TGF-β1 Promotes Migration and Invasion of Salivary Adenoid Cystic Carcinoma

L. Dong, Y.X. Wang, S.L. Li, G.Y. Yu, Y.H. Gan, D. Li and C.Y. Wang

J DENT RES 2011 90: 804 originally published online 24 March 2011
DOI: 10.1177/0022034511401407

RESEARCH REPORTS
Biological

L. Dong[1], Y.X. Wang[2,1], S.L. Li[2,1]*, G.Y. Yu[3]*, Y.H. Gan[2], D. Li[1], and C.Y. Wang[4]

[1]Laboratory of Oral and Maxillofacial Surgery, [2]Central Laboratory, and [3]Department of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology, 22 Zhongguancun Nandajie, Haidian District, Beijing 100081, P.R. China; and [4]Division of Oral Biology and Medicine, UCLA School of Dentistry, Los Angeles, CA 90095, USA; *co-corresponding authors, kqshlli@bjmu.edu.cn and gyyu@263.net

J Dent Res 90(6):804-809, 2011

TGF-β1 Promotes Migration and Invasion of Salivary Adenoid Cystic Carcinoma

ABSTRACT

Salivary adenoid cystic carcinoma (SACC) is one of the most common subtypes of salivary gland carcinomas and frequently metastasizes to distant organs. However, little is known about the molecular mechanisms that promote SACC metastasis. In this study, we report that transforming growth factor (TGF)-β1 was highly expressed in the highly metastatic SACC-LM cell line as compared with its parental low-metastatic SACC-83 cell line. Exogenous addition of TGF-β1 induced Smad2 phosphorylation and promoted the migration and invasion of SACC-83 cells. Consistently, the inhibition of endogenous TGF-β1 signaling in SACC-LM cells by an inhibitor specific to the type I TGF-β1 receptor (TβRI) suppressed cell migration and invasion. Moreover, we found that TGF-β1 expression was significantly increased in human primary SACC samples with metastasis. Taken together, our results suggest that TGF-β1 may play a crucial role in SACC metastasis.

KEY WORDS: salivary adenoid cystic carcinoma, TGF-β1, migration and invasion, Smad2, SB431542.

DOI: 10.1177/0022034511401407

Received November 23, 2010; Last revision January 11, 2011; Accepted January 12, 2011.

A supplemental appendix to this article is published electronically only at http://jdr.sagepub.com/supplemental.

INTRODUCTION

Salivary adenoid cystic carcinoma (SACC) is remarkable for its slow but progressive clinical course, high rates of recurrence, and lung metastasis (Kokemueller *et al*., 2004). Numerous studies have identified factors related to the prognosis and outcome of SACC (Li *et al*., 1990; He *et al*., 2008; Vékony *et al*., 2008), but little is known regarding the underlying molecular mechanisms that control SACC metastasis (Fordice *et al*., 1999; Takata *et al*., 1999; Muller *et al*., 2006). To decrease both morbidity and mortality in SACC patients, it is important that we understand the process of SACC metastasis and identify the molecular factors that contribute to this process.

Transforming growth factor (TGF)-β is a multifunctional polypeptide that plays a critical role in cell proliferation, apoptosis, and epithelial-mesenchymal transition (Ma *et al*., 2007; Massagué, 2008; Liu *et al*., 2009). It is well known that TGF-β binds to TGF-β receptors to induce the phosphorylation and activation of the transcription factor Smad. While some studies suggest that TGF-β signaling promotes the invasive growth and metastasis of breast cancer and glioma (Platten *et al*., 2000; Wei *et al*., 2008; Zheng *et al*., 2008), the role of TGF-β in head and neck cancer is controversial (Lu *et al*., 2006; Takayama *et al*., 2009). Moreover, there have been no studies examining whether TGF-β regulated invasive growth of SACC cells.

To understand the molecular mechanisms regulating SACC metastasis, we previously established SACC cell lines, named SACC-83 and SACC-LM, obtained from a lung-metastatic tumor of SACC-83 cells in a nude mouse. The lung-metastatic rates of SACC-83 cells and SACC-LM cells were 33.3% and more than 85%, respectively (Li *et al*., 1990; Hu *et al*., 2009). Because of the important role of TGF-β1 in tumor progression, we sought to examine whether TGF-β1 was associated with SACC metastasis in this study. We found that TGF-β1 was more highly expressed in SACC-LM cells than in SACC-83 cells. Exogenous addition of TGF-β1 potently induced Smad2 phosphorylation and promoted the migration and invasion of SACC-83 cells. In contrast, inhibition of elevated endogenous TGF-β1 signaling in SACC-LM cells blocked cell migration and invasion. Moreover, we found that TGF-β1 expression was significantly elevated in human primary SACC with metastasis. Our results suggest that TGF-β1 may be an important factor that promotes SACC metastasis.

