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Enhancement of Metastatic and Invasive Capacity of Gastric Cancer Cells by Transforming Growth Factor-β1

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Abstract Transforming growth factor-β (TGF-β), a multifunctional cytokine, exerts contradictory roles in different kinds of cells. A number of studies have revealed its involvement in the progression of many types of tumors. To investigate the effect of TGF-β on gastric carcinoma, SGC7901, BGC823 and MKN28 (a TGF-β-resistant cell line) adenocarcinoma clones were used. After pretreatment in serum-free medium with or without 10 ng/ml TGF-β1, their experimental metastatic potential, chemotaxis, and invasive and adhesive ability were measured. Furthermore, zymography for gelatinase was processed. Liver colonies were also measured 4 weeks after inoculation of SGC7901, BGC823 and MKN28 in Balb/c nude mice, and an increase in the number of surface liver metastases was seen in SGC7901 (from 11.0±3.0 to 53.3±3.3) and BGC823 (from 9.3±2.5 to 60.0±2.8) groups, whereas there was no difference between MKN28 groups (from 35. 2±3.8 to 38.5±2.7). *In vitro* experiments showed that TGF-β1 increased the adhesion capacity of SGC7901 and BGC823 cells to immobilized reconstituted basement membrane/fibronectin matrices and promoted their penetration through reconstituted basement membrane barriers. Zymography demonstrated that enhanced invasive potential was partly due to the increased type IV collagenolytic (gelatinolytic) activity, but there was no difference in type IV collagenolytic activity and other biological behaviors between MKN28 groups. These results suggested that TGF-β1 might modulate the metastatic potential of gastric cancer cells by promoting their ability to break down and penetrate basement membrane barriers and their adhesive and motile activities. We speculated that TGF-β1 might act as a progression-enhancing factor in gastric cancer. Therefore blockage of TGF-β or TGF-β signaling might prevent gastric cancer cells from invading and metastasizing.

Key words gastric cancer; transforming growth factor-β; invasion; metastasis

Transforming growth factor-β (TGF-β), a family of 25 kDa homodimeric multifunctional regulatory peptides, is involved in various processes, including development, wound healing, and carcinogenesis [1]. Of the three isoforms of human TGF-β, TGF-β1 is the predominant form [2,3]. *In vitro*, TGF-β1 potently inhibits proliferations of nearly all epithelia and promotes adhesion by enhancing matrix production and decreasing proteolysis [1]. In addition, TGF-β1 is also involved in the events of apoptosis, replicative senescence, genomic stability, a negative angiogenic regulator profile and cellular immortalization [4,5]. Resistances to the negative growth-regulating properties of TGF-β1 have been observed in epithelial and mesenchymal tumors [6]. Tumor cell lines that lack TGF-β receptors lost responsiveness to TGF-β, and the escape of cells from TGF-β-mediated negative regulation was linked to tumor progression [7,8]. Furthermore, TGF-β1 has also been found to suppress the invasive potential of many types of cancer cell lines *in vitro*, such as renal cell carcinomas, fibrosarcoma and thyroid cancer cell lines [9–12]. All these indicated that TGF-β1 might serve as a tumor suppressor.

But interestingly, the more aggressive forms of tumors are growth-stimulated by TGF-β [13]. In breast cancer, expression of TGF-β1 was positively associated with invasion and metastasis [14,15]. Huang *et al*. reported that transfection of a TGF-β1 antisense expression plasmid

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decreased the tumorigenicity of colon cancer cells [3]. These observations suggested that TGF-β1 synthesized by tumor cells might facilitate tumorigenesis and hence regulate tumor cell behavior.

The mortality of gastric cancer patients is quite high in China. In clinical studies, it was reported that an elevated serum level and overexpression of TGF-β1 in primary gastric cancer were significantly correlated with lymph node metastasis and poor prognosis in patients with gastric carcinoma [5,16,17]. However, whether TGF-β1 influences the invasive and metastatic abilities of gastric cancer cells remains unknown. Our study was designed to determine the exact role of TGF-β1 on gastric cancer invasion and metastasis and to explore its possible mechanism through experiments both *in vitro* and *in vivo*. The results indicated that a brief exposure to TGF-β1 resulted in enhanced abilities of tumor cells to adhere to the epithelium monolayer (and matrix), penetrate a basement membrane-like matrix and migrate towards chemotactic factors *in vitro*. *In vivo*, TGF-β1 enhanced the formation of liver colonies in Balb/c nude mice.

Materials and Methods

Cell lines and tissue culture

Gastric cancer cell lines SGC7901 and BGC823 were purchased from Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The TGF-β-resistant gastric carcinoma cell line, MKN28 [18], was kindly provided by Dr. Shuyu JI (Kunming Medical College, Kunming, China). The human umbilican vein endothelial cells (HUVEC) were kindly provided by Dr. Dan LUO (Central South University, Changsha, China). The cells were maintained in RPMI 1640 supplemented with 10% calf serum in a CO_2 incubator (5% CO_2 and 95% air) at 37 ºC.

