). We found that Anti-Sema-7A-treated decreased Snail, N-cadherin, and Vimentin expression in tumors. These results suggested that Semaphorin-7A was a tumor-promoted protein that enhances tumor growth, migration, invasion and tumor angiogenesis in OTSCC

Discussion

Semaphorins were originally identified as axon guidance molecules in neural development. However, the accumulated evidence indicates that several semaphorins play crucial roles in physiological and pathological immune responses[18,19]. Semaphorin-7A is one of Semaphorins members that was identified as a signaling molecule to induce termination of protein synthesis through controlling of many biochemical reactions to regulate metabolism[11]. Previous study has showed that inhibition of axonal outgrowth in the tumor environment selectively inhibited melanoma cells growth[20,21]. In addition, Semaphorin-7A stimulated melanocyte adhesion and dendricity through opposing actions of beta1-integrin and Plexin C1 receptors[15]. Furthermore, Semaphoring-7A was identified as a potent driver of ductal carcinoma in situ progression, which occurred in a large percentage of breast cancers and was associated with decreased overall and distant metastasis-free survival[11].

The clinical value of OTSCC-target molecular not only predicts the degree of aggressive tumor behaviors, but also provides tumor target ther-

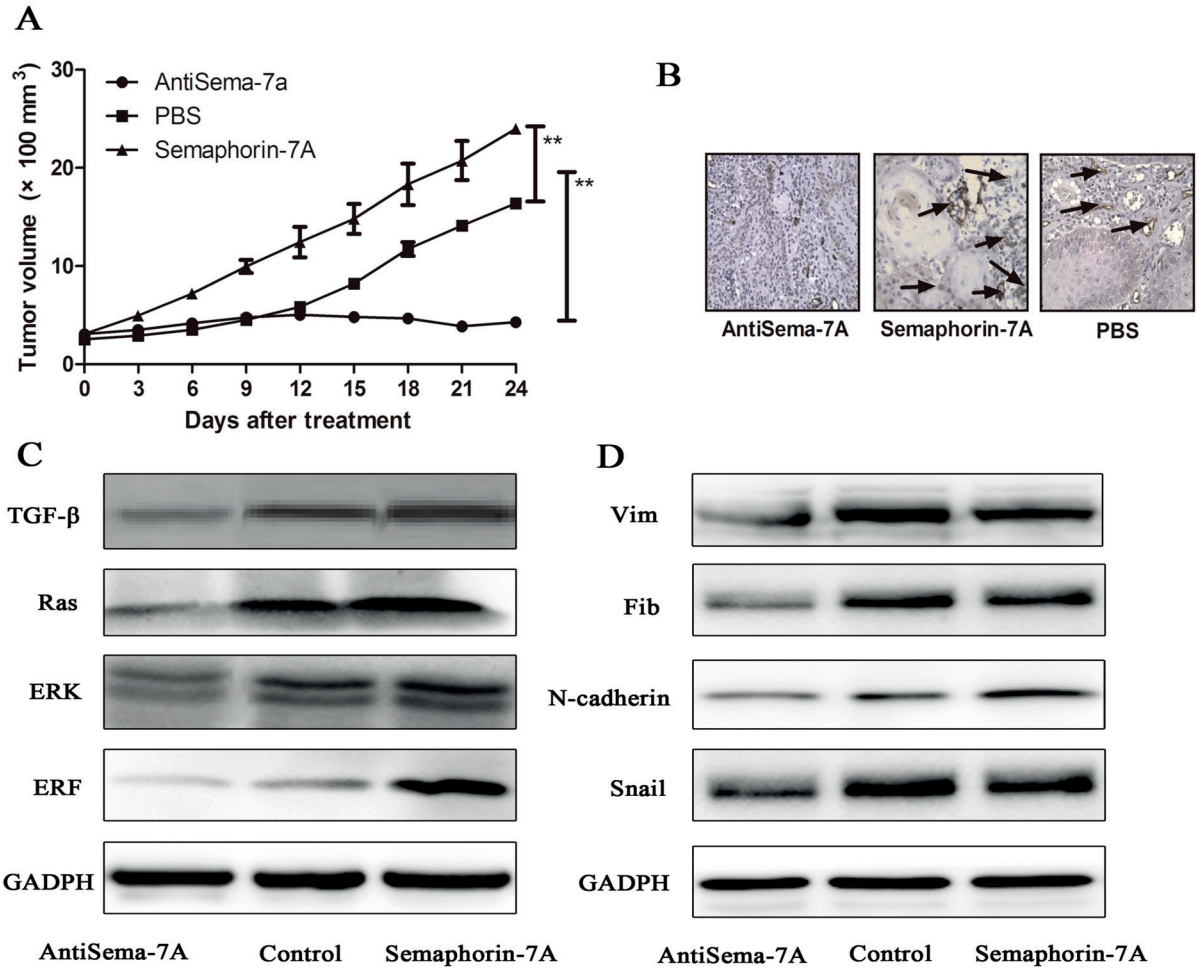

Figure 4. Inhibition of tumor metastasis through targeting for Semaphorin-7A in HSC-3-bearing mice. ***A,*** Tumor growth was analyzed after AntiSema-7A, Semaphorin-7A or PBS treatment in 18-day short term observation. ***B,*** Tumor angiogenesis was analyzed in tumors from experimental mice on day 25. ***C,*** TGF-β, Ras, ERK and ERF expression levels were analyzed *in vivo* in AntiSema-7A, Semaphorin-7A or PBS-treated tumors. ***D,*** Fibronectin, Snail, N-cadherin and Vimentin expression levels were analyzed *in vivo* in AntiSema-7A, Semaphorin-7A or PBS-treated tumors. Student *t*-test revealed a significant effect. ***p* < 0.01, *vs.* control.