MATERIALS & METHODS

Cell Culture

The parental, low-metastatic SACC-83 cell line was derived from a patient pathologically diagnosed in 1983 with SACC in the sublingual gland (Li *et al*., 1990). To generate the SACC-LM cell line, we injected the parental SACC-83 cells into the tail veins of nude mice. Metastatic tumor cells were isolated from the lung and injected into the tail vein again. After 5 rounds of selection, the highly metastatic SACC-LM cell line was generated from the lung-metastatic foci. Both SACC-83 and SACC-LM were grown in RPMI 1640 medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (Gibco) at 37°C, 5% CO_2.

RNA Isolation and Reverse-transcription Polymerase Chain-reaction (RT-PCR)

Total RNA was isolated from cells with Trizol reagent (Invitrogen, Carlsbad, CA, USA). RT-PCR was performed with a two-step RT-PCR kit (Invitrogen). The primers were as follows: (TGF-β1) sense 5′-GACTACTACGCCAAGGAGGTCA-3′, antisense 5′-AGTCAATGTACAGCTGCCGCAC-3′; and (GAPDH) sense 5′-CCATGGAGAAGGCTGGG-3′, antisense 5′-CAAAG TTGTCATGGATGACC-3′.

Enzyme-linked Immunosorbent Assay (ELISA) and Western Blot Analysis

Cells were cultured with complete medium for 24 hrs and then deprived of serum for an additional 24 hrs. Supernatants were collected, and cell numbers were used to normalize the TGF-β1 expression level. Half of each supernatant sample was used to determine activated TGF-β1 with a TGF-β1 ELISA kit (R&D Systems, Minneapolis, MN, USA). The other half of each supernatant sample was acidified with 20 μL 1 N HCl *per* 100 μL of supernatant to activate the latent TGF-β1 and render it immunoreactive, then neutralized with an equal volume of 1.2 N NaOH and assayed for total TGF-β1 content.

For Western blot, SACC-83 cells were treated with human recombinant TGF-β1 (5 ng/mL; R&D Systems) for the indicated times (0-24 hrs) or with TGF-β1 (0-10 ng/mL) for 1 hr. For inhibition assays, cells were pre-incubated with SB431542 (Sigma-Aldrich, St. Louis, MO, USA), a specific TGF-β receptor type I (TβRI) inhibitor, at a concentration of 10 μmol/L for 30 min prior to TGF-β1 treatment. Whole-cell protein extracts were subjected to 12% SDS-PAGE. The membranes were probed with the following antibodies at 4°C overnight: anti-Smad2/3, anti-phospho-Smad2, anti-extracellular signal-regulated kinase1/2 (ERK1/2), anti-phospho-ERK1/2, anti-p38, anti-phospho-p38, anti-c-Jun N-terminal kinase (JNK), and anti-phospho-JNK (1:1000, Cell Signaling, Beverly, MA, USA). Immunocomplexes were detected with an enhanced chemiluminescence blotting kit (Applygen Technology Inc., Beijing, China).

Wound-healing Assay

To assess cell migration, we performed a wound-healing assay as described previously (Saadoun *et al*., 2005). Briefly, cells were cultured as confluent monolayers, synchronized in RPMI 1640 medium containing 0.5% fetal bovine serum for 6 hrs, and wounded by removal of a 300-400 μm strip across the well. The cells were then treated with SB431542 and TGF-β1. Wounded monolayers were photographed at 10x magnification (TE-2000 U, Nikon, Japan) 0 and 24 hrs after wounding. Wound healing was quantified by measurement of the average linear speed of movement of the wound edges over 24 hrs.

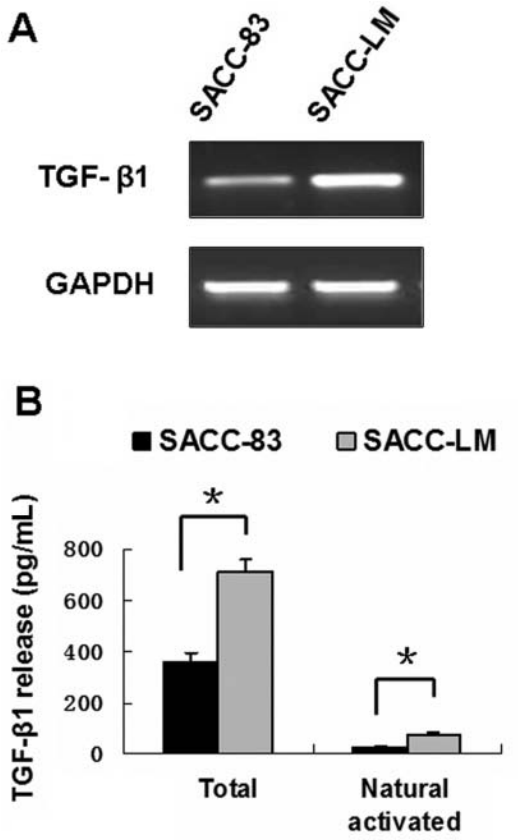

Figure 1. TGF-β1 expression in SACC cells. **(A)** The levels of TGF-β1 mRNA in SACC-83 and SACC-LM cells were examined by RT-PCR. **(B)** An ELISA assay was performed to measure normalized total TGF-β1 and naturally activated TGF-β1 in SACC-83 cells (dark gray) and SACC-LM cells (light gray, $*P < 0.05$). Each datapoint represents the mean ± SD of triplicate samples, and similar results were seen in 3 independent experiments.