Expressions of TGF-β1 and TGF-β type II serine/threonine (Ser/Thr) receptor in untreated SGC7901, BGC823 and MKN28 cells

Expression of TGF-β1 and TGF-β type II Ser/Thr receptor in untreated SGC7901, BGC823 and MKN28 cells was measured by Western blotting. Briefly, total proteins were measured using a BCA protein assay kit (Pierce, Rockford, USA) according to the manufacturer's protocol. Forty micrograms of total proteins was electrophoresed on a 12.5% denaturing sodium dodecyl sulphate gel and transferred to a nitrocellulose membrane. The nitrocellulose membrane was then blocked with phosphate-buffered saline (PBS) containing 5% non-fat milk for 2 h at room temperature and with rabbit polyclonal antibody against TGF-β1 (Santa Cruz Biotechnology, Santa Cruz, USA) and TGF-β type II Ser/Thr receptor (Santa Cruz Biotechnology) overnight with gentle shaking. The membrane was washed with PBS twice for 5 min, and then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Zhongshan, Beijing, China) diluted at 1:3000 for 2 h at room temperature. After washing, TGF-β1 and TGF-β type II Ser/Thr receptor was detected using 3,3′-diaminobenzidine tetra-hydrochloride (DAB). Ponceau S staining was used as a loading control. Experiments were repeated three times.

Treatment with TGF-β1

Subconfluent cultures were seeded and, at the desired confluence (80%), the medium was removed. The cells were washed with pre-warmed D-Hanks solution and the medium was replaced with serum-free RPMI 1640 with or without 10 ng/ml of TGF-β1. The cells were cultured for 24 h before being used in other assays [19].

Experimental metastasis

Forty-eight Balb/c nude mice aged 4–6 weeks were purchased from Shanghai Laboratory Animal Company (Shanghai, China). They were divided randomly into six groups (eight mice in each group). Cancer cells were detached and resuspended in PBS. Subsequently, 0.2 ml of suspension containing 2×10^6 cells was injected into the abdominal cavity of each animal. The mice were killed after 4 weeks. All organs were examined for metastasis formation. The livers were removed and fixed in 10% formalin. The number of liver tumor colonies was counted under a magnifier. The representative liver tumors were removed, fixed, and embedded in paraffin, which were then sectioned into 4 μm layers and stained with hematoxylin-eosin for histological analysis [20–22].

In vitro invasion assay

The Transwell chamber (Corning, New York, USA) was used to measure the invasive abilities of cell lines, according to the modified protocol [20,23]. Briefly, a polycarbonate membrane containing 8 μM pores was coated with 100 μl 1:20 diluted Matrigel (Becton, Dickinson and Company, New York, USA) in cold RPMI 1640. Eight-hundred microliters of medium conditioned with 10 μg/ml fibronectin was placed in the lower compartment of the Transwell chamber as a chemoattractant. Subsequently, cells were resuspended in RPMI 1640 containing 1% calf serum and seeded into the upper wells of the chamber (5×10^4 cells/well). After incubation for 48 h at 37 ºC, the

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cells were fixed with 4% paraformaldehyde and stained with hematoxylin-eosin. After removing the cells attached on the upper side of the membrane by wiping with a wet cotton swab, those attached on the lower side of the membrane were counted under a microscope. Invasiveness was evaluated by the number of cells penetrating through the membrane per field (magnification, 400×).

Chemotaxis assay

Chemotaxis assay was performed with Transwell chambers containing 8 μM pores [24]. Eight-hundred microliters of RPMI 1640 containing 10 μg/ml fibronectin was placed in the lower compartment of the Transwell chamber. Tumor cells (1×10^5 per well) pretreated with or without TGF-β1 were added into the upper compartment and incubated for 6 h, and then the migrated cells were counted as described above.

Assay for adhesion of SGC7901, BGC823 and MKN28 cells to HUVEC monolayer

Adhesion of SGC7901, BGC823 and MKN28 cells to the HUVEC monolayer were assayed according to the method described in previous reports with some modification [23, 25]. Cells treated with or without TGF-β1 for 24 h were detached from culture dishes and suspended in RPMI 1640 containing 1% calf serum. When HUVEC seeded on 96-well plates reached confluency, the medium was replaced with previous cancer cell suspensions. The mixture was incubated at 37 ºC for 30 min, then fixed with 4% paraformaldehyde and stained with low keratin in an immunocytochemical way. Adhesion was evaluated by the number of labeled cells per field (magnification, 400×).

Adhesion to tissue culture plates coated with Matrigel or fibronectin

Each well of the 96-well tissue culture plate was coated with 50 μl of Matrigel (1:20 dilution), 10 mg/ml bovine serum albumin or 10 μg/ml fibronectin and left to air-dry in a hood overnight. Before use, the plate was incubated with 50 μl of serum-free RPMI 1640 containing 10 mg/ml bovine serum albumin per well for 1 h. One-hundred microliters of suspended tumor cells pretreated with or without TGF-β1 were seeded into each well and incubated in a CO_2 incubator. After incubation for 1 h, suspended cells that had not adhered to the coated plate were washed away, and the amounts of adhered cells were evaluated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay [23].