apeutic agents for patients with OTSCC, which achieved early diagnosis and efficient treatment for patients in the mild stage in clinical[22,24]. Therefore, the identification of selective and specific tumor markers can define the OTSCC cells phenotype and achieves more efficient target therapy for patients with OTSCC. In this investigation, we identified Semaphorin-7A was a contributor for OTSCC metastasis both *in vitro* and *in vivo*. Our results presented that Semaphorin-7A promoted tumor cells growth, migration and the invasive abilities of OTSCC cells through EMT signaling pathway. EMT signaling pathway is a key process in tumor cells progression and metastasis stimulated by epidermal growth factor (EGF)/Ras and TGF-β signaling pathways, which lead to a complex biochemical reaction processes in tumor cells[25,26]. Previous work[27] has explained the molecular mechanisms of EMT signaling pathway by Ras-induce signaling, which regulated EMT process in human tumor cells. Marylineet al[17] demonstrated that Semaphorin-7a reversed the ERF-induced inhibition of EMT signaling pathways in Ras-dependent mouse mammary epithelial cells. These results identified that Semaphorin-7a was a potential tumor-target suppressor protein in progression of neoplasm, and suggested inhibition of Semaphorin-7a expression may suppress migration, invasion and metastasis through loss of inhibitory signaling on TGF-β-mediate EMT signaling pathway.

EMT signaling pathway has been indicated as a developmental process in tumors progression and metastasis, which has been extensively studied in previous researches[27-29]. TGF-β and Ras are essential proteins for EMT signaling pathway that often depend on tumor cell types[27]. Our findings suggested that Semaphorin-7A overexpression promoted TGF-β-induced EMT signaling pathway.

In addition, our results have showed that the levels of the epithelial marker E-cadherin were downregulated and the mesenchymal marker Fibronectin were up-regulated in OTSCC cells. Furthermore, Vim, CT-I and Slug expression levels were also up-regulated after Semaphorin-7A treatment. These results suggested that Semaphorin-7A expression contributed to OTSCC cells growth and metastasis through TGF-β-induced EMT signaling pathway, which indicated blocking Semaphorin-7A expression may play a vital role in regulating EMT process. Noteworthy, Semaphorin-7A enhanced density of blood vessels in tumor tissues, and this assumed that Semaphorin-7A played an important role in the progression, dissemination and metastasis of OTSCC.