Transwell Invasion Assays

Cell invasion assays were performed with the use of transwell chambers with a polycarbonate membrane (Millipore, Bedford, MA, USA) coated with 20 μg extracellular matrix (ECM) gel (Sigma-Aldrich). Cells were pre-incubated with or without SB431542 (10 μmol/L) for 30 min, stimulated with TGF-β1 (5 ng/mL) for 24 hrs, and then seeded at 1×10^5 cells/well in the upper chambers. Sixteen hrs after incubation, cells on the upper surface of the membrane were wiped off, and the membranes were fixed with 95% ethanol and stained with 1% crystal violet (Sigma-Aldrich). The invaded cells were counted by light microscopy at 10x magnification.

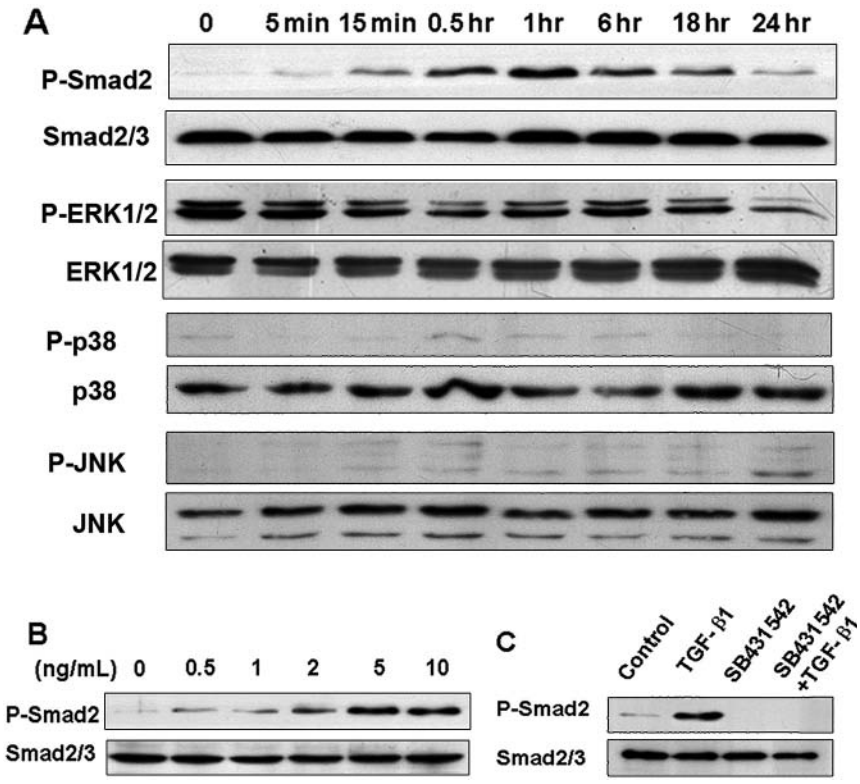

Figure 2. TGF-β1 induces the phosphorylation of Smad2, but not ERK1/2, p38, or JNK in SACC-83 cells. **(A)** TGF-β1 induced the phosphorylation of Smad2, but not ERK1/2, p38, or JNK, in a time-dependent manner. Cells were treated with 5 ng/mL TGF-β1 at the indicated time-points. Blots were stripped and reprobed to detect the total amounts of Smad2/3, ERK1/2, p38, and JNK as controls. Results represent 1 of 3 independent sets of experiments. **(B)** TGF-β1 induced the phosphorylation of Smad2 in a dose-dependent manner. **(C)** SB431542 inhibited TGF-β1-induced Smad2 phosphorylation in SACC-83 cells.