Zymography for gelatinase

Zymographic analysis of gelatinase activity in secreted medium was performed in 10% sodium dodecyl sulphate-polyacrylamide gels containing 1 mg/ml gelatin, as described in previous reports with some modification [20, 26]. Briefly, cells were subcultured in 50 ml tissue culture dishes. The next day, the cells were washed extensively and cultured in serum-free RPMI 1640 with or without 10 ng/ml TGF-β1. After incubating for 24 h, supernatant was collected and used for electrophoresis. The gels were incubated for 60 min in 50 mM Tris-HCl containing 2.5% Triton X-100 (pH 7.6), followed by incubation in incubation buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM $CaCl_2$, pH 7.8) at 37 ºC for 18 h. After they were stained with Coomassie blue, the gels were destained in a 45% (*V*/*V*) methanol/10% (*V*/*V*) acetic acid solution until the transparent bands were shown on the blue background. The gels were scanned in a gray-scale model and printed. Densitometric analysis (Uthscsa ImageTool version 2.0, S. Brent Dove, San Antonio, USA) was performed to quantify the results of zymography. The enzyme activity was measured quantitively by comparing the relative strap hydrolytic amount to that of matrix metalloproteinases (MMP)-9 in untreated SGC7901.

Statistical analysis

Results were expressed as mean±SD. Student's *t*-test was used for statistical analysis. In all statistical comparisons, $P<0.05$ was used to indicate a statistically significant difference.

Results

Expression of TGF-β1 and TGF-β type II Ser/Thr receptor in untreated SGC7901, BGC823 and MKN28 cells

Western blotting analysis showed no definite basal expression of active TGF-β1 in SGC7901, BGC823 or MKN28 cells. The expression of TGF-β type II receptor occurred in every kind of cell, even though MKN28 is a TGF-β-resistant gastric carcinoma cell line (**Fig. 1**).

Effect of TGF-β1 on liver metastasis

TGF-β1 (10 ng/ml) pretreatment increased liver metastases in Balb/c nude mice in SGC7901 and BGC823 groups compared with the untreated control (liver metastases numbers: 11.0±3.0 vs. 53.3±3.3 in SGC7901 groups and 9.3±2.5 vs. 60.0±2.8 in BGC823 groups; $P<0.05$). Of note, one mouse in the untreated SGC7901 group had no

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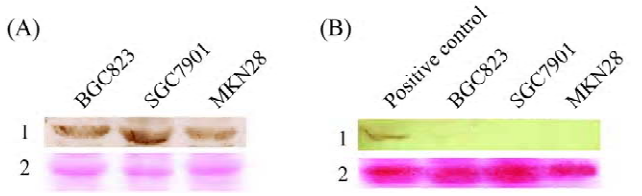

Fig. 1 Expression of transforming growth factor (TGF)-β1 and TGF-β type II serine/threonine (Ser/Thr) receptor in untreated SGC7901, BGC823 and MKN28 cells

(A) Expression of TGF-β type II Ser/Thr receptor. Protein stained by Ponceau S after electrophoresis. 1, Western blot analysis result; 2, loading control. (B) Expression of TGF-β1. Rat adrenal extract was used as the positive controls. 1, Western blot analysis result; 2, loading control.

metastasis. The experimental metastatic potential of MKN28 showed no alteration after pretreatment with TGF-β1 (35.2±3.8 vs. 38.5±2.7; $P>0.05$) (**Figs. 2** and **3**).

Effect of TGF-β1 on gastric cancer cell invasion across reconstituted basement membrane

Matrigel is a kind of basement membrane matrix extract, which is rich in laminin, type IV collagen, and heparan sulfate proteoglycans. Its structural and functional characteristics are similar to those of basement membranes *in vivo*, therefore it constitutes a valuable *in vitro* basis for assessing the invasive potential of tumor cells. Only cells that can digest the Matrigel matrix are capable of moving across this artificial basement membrane. To investigate the potential of TGF-β1 in promoting gastric cancer cell invasion through basement membranes, we used SGC7901, BGC823 and MKN28 cell lines in Transwell invasion assays. TGF-β1 (10 ng/ml) significantly promoted the invasiveness of cells in the SGC7901 groups (60±5 vs. 106±7; $P<0.05$) and BGC823 groups (49±5 vs. 88±6; $P<0.05$). The invasive abilities of the control and TGF-β1 pretreated MKN28 groups showed no difference (65±3 vs. 58±4; $P>0.05$) (**Figs. 4** and **5**).