Conclusions

We showed that the overexpression of Semaphorin-7A in OTSCC cell lines, and Semaphorin-7A promoted growth, migration and invasion of OTSCC cells. Semaphorin-7A-overexpressing accelerated TGF-β-induced EMT, retained expression of epithelial markers and formed blood vessel density in OTSCC. Our data suggested that the Semaphorin-7A/TGF-β/EMT signaling network contributed to TGF-β-induced apoptosis resistance, tumor progression and metastasis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Kolokythas A, Park S, Schlieve T, Pytynia K, Cox D. Squamous cell carcinoma of the oral tongue: histopathological parameters associated with outcome. Int J Oral Maxillofac Surg 2015; 44: 1069-1074.
2) Wong WM, Parvathaneni U, Jewell PD, Martins RG, Futran ND, Laramore GE, Liao LJ. Squamous cell carcinoma of the oral tongue in a patient with Fanconi anemia treated with radiotherapy and concurrent cetuximab: a case report and review of the literature. Head Neck 2013; 35: E292-298.
3) Amichetti M. Squamous cell carcinoma of the oral tongue in patients less than fifteen years of age: report of a case and review of the literature. J Craniomaxillofac Surg 1989; 17: 75-77.
4) Joseph LJ, Goodman M, Higgins K, Pilai R, Ramalingam SS, Magliocca K, Patel MR, El-Deiry M, Wadsworth JT, Owonikoko TK, Beitler JJ, Khuri FR, Shin DM, Saba NF. Racial disparities in squamous cell carcinoma of the oral tongue among women: a SEER data analysis. Oral Oncol 2015; 51: 586-592.
5) Shakeel UZ, Adeel M, Suhail A. Squamous cell carcinoma of oral tongue in young patients - A 10 years tertiary care experience. J Pak Med Assoc 2016; 66: 155-158.
6) Kwon M, Moon H, Nam SY, Lee JH, Kim JW, Lee YS, Roh JL, Choi SH, Kim SY. Clinical significance of three-dimensional measurement of tumour thickness on magnetic resonance imaging in patients with oral tongue squamous cell carcinoma. Eur Radiol 2016; 26: 858-865.
7) Martinez C, Hernandez M, Martinez B, Adorno D. Frequency of oral squamous cell carcinoma and oral epithelial dysplasia in oral and oropharyngeal mucosa in Chile. Rev Med Chil 2016;144: 169-174.
8) Maxwell JH, Thompson LD, Brandwein-Gensler MS, Weiss BG, Canis M, Purqina B, Prabhu AV, Lai C, Shuai Y, Carroll WR, Morland A, Duvvuri U, Kim S, Johnson JT, Ferris RL, Seethala R, Chiosea SI. Early Oral tongue squamous cell carcinoma: sampling of margins from tumor bed and worse local control. JAMA Otolaryngol Head Neck Surg 2015; 141: 1104-1110.
9) Mehrotra R, Yadav S. Oral squamous cell carcinoma: etiology, pathogenesis and prognostic value of genomic alterations. Indian J Cancer 2006; 43: 60-66.
10) Gras C, Eiz-Vesper B, Seltsam A, Immenschuh S, Blasczyk R, Figueiredo C. Semaphorin 7A protein variants differentially regulate T-cell activity. Transfusion 2013; 53: 270-283
11) Black SA, Nelson AC, Gurule NJ, Futscher BW, Lyons TR. Semaphorin 7a exerts pleiotropic effects to promote breast tumor progression. Oncogene 2016; 35: 5170-5178.
12) Mizutani N, Nabe T, Yoshino S. Semaphorin 7A plays a critical role in IgE-mediated airway inflammation in mice. Eur J Pharmacol 2015; 764: 149-156.
13) Azar DT. Corneal angiogenic privilege: angiogenic and antiangiogenic factors in corneal avascularity, vasculogenesis, and wound healing (an American Ophthalmological Society thesis). Tran Am Ophthalmol Soc 2006; 104: 264-302.