Human Primary SACC Samples and Immunostaining

Fifty SACC patients, who were diagnosed and surgically treated at the Department of Oral and Maxillofacial Surgery, Peking University School of Stomatology, from 1967 to 2008, were included in this study. Twenty-two patients were male and 28 female, with a median age of 46.5 yrs (range from 17 to 71 yrs). All patients were followed up for 6 to 240 mos, with a mean follow-up duration of 74.4 mos (of note, one case was found to have metastasis in 6 mos). Lung metastases occurred in 27 cases during the follow-up period. No metastases were found in 23 patients during the follow-up period of a mean duration of 85.7 mos (52.2% were followed up for more than 5 yrs, and 22% for more than 10 yrs). The human primary tumors were sectioned into 4-μm-thick slices and stained with mouse anti-TGF-β1 monoclonal antibody (1:50, Abcam Ltd., Cambridge, MA, USA) or IgG control at 4°C overnight and subsequently reacted with biotinylated secondary antibody (1:200) for 1 hr. The immunocomplexes were visualized with diaminobenzidine (Zhongshan Golden Bridge Biological Technology CO., LTD, Beijing, China). The immunostaining was analyzed semi-quantitatively according to a histological score (H-score) based on both labeled cell numbers and intensity. The H-score was calculated by the formula HS = ∑ (Pi x i)/100, where Pi is the percentage of labeled cells and i is the labeling intensity ranging from 1 to 3 (Huang *et al*., 1996). Scores of 1, 2, and 3 indicate weak, moderate, and strong staining, respectively.

Statistical Analysis

Quantitative data were expressed as means ± SD and compared by a Student's *t* or Mann-Whitney U test. $P < 0.05$ was considered as statistically significant.

RESULTS

SACC-LM Cells Expressed Higher Levels of TGF-β1 Than Did SACC-83 Cells

To determine whether TGF-β1 might be associated with the invasive growth of SACC cells, we compared the expression levels of TGF-β1 in both SACC-83 and SACC-LM cells. RT-PCR revealed that the highly metastatic SACC-LM cells significantly expressed higher levels of TGF-β1 mRNA than did SACC-83 cells (Fig. 1A). ELISA also confirmed that the protein levels of the activated TGF-β1 in SACC-LM cells (71.8 ± 9.5 pg/mL) were significantly higher than those in SACC-83 cells (25.5 ± 2.0 pg/mL). Moreover, the total TGF-β1 content in SACC-LM cells (709.4 ± 71.8 pg/mL), including active and latent TGF-β1, was also significantly higher than that in SACC-83 cells (360.2 ± 43.9 pg/mL; Fig. 1B). Recently, it was reported that SACC cell lines from different sources were contaminated with HeLa cells (Phuchareon *et al*., 2009). To confirm the authenticity of SACC-83 and SACC-LM, we performed short tandem-repeat analysis (STR) to compare DNA fingerprinting of SACC-83 and SACC-LM with HeLa cells. STR revealed that, while DNA fingerprinting of SACC-83 and SACC-LM cells was identical, neither line was contaminated with HeLa cells (Appendix Fig. 1 and Table). Also, we searched the ATCC Profile Database and found that the STR of SACC-83 and SACC-LM cells did not match that of any known human tumor cell lines. To rule out the possibility that the cell lines might be derived from oral squamous cell carcinoma (SCC), we performed immunostaining to compare the expression pattern of keratins that have been used to differentiate squamous epithelia from salivary gland epithelia. In general, oral squamous epithelia do not express cytokeratin (CK) 7, CK8/18, and CK20 (Moll *et al*, 1982; Chu and Weiss, 2002). Immunostaining showed that both SACC-83 and SACC-LM tumors derived from nude mice, like human primary SACC,

expressed all of these markers. In contrast, human primary oral SCC did not express these markers (Appendix Fig. 2). Taken together, our tests confirmed that SACC-83 and SACC-LM are authentic ACC cell lines.

Activation of Smad Signaling by TGF-β1 in SACC-83 Cells

TGF-β family proteins have been found to induce intracellular signaling cascades involving Smad and/or mitogen-activated protein kinase (MAPK). To determine whether TGF-β1 affected SACC cell function, we first examined whether TGF-β1 activated Smad and MAPK in SACC cells. Western blot revealed that the levels of phosphorylated Smad2 were increased in a time-dependent manner from 5 min to 1 hr and then decreased upon TGF-β1 treatment (Fig. 2A). In contrast, the phosphorylated ERK1/2, p38, and JNK were not induced by TGF-β1. Additionally, the phosphorylation of Smad2 induced by TGF-β1 was dose-dependent, since concentrations of TGF-β1 increased from 0.5 ng/mL to 5-10 ng/mL (Fig. 2B). An inhibitor specific to TβRI, SB431542, prevented TGF-β1-induced Smad2 phosphorylation, but did not affect total Smad2/3 levels (Fig. 2C).