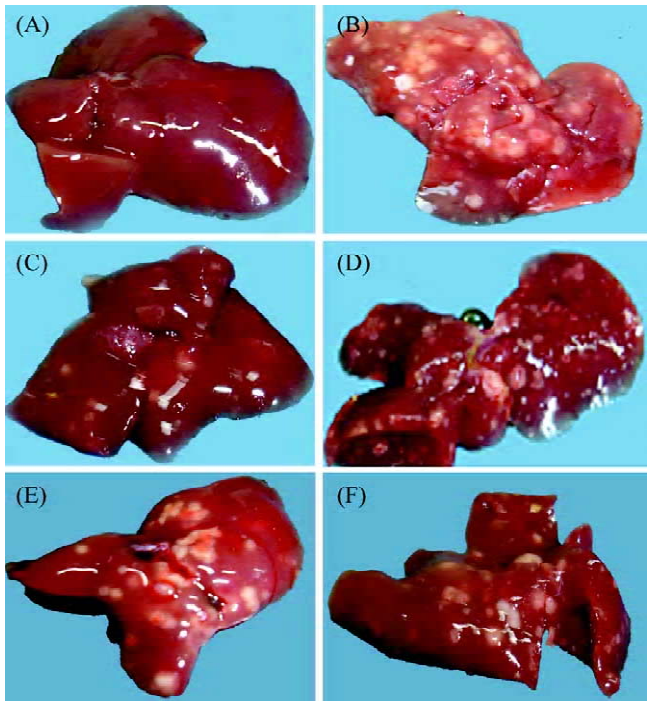

Fig. 3 Effect of transforming growth factor (TGF)-β1 on liver metastasis of SGC7901, BGC823 and MKN28 cells

(A) SGC7901 treated with 0 ng/ml TGF-β1. (B) SGC7901 treated with 10 ng/ml TGF-β1. (C) BGC823 treated with 0 ng/ml TGF-β1. (D) BGC823 treated with 10 ng/ml TGF-β1. (E) MKN28 treated with 0 ng/ml TGF-β1. (F) MKN28 treated with 10 ng/ml TGF-β1.

Effects of TGF-β1 on adhesion to Matrigel and fibronectin

Pretreatment with TGF-β1 promoted the adhesiveness

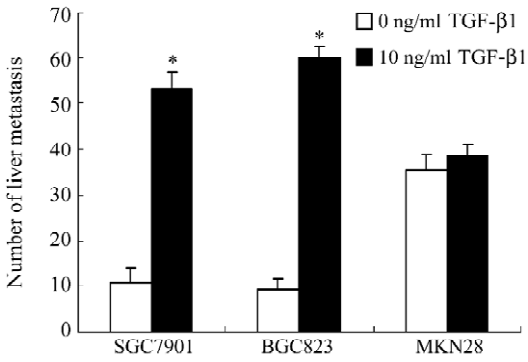

Fig. 2 Effect of transforming growth factor (TGF)-β1 on liver metastasis in nude mice

* $P<0.05$ vs. 0 ng/ml TGF-β1.

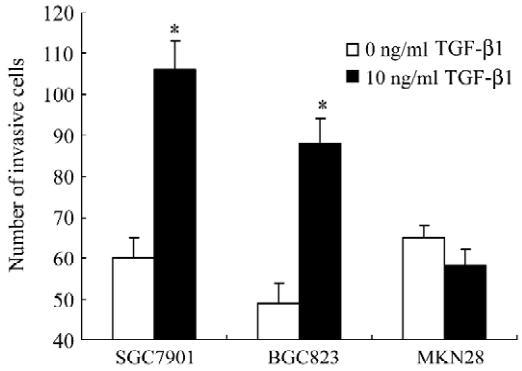

Fig. 4 Effect of transforming growth factor (TGF)-β1 on invasion of SGC7901, BGC823 and MKN28 cells

* $P<0.05$ vs. 0 ng/ml TGF-β1.

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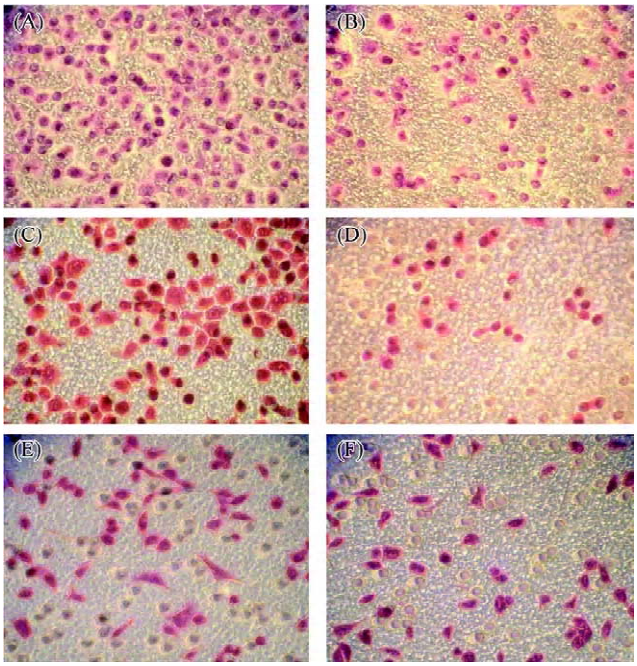

Fig. 5 Effect of transforming growth factor (TGF)-β1 on invasion of SGC7901, BGC823 and MKN28 cells

(A) SGC7901 treated with 0 ng/ml TGF-β1. (B) SGC7901 treated with 10 ng/ml TGF-β1. (C) BGC823 treated with 0 ng/ml TGF-β1. (D) BGC823 treated with 10 ng/ml TGF-β1. (E) MKN28 treated with 0 ng/ml TGF-β1. (F) MKN28 treated with 10 ng/ml TGF-β1.

to Matrigel and fibronectin significantly in SGC7901 and BGC823 groups, but it had no effect on adhesion of MKN28 cells to the matrix (**Table 1**).