14) Albe E, Chang JH, Azar NF, Ivanov AR and Azar DT. Proteomic analysis of the hyaloid vascular system regression during ocular development. J Proteome Res 2008; 7: 4904-4913.

15) Scott GA, McClelland LA, Fricke AF, Fender A. Plexin C1, a receptor for semaphorin 7a, inactivates cofilin and is a potential tumor suppressor for melanoma progression. J Invest Dermatol 2009; 129: 954-963.

16) Allegra M, Zaragkoulias A, Vorgia E, Loannou M, Litos G, Beuq H, Mavrothalassitis G. Semaphorin-7a reverses the ERF-induced inhibition of EMT in Ras-dependent mouse mammary epithelial cells. Mol Biol Cell 2012; 23: 3873-3881

17) Sawicka D, Chojnacka-Puchta L, Zielinski M, Plucienniczak G, Plucienniczak A, Bednarczyk M. Flow cytometric analysis of apoptosis in cryoconserved chicken primordial germ cells. Cell Mol Biol lett 2015; 20: 143-159.

18) Lindholm T, Risling M, Caristedt T, Hammarberg H, Wallguist W, Cullheim S, SkÐld MK. Expression of semaphorins, neuropilins, VEGF, and tenascins in rats and human primary sensory neurons after a dorsal root injury. Front Neurol 2017; 8: 49.

19) Serini G, Maione F, Giraudo E, Bussolino F. Semaphorins and tumor angiogenesis. Angiogenesis 2009; 12: 187-193.

20) Bielenberg DR, Klagsbrun M. Targeting endothelial and tumor cells with semaphorins. Cancer Metastasis Rev 2007; 26: 421-431.

21) Vachkov IH, Huang X, Yamada Y, Tonchev AB, Yamashima T, Kato S, Takakura N. Inhibition of axonal outgrowth in the tumor environment: involvement of class 3 semaphorins. Cancer Sci 2007; 98: 1192-1197.

22) Keski-Santti H, Atula T, Tornwall J, Koivunen P, Makitie A. Elective neck treatment versus observation in patients with T1/T2 No squamous cell carcinoma of oral tongue. Oral Oncol 2006; 42: 96-101.

23) Schiff BA, Roberts DB, El-Naggar A, Garden AS, Myers JN. Selective vs modified radical neck dissection and postoperative radiotherapy vs observation in the treatment of squamous cell carcinoma of the oral tongue. Arch Otolaryngol Head Neck Surg 2005; 131: 874-878.

24) Umeda M, Komatsubara H, Ojima Y, Minamikawa T, Shibuya Y, Yokoo S, Ishil J, Komori T. A comparison of brachytherapy and surgery for the treatment of stage I-II squamous cell carcinoma of the tongue. Int J Oral Maxillofa Surg 2005; 34: 739-744.

25) Zhang K, Li XY, Wang ZM, Han ZF, Zhao YH. Mir-22 inhibits lung cancer cell EMT and invasion through targeting Snail. Eur Rev Med Pharmacol Sci 2017; 21: 3598-3604.

26) Elsum IA, Martin C and Humbert PO. Scribble regulates an EMT polarity pathway through modulation of MAPK-ERK signaling to mediate junction formation. J Cell Sci 2013; 126: 3990-3999.

27) Yang X, Duan B, Zhou X. Long non-coding RNA FOXD2-AS1 functions as a tumor promoter in colorectal cancer by regulating EMT and Notch signaling pathway. Eur Rev Med Pharmacol Sci 2017; 21: 3586-3591.

28) Pasterkamp RJ, Peschon JJ, Spriggs MK, Kolodkin AL. Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. Nature 2003; 424: 398-405.

29) Wang Z, Li Y, Kong D, Sarkar FH. The role of Notch signaling pathway in epithelial-mesenchymal transition (EMT) during development and tumor aggressiveness. Curr Drug Targets 2010; 11: 745-751.