Promotion of SACC Cell Migration and Invasion by TGF-β1

Since TGF-β1 induced Smad2 phosphorylation, we used a wound-healing assay to examine whether TGF-β1 treatment could induce SACC-83 migration. Upon TGF-β1 treatment, SACC-83 cells exhibited increased motility, with a speed of 8.96 ± 2.06 μm/hr, as compared with the control group with a speed of 5.58 ± 1.07 μm/hr ($P < 0.05$) (Figs. 3A, 3B). When the cells were pre-treated with SB431542 prior to TGF-β1 treatment, the increased motility was significantly reduced to 5.16 ± 0.88 μm/hr ($P < 0.05$). Moreover, cells treated with SB431542 in the absence of TGF-β1 had a speed of 3.87 ± 0.58 μm/hr, which was even slower than that of the untreated control ($P < 0.05$). Similarly, we found that TGF-β1 also induced the invasion of SACC-83 cells, as determined by a transwell invasion assay (Fig. 3E). SB431542 potently inhibited TGF-β1-induced SACC-83 cell invasion (Fig. 3F).

Since SACC-LM cells expressed high levels of TGF-β1, we examined whether SACC-LM cells were more invasive than SACC-83 cells. The wound-healing assay revealed that SACC-LM cells exhibited significantly stronger migratory abilities than SACC-83 cells (11.17 ± 1.60 μm/hr *vs*. 5.58 ± 1.07 μm/hr; Figs. 3A, 3C). The transwell invasion assay showed that SACC-LM cells were more invasive than SACC-83 cells (183 ± 7 *per* field *vs*. 105 ± 8; Figs. 3E, 3G). To determine whether TGF-β1 was responsible for the higher invasive ability of SACC-LM cells, we treated SACC-LM cells with SB431542. The migratory speed of SACC-LM cells was reduced from 11.17 ± 1.60 μm/hr to 5.03 ± 0.76 μm/hr ($P < 0.05$, Fig. 3D). Similarly, SB431542 significantly inhibited SACC-LM cell invasion (183 ± 7 *vs*. 85 ± 16 *per* field; $P < 0.05$, Fig. 3H). Taken together, our results suggest that TGF-β1 promotes SACC cell migration and invasion.