Effect of TGF-β1 on adhesion of cancer cells to HUVEC

SGC7901 and BGC823 cells pretreated with TGF-β1 attached to the HUVEC monolayer at significantly higher rates than the untreated control (15.97±1.38 vs. 6.57±1.14 in SGC7901 groups and 19.07±2.01 vs. 11.12±1.75 in BGC823 groups; $P<0.05$). The number of untreated and treated MKN28 cells was 7.58±1.12 and 8.67±1.23, respectively ($P>0.05$) (**Fig. 6**).

Table 1 Adhesion of SGC7901, BGC823 and MKN28 cells to fibronectin and matrigel matrices after treatment with transforming growth factor (TGF)-β1

Cell line	Fibronectin		Matrigel	
	TGF-β1[a]	TGF-β1[b]	TGF-β1[a]	TGF-β1[b]
SGC7901	12.3%	46.5%*	33.8%	77.8%*
BGC823	11.2%	53.9%*	29.4%	68.7%*
MKN28	12.1%	13.5%	30.8%	33.2%

[a] 0 ng/ml TGF-β1; [b] 10 ng/ml TGF-β1. *$P<0.05$ vs. 0 ng/ml TGF-β1.

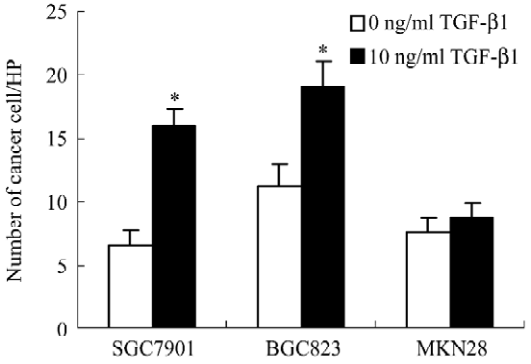

Fig. 6 Effect of transforming growth factor (TGF)-β1 on adhesion of SGC7901, BGC823 and MKN28 cells to human umbilican vein endothelial cells

*$P<0.05$ vs. 0 ng/ml TGF-β1. HP, high power objective.

Effect of TGF-β1 on chemotaxis of gastric cancer cells

The motility of tumor cells is an essential property to invade the endothelium or extracellular matrix. Compared with untreated SGC7901 and BGC823 cells, TGF-β1-treated SGC7901 and BGC823 cells showed higher chemotactic activity to fibronectin (78±8 vs. 35±6 in SGC7901 groups and 85±9 vs. 40±7 in BGC823 groups; $P<0.05$). However, TGF-β1 had no effect on chemotaxis of MKN28 to fibronectin (41±3 and 46±2, respectively; $P>0.05$) (**Figs. 7** and **8**).

Zymography for gelatinase

To further elucidate the phenotypic difference between

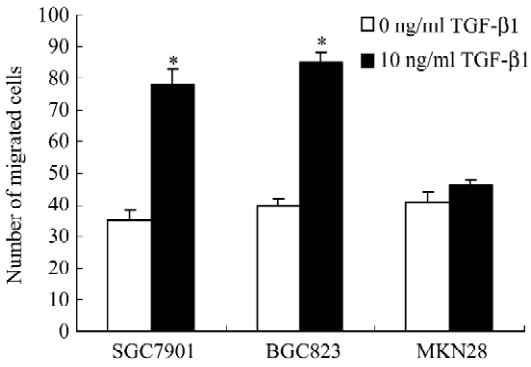

Fig. 7 Alteration of migration of SGC7901, BGC823 and MKN28 cells after treatment with transforming growth factor (TGF)-β1

*$P<0.05$ vs. 0 ng/ml TGF-β1.

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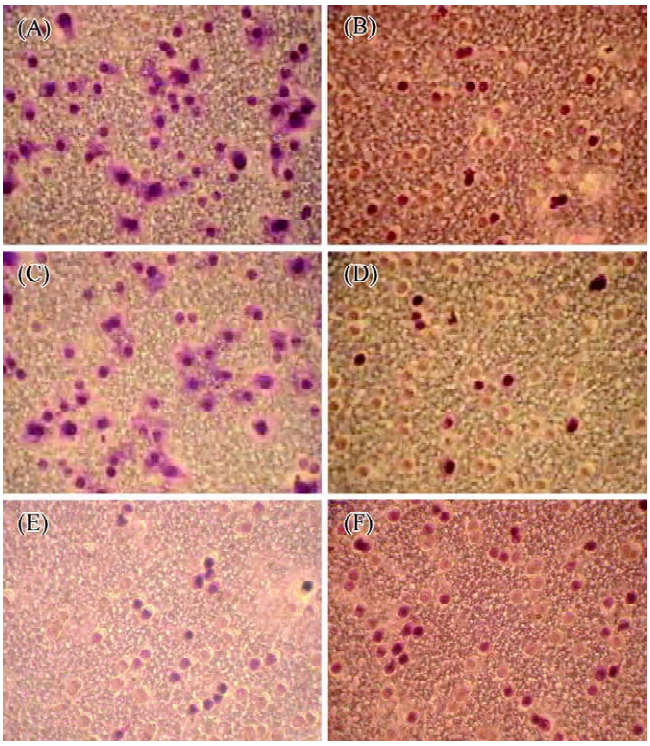