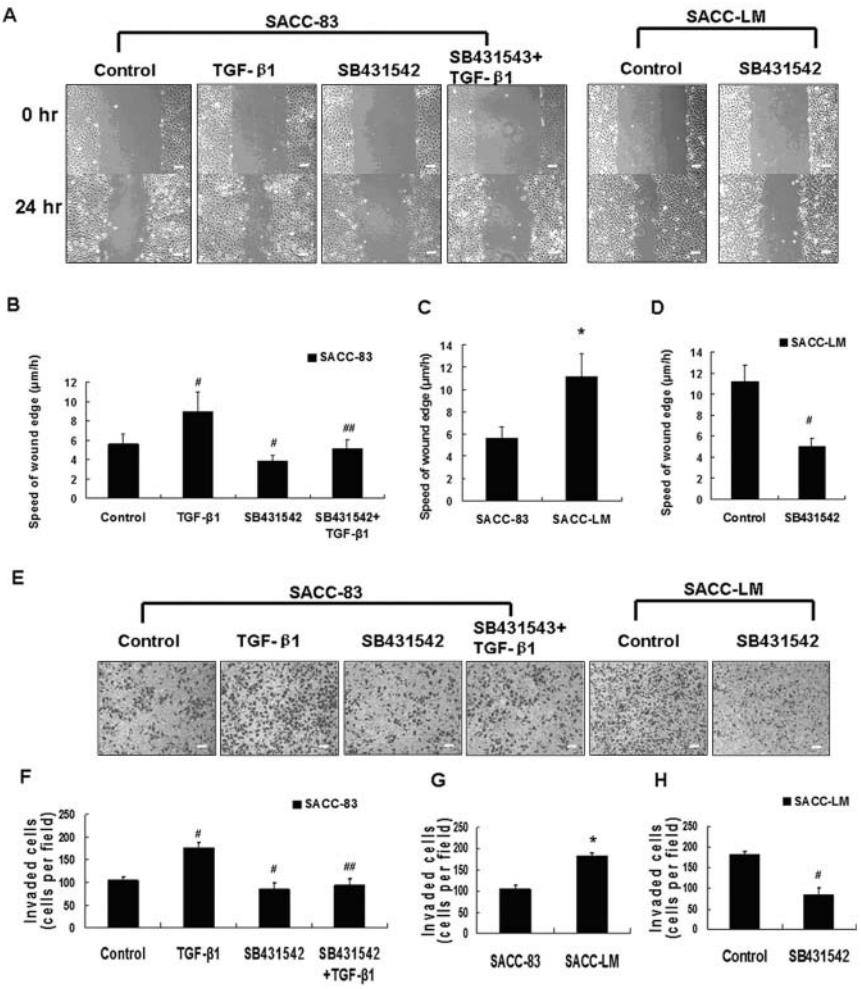

Figure 3. TGF-β1 promotes migration and invasion of SACC cells. **(A)** TGF-β1 promoted migration of SACC-83 and SACC-LM cells. SACC-83 cells were pre-treated with SB431542 or a vehicle control and then treated with TGF-β1. SACC-LM cells were treated with SB431542 or vehicle control only. Bars, 50 μm. **(B-D)** Qualitative measurement of the speed of SACC-83 and SACC-LM migration. $^{\#}P < 0.05$, in comparison with controls; $^{\#\#}P < 0.05$, in comparison with TGF-β1; $^{*}P < 0.05$ in comparison with SACC-83 cells. **(E)** TGF-β1 promoted invasion of SACC-83 and SACC-LM cells. SACC-83 cells were pre-treated with SB431542 or a vehicle control and then treated with TGF-β1. SACC-LM cells were treated with SB431542 or a vehicle control only. Bars, 50 μm. **(F-H)** Qualitative measurement of invasive SACC-83 and SACC-LM cells. $^{\#}P < 0.05$, in comparison with controls; $^{\#\#}P < 0.05$, in comparison with TGF-β1; $^{*}P < 0.05$ in comparison with SACC-83 cells.

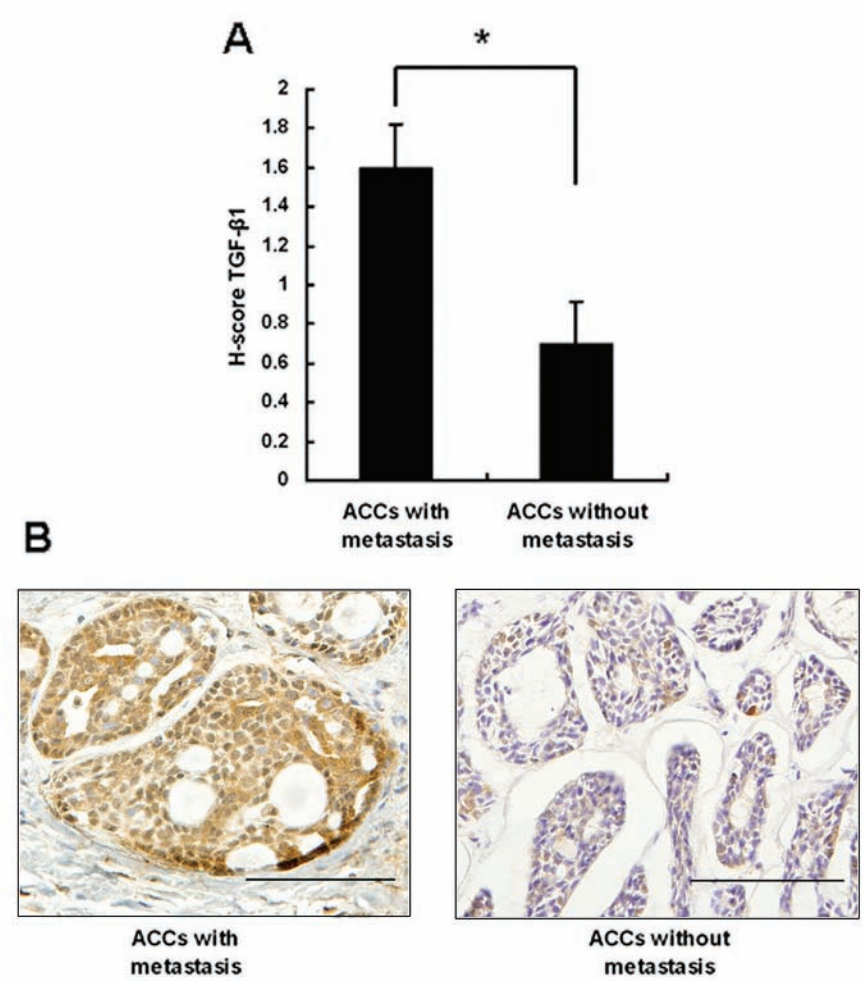

Figure 4. TGF-β1 is highly expressed in human primary SACC with metastasis. **(A)** TGF-β1 expression was significantly higher in human primary SACC with metastasis as compared with human primary SACC without metastasis. TGF-β1 staining was semi-qualitatively measured by the H-score. The statistical significance was determined by the Mann-Whitney U test. **(B)** Representative staining of TGF-β1 in human primary SACC with or without metastasis. Bars, 100 μm.

Increased TGF-β1 Expression in Human Primary SACC with Metastasis

Since our *in vitro* studies suggested that TGF-β1 promotes the migration and invasion of SACC cells, we performed immunostaining to determine whether TGF-β1 expression in primary tumors was associated with human SACC metastasis. Fifty human primary SACC tumor samples, including 23 samples without metastasis and 27 samples with metastasis, were immunostained with anti-TGF-β1 antibodies. The Mann-Whitney U test showed that TGF-β1 expression was significantly higher in human primary SACC samples with metastasis than in SACC samples without metastasis ($P < 0.05$; Fig. 4A). The representative staining showed that TGF-β1 was more strongly expressed in human primary SACC with metastasis as compared with human primary SACC without metastasis (Fig. 4B). There was no significant difference in TGF-β1 expression among tumor grades or ages (data not shown).

DISCUSSION

SACC has several unique characteristics, including a slow growth rate and frequent metastasis to distant organs such as the lung. Once distant metastasis has occurred, the prognosis of SACC is poor (Spiro *et al*., 1974; van der Wal *et al*., 2002). In this study, we demonstrated that TGF-β1 expression was at increased levels in highly metastatic SACC-LM cells. TGF-β1 promoted SACC migration and invasion. Moreover, we found that TGF-β1 was highly expressed in human SACC with metastasis, suggesting that TGF-β1 may play an important role in SACC metastasis. To the best of our knowledge, this is the first demonstration that TGF-β1 is associated with SACC metastasis.

TGF-β can be a tumor suppressor or a tumor promoter, depending on the stage of tumor development (Massagué, 2008). It is well accepted that TGF-β inhibits cell proliferation or induces apoptosis in normal epithelial cells. Consistently, inhibition of the TGF-β/Smad signaling pathway in normal epithelial cells and/or stromal cells has been found to promote epithelial cell transformation and oncogenesis. In contrast, metastatic tumor cells have been found to highly express TGF-β1. The inhibition of TGF-β/Smad signaling attenuates tumor invasive growth and metastasis (Massagué, 2008). TGF-β can promote tumor cell invasive growth through both cell-autonomous and non-cell-autonomous mechanisms. In this study, TGF-β1 potently stimulated the migration and invasion of SACC cells, suggesting that TGF-β1 can function in a cell-autonomous fashion. However, it is possible that TGF-β1 produced by SACC cells can also facilitate tumor growth and metastasis by modulating the tumor microenvironment. In future studies, it will be interesting to determine whether excess production of TGF-β1 by SACC cells can promote tumor angiogenesis and inhibit immune surveillance (Thomas and Massagué, 2005; Ma *et al*., 2007).

TGF-β1 may regulate tumor cell growth and survival through Smad-dependent or Smad-independent mechanisms (Derynck and Zhang, 2003; Moustakas and Heldin, 2005). We found that TGF-β1 induced Smad2 phosphorylation in SACC-83 cells in a time- and dose-dependent manner. TGF-β1 did not induce the phosphorylation of ERK1/2, p38, and JNK in SACC-83 cells. Our results suggest that TGF-β1-induced invasive growth in SACC cells is dependent on Smad signaling. It has been

reported that TGF-β1 can promote breast cancer cell motility through activation of the MAPK pathway, independent of Smad signaling (Dumont *et al*., 2003; Walsh *et al*., 2008). These different manners of activation may be attributed to the specific biological traits of SACC cells, which differ from those of other tumor cells. We observed that TGF-β1-induced Smad2 phosphorylation was decreased in SACC-83 cells in 6 hrs. This could be due to the short half-life of the exogenous TGF-β1. However, it was also possible that Smad signaling might induce its own signal decay and termination (Massagué, 2008). Our immunostaining confirmed that TGF-β1 was highly expressed in human primary SACC with metastasis as compared with SACC without metastasis. Since TGF-β1 is a secreted protein, it will be important to determine in future studies whether the levels of TGF-β1 are significantly elevated in the blood sera or saliva in patients with SACC metastasis. Positive outcomes may help to develop a biomarker to predict SACC metastasis.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (No. 39270723, 30572050, 30228028, and 39470754) and the Beijing Natural Science Foundation (No.7062069).

REFERENCES

Chu PG, Weiss LM (2002). Keratin expression in human tissues and neoplasms. *Histopathology* 40:403-439.

Derynck R, Zhang YE (2003). Smad-dependent and Smad-independent pathways in TGF-β family signaling. *Nature* 425:577-584.

Dumont N, Bakin AV, Arteaga CL (2003). Autocrine transforming growth factor-β1 signaling mediates Smad-independent motility in human cancer cells. *J Biol Chem* 278:3275-3285.

Fordice J, Kershaw C, El-Naggar A, Goepfert H (1999). Adenoid cystic carcinoma of the head and neck: predictors of morbidity and mortality. *Arch Otolaryngol Head Neck Surg* 125:149-152.

He JF, Ge MH, Zhu X, Chen C, Tan Z, Li YN, *et al*. (2008). Expression of RUNX3 in salivary adenoid cystic carcinoma: implications for tumor progression and prognosis. *Cancer Sci* 99:1334-1340.

Hu K, Li SL, Gan YH, Wang CY, Yu GY (2009). Epiregulin promotes migration and invasion of salivary adenoid cystic carcinoma cell line SACC-83 through activation of ERK1/2 and Akt. *Oral Oncol* 45:156-163.

Huang A, Pettigrew NM, Watson PH (1996). Immunohistochemical assay for oestrogen receptors in paraffin wax sections of breast carcinoma using a new monoclonal antibody. *J Pathol* 180:223-227.