Fig. 8 Alteration of migration of SGC7901, BGC823 and MKN28 cells after treatment with transforming growth factor (TGF)-β1

(A) 10 ng/ml TGF-β1 SGC7901. (B) 0 ng/ml TGF-β1 SGC7901. (C) 10 ng/ml TGF-β1 BGC823. (D) 0 ng/ml TGF-β1 BGC823. (E) 10 ng/ml TGF-β1 MKN28. (F) 0 ng/ml TGF-β1 MKN28.

treated and untreated cells, zymographic analysis was used to assess whether their invasive nature correlated with their gelatinase activity. Activity of 72 kDa and 92 kDa gelatinase was observed in all cells. Compared with the untreated control, the activity of 72 kDa and 92 kDa gelatinase increased in treated SGC7901 and BGC823 groups ($P<0.05$). However, there were no differences between MKN28 groups (**Table 2** and **Fig. 9**).

Table 2 Alteration of type IV collagenolytic activity of SGC7901, BGC823 and MKN28 cells after treatment with transforming growth factor (TGF)-β1

Cell line	MMP-9		MMP-2	
	TGF-β1[a]	TGF-β1[b]	TGF-β1[a]	TGF-β1[b]
SGC7901	1.00±0.00	3.36±0.05*	2.20±0.23	4.09±0.09*
BGC823	1.12±0.02	3.46±0.18*	1.36±0.08	3.78±0.23*
MKN28	0.55±0.03	0.63±0.05	0.85±0.09	0.97±0.11

Data were represented as mean±SD. [a] 0 ng/ml TGF-β1; [b] 10 ng/ml TGF-β1. *$P<0.05$ vs. 0 ng/ml TGF-β1.

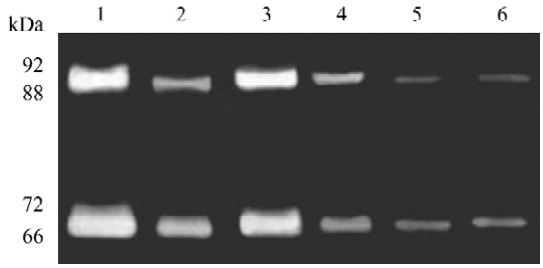

Fig. 9 Type IV collagenolytic activity of SGC7901, BGC823 and MKN28 cells after treatment with transforming growth factor (TGF)-β1

1, 10 ng/ml TGF-β1 on SGC7901; 2, 0 ng/ml TGF-β1 on SGC7901; 3, 10 ng/ml TGF-β1 on BGC823; 4, 0 ng/ml TGF-β1 on BGC823; 5, 10 ng/ml TGF-β1 on MKN28; 6, 0 ng/ml TGF-β1 on MKN28.

Discussion

Invasion and metastasis are the major obstacles in the treatment of malignancy, and are the major causes of death. Investigations showed that TGF-β participated in invasion and metastasis by modulating the ability of cells in invading the extracellular matrix and basement membrane [27]. For example, TGF-β could promote the invasion and metastasis of mammary tumors [28]. But TGF-β was also found to suppress the invasive potential of many kinds of cancer cell lines *in vitro* [9–12]. Recent studies indicated that TGF-β upregulates the expression of the cyclin-dependent kinase inhibitors p15 and p21 and inhibits cell proliferation by causing cell cycle arrest at the G1 phase [29]. So the exact role of TGF-β in tumor cells is still unclear. It seemed that TGF-β might exert different, even contradictory biological effects on different tumors. In this study we focused on what kind of role TGF-β may exert during the progression of gastric cancer, suppressor or accelerator?

SGC7901 and BGC823 are sensitive to TGF-β [30,31]; however, MKN28 is a TGF-β-resistant gastric carcinoma cell line. It is further confirmed by our data that liver metastatic and invasive capacities of SGC7901 and BGC823 were enhanced by pretreatment with TGF-β1, and the metastatic and invasive potential of MKN28 did not alter after pretreatment with TGF-β1. It is well known that TGF-β initiates its signal through binding the TGF-β type II Ser/Thr receptor kinase, which in turn phosphorylates the type I Ser/Thr receptor, forming an activated receptor complex. Then the activated receptor complex transmits the TGF-β signal to a set of intracellular mediators, known as the Smad proteins. Based on these facts, we speculated that the TGF-β signal pathway might be intact in SGC7901 and BGC823, which was further confirmed by our find-

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ing that there was high-level expression of the TGF-β type II Ser/Thr receptor in SGC7901 and BGC823. However, expression of the TGF-β type II Ser/Thr receptor was also detected in MKN28. So the resistance of MKN28 to TGF-β might be due to other mechanisms such as low binding activity of the type I receptor to TGF-β [18], mutation of RUN-X3 [32] and so on, which are crucial in the transmission of the TGF-β signal.

Blood-borne tumor cells must attach to the endothelium and matrix to establish metastatic colonies in a distant organ, therefore the interaction of the tumor cells with the endothelium and matrix is very important. The adhesion assay *in vitro* using the endothelial cell monolayer revealed that TGF-β1 increased the adherent capacity of SGC7901 and BGC823. We speculated that the augmented adhesion to the endothelium monolayer by TGF-β might be due in part to increased expression of CD44H and β1 integrin [21]. Furthermore, our data showed that pretreatment with TGF-β1 significantly promoted the adhesiveness of SGC7901 and BGC823 cells to Matrigel and fibronectin.

It is well known that a defect of the basement membrane of the epithelium is the signal of invasion and that tumor cells invading the extracellular matrix rely on three sequential biological events [33,34]: attachment to components of the matrix; production of matrix degradative enzymes; and tumor cell locomotion into the region of proteolyzed matrix. In this study, we focused on the interaction between these three cell lines and the matrix *in vitro* and analyzed mechanisms of TGF-β1 on metastasis of SGC7901, BGC823 and MKN28 cells.

Degradation of the matrix is the key point of invasion. During this process, matrix metalloproteinases exert critical roles. Overexpression of gelatinase A significantly correlated with the invasion of esophageal carcinoma, metastasis of esophageal carcinoma and gastric cancer. Zymography indicated that activity of gelatinase A positively correlated with the grading of breast carcinoma and local invasion of gastric cancer. Compared with non-metastasis cases, pulmonary carcinoma with nodal metastasis displayed higher gelatinase A activity [35]. The expression and activity of gelatinase B also positively correlated with the grading of breast carcinoma [36]. In our study, higher levels of MMP-2 and MMP-9 activity were observed in SGC7901 and BGC823 cells after pretreatment with TGF-β1, indicating TGF-β1 facilitates MMP production. Extracellular matrix degradation of similar results have been reported by Welch *et al.* [19].

Migration of tumor cells is the rate-limiting step of metastasis. Generally, migration ability positively correlated with metastasis potential. Yoshinaga *et al.* found that the migratory ability of metastatic melanoma cells in lamin and collagen was much greater than that of primary melanoma cells [37]. With their chemotaxis and haptotaxis to stimulus, tumor cells metastasize to remote sites. Studies showed that many kinds of substances, such as growth factors, could stimulate the migration of tumor cells. Our research revealed that TGF-β1 could enhance the migration of both kinds of TGF-β-sensitive gastric cancer cells.

In this study, we found that TGF-β1 was involved in gastric cancer progression and metastases, in spite of the fact that TGF-β signaling is impaired in most gastric cancers [38]. Recently, Santibanez *et al.* and Kim *et al.* found that TGF-β could mediate the expression of MMP-2 and MMP-9 through the mitogen-activated protein kinase signaling pathway [39,40]. Therefore the cross-talk between TGF-β-signaling and other signaling pathways might be the mechanism responsible for TGF-β1-involved gastric cancer progression and metastases. However, to clarify the issue, further research is needed.

In conclusion, our study postulated that TGF-β might be regarded as one of the factors that enhance malignant progression of gastric cancer cell lines. In clinical gastric cancer cases, TGF-β-positive tumor cells seemed to express more malignant phenotypes, for example, serosal invasion, infiltrative growth and lymph node metastasis were more prominent in TGF-β1-positive cases [16]. So it is possible for TGF-β to serve as a potential target for gene therapy in TGF-β positive gastric cancer and blockage of TGF-β or TGF-β signaling might prevent gastric cancer cells from invading and metastasizing. More evidence is needed to confirm this postulation.

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References

1 Massague J. The transforming growth factor-beta family. Annu Rev Cell Biol 1990, 6: 597–641
2 Tsushima H, Kawata S, Tamura S, Ito N, Shirai Y, Kiso S, Imai Y *et al.* High levels of transforming growth factor beta 1 in patients with colorectal cancer: Association with disease progression. Gastroenterology 1996, 110: 375–382
3 Huang F, Newman E, Theodorescu D, Kerbel RS, Friedman E. Transforming growth factor β1 (TGFβ1) is an autocrine positive regulator of colon carcinoma U9 cells *in vivo* as shown by transfection of a TGFβ1 antisense expression plasmid. Cell Growth Differ 1995, 6: 1635–1642
4 Wakefield LM, Roberts AB. TGF-β signaling: Positive and negative effects on tumorigenesis. Curr Opin Genet Dev 2002, 12: 22–29

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5 Derynck R, Akhurst RJ, Balmain A. TGF-beta signaling in tumor suppression and cancer progression. Nat Genet 2001, 29: 117–129

6 Lahm H, Odartchenko N. Role of transforming growth factor beta in colorectal cancer. Growth Factors 1993, 9: 1–9

7 Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS *et al*. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science 1995, 268: 1336–1338

8 Parsons R, Myeroff LL, Liu B, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. Cancer Res 1995, 55: 5548–5550

9 Pavelic K, Despot N, Levanat S, Casl T. Protective role of transforming growth factor beta (TGF beta) in tumor-induced degradation of basement membranes. Biol Chem Hoppe Seyler 1990, 371: 687–692

10 Holting T, Zielke A, Siperstein AE, Clark OH, Duh QY. Transforming growth factor-beta 1 is a negative regulator for differentiated thyroid cancer: Studies of growth, migration, invasion, and adhesion of cultured follicular and papillary thyroid cancer cell lines. J Clin Endocrinol Metab 1994, 79: 806–813

11 Otani N, Tsukamoto T, Masumori N, Saiki I, Yoneda J, Kumamoto Y. Influence of growth factors on *in vitro* invasiveness and type IV collagenolysis of human renal cell carcinoma cells. J Urol 1994, 151: 223–226

12 Otani N, Tsukamoto T, Kumamoto Y, Miyao N. Study on *in vitro* invasive potential of renal cell carcinoma cell lines and effect of growth factors (EGF and TGF-beta 1) on their in invasions. Nippon Hinyokika Gakkai Zasshi 1991, 82: 613–619

13 Mooradian DL, Purchio AF, Furcht LT. Differential effects of transforming growth factor beta 1 on the growth of poorly and highly metastatic murine melanoma cells. Cancer Res 1990, 50: 273–277

14 Walker RA, Dearing SJ, Gallacher B. Relationship of transforming growth factor beta 1 to extracellular matrix and stromal infiltrates in invasive breast carcinoma. Br J Cancer 1994, 69: 1160–1165

15 Gorsch SM, Memoli VA, Stukel TA, Gold LI, Arrick BA. Immunohistochemical staining for transforming growth factor beta 1 associates with disease progression in human breast cancer. Cancer Res 1992, 52: 6949–6952

16 Maehara Y, Kakeji Y, Kabashima A, Emi Y, Watanabe A, Akazawa K, Baba H *et al*. Role of transforming growth factor-beta 1 in invasion and metastasis in gastric carcinoma. J Clin Oncol 1999, 17: 607–614

17 Saito H, Tsujitani S, Oka S, Kondo A, Ikeguchi M, Maeta M, Kaibara N. An elevated serum level of transforming growth factor-beta 1 (TGF-beta 1) significantly correlated with lymph node metastasis and poor prognosis in patients with gastric carcinoma. Anticancer Res 2000, 20: 4489–4493

18 Ito M, Yasui W, Kyo E, Yokozaki H, Nakayama H, Ito H, Tahara E. Growth inhibition of transforming growth factor beta on human gastric carcinoma cells: Receptor and postreceptor signaling. Cancer Res 1992, 52: 295–300

19 Welch DR, Fabra A, Nakajima M. Transforming growth factor β stimulates mammary adenocarcinoma cell invasion and metastatic potential. Proc Natl Acad Sci USA 1990, 87: 7678–7682

20 Chu YW, Yang PC, Yang SC, Shyu YC, Hendrix MJ, Wu R, Wu CW. Selection of invasive and metastatic subpopulations from a human lung adenocarcinoma cell line. Am J Respir Cell Mol Biol 1997, 17: 353–360

21 Nakashio T, Narita T, Akiyama S, Kasai Y, Kondo K, Ito K, Takagi H *et al*. Adhesion molecules and TGFβ1 are involved in the peritoneal dissemination of NUGC-4 human gastric cancer cells. Int J Cancer 1997, 70: 612–618

22 Nishimura S, Chung YS, Yashiro M, Inoue T, Sowa M. CD44H plays an important role in peritoneal dissemination of scirrhous gastric cancer cells. Jpn J Cancer Res 1996, 87: 1235–1244

23 Situ ZQ, Wu JZ. Cell Culture. Modified edition. Xi'an: Xi'an World Publication Corporation 2003

24 Hamada J, Nagayasu H, Takayama M, Kawano T, Hosokawa M, Takeichi N. Enhanced effect of epidermal growth factor on pulmonary metastasis and *in vitro* invasion of rat mammary carcinoma cells. Cancer Lett 1995, 89: 161–167

25 Wu JZ, Situ ZQ, Liu ZB. Selection and characterization of a highly metastatic cell clone from mucoepidermoid carcinoma cell line derived from human salivary gland. Chin J Dental Res 1998, 1: 71–77

26 Wang L, Zhang LH, Li YL, Li YL, Liu Z. Expression of MMP-9 and MMP-9 mRNA in gastric carcinoma and its correlation with angiogenesis. Zhonghua Yi Xue Za Zhi 2003, 83: 782–786

27 Kretzschmar M, Massague J. SMADs: Mediators and regulators of TGF-beta signaling. Curr Opin Genet Dev 1998, 8: 103–111

28 Nakata D, Hamada J, Ba Y, Matsushita K, Shibata T, Hosokawa M, Moriuchi T. Enhancement of tumorigenic, metastatic and *in vitro* invasive capacity of rat mammary tumor cells by transforming growth factor-β. Cancer Lett 2002, 175: 95–106

29 Ravitz MJ, Wenner CE. Cyclin-dependent kinase regulation