Kokemueller H, Eckardt A, Brachvogel P, Hausamen JE (2004). Adenoid cystic carcinoma of the head and neck: a 20 years experience. *Int J Oral Maxillofac Surg* 33:25-31.

Li SL, Liu XP, Zhang KH (1990). Establishment of a human cancer cell line from adenoid cystic carcinoma of the minor salivary gland. *Zhonghua Kou Qiang Yi Xue Za Zhi* 25:29-31 (article in Chinese).

Liu C, Wu Z, Sun H (2009). The effect of simvastatin on mRNA expression of transforming growth factor-β1, bone morphogenetic protein-2 and vascular endothelial growth factor in tooth extraction socket. *Int J Oral Sci* 1:90-98.

Lu SL, Herrington H, Reh D, Weber S, Bornstein S, Wang D, *et al*. (2006). Loss of transforming growth factor-beta type II receptor promotes metastatic head-and-neck squamous cell carcinoma. *Genes Dev* 20: 1331-1342.

Ma J, Wang Q, Fei T, Han JD, Chen YG (2007). MCP-1 mediates TGF-β1–induced angiogenesis by stimulating vascular smooth muscle cell migration. *Blood* 109:987-994.

Massagué J (2008). TGF-β in cancer. *Cell* 134:215-230.

Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982). The catalog of human cytokeratins: pattern of expression in normal epithelia, tumors and cultured cells. *Cell* 31:11-24.

Moustakas A, Heldin CH (2005). Non-Smad TGF-β signals. *J Cell Sci* 118(Pt 16): 3573-3584.

Muller A, Sonkoly E, Eulert C, Gerber PA, Kubitza R, Schirlau K, *et al*. (2006). Chemokine receptors in head and neck cancer: association with metastatic spread and regulation during chemotherapy. *Int J Cancer* 118:2147-2157.

Phuchareon J, Ohta Y, Woo JM, Eisele DW, Tetsu O (2009). Genetic profiling reveals cross-contamination and misidentification of 6 adenoid cystic carcinoma cell lines: ACC2, ACC3, ACCM, ACCNS, ACCS, CAC2. *PLoS One* 4:e6040.

Platten M, Wick W, Wild-Bode C, Aulwurm S, Dichgans J, Weller M (2000). Transforming growth factors beta1 (TGF-beta1) and TGF-beta2 promote glioma cell migration via up-regulation of alphaVbeta3 integrin expression. *Biochem Biophys Res Commun* 268:607-611.

Saadoun S, Papadopoulos MC, Hara-Chikuma M, Verkman AS (2005). Impairment of angiogenesis and cell migration by targeted aquaporin-1 gene disruption. *Nature* 434:786-792.

Spiro RH, Huvos AG, Strong EW (1974). Adenoid cystic carcinoma of salivary origin: a clinicopathologic study of 242 cases. *Am J Surg* 128:512-520.

Takata T, Kudo Y, Zhao M, Ogawa I, Miyauchi M, Sato S, *et al*. (1999). Reduced expression of p27Kip1 protein in relation to salivary adenoid cystic carcinoma metastasis. *Cancer* 86:928-935.

Takayama S, Hatori M, Kurihara Y, Kinugasa Y, Shirota T, Shintani S (2009). Inhibition of TGF-beta1 suppresses motility and invasiveness of oral squamous cell carcinoma cell lines via modulation of integrins and down-regulation of matrix-metalloproteinases. *Oncol Rep* 21: 205-210.

Thomas DA, Massagué J (2005). TGF-β directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell* 8:369-380.

van der Wal JE, Becking AG, Snow GB, van der Waal I (2002). Distant metastases of adenoid cystic carcinoma of the salivary glands and the value of diagnostic examinations during follow-up. *Head Neck* 24:779-783.

Vékony H, Raaphorst FM, Otte AP, van Lohuizen M, Leemans CR, van der Waal I, *et al*. (2008). High expression of polycomb group protein EZH2 predicts poor survival in salivary gland adenoid cystic carcinoma. *J Clin Pathol* 61:744-749.

Walsh MF, Ampasala DR, Hatfield J, Vander Heide R, Suer S, Rishi AK, *et al*. (2008). Transforming growth factor-β stimulates intestinal epithelial focal adhesion kinase synthesis via Smad- and p38-dependent mechanisms. *Am J Pathol* 173:385-399.

Wei YY, Chen YJ, Hsiao YC, Huang YC, Lai TH, Tang CH (2008). Osteoblasts-derived TGF-β1 enhance motility and integrin upregulation through Akt, ERK1/2, and NF-κB-dependent pathway in human breast cancer cells. *Mol Carcinog* 47:526-537.

Zheng Q, Safina A, Bakin AV (2008). Role of high-molecular weight tropomyosins in TGF-β1-mediated control of cell motility. *Int J Cancer* 122:78-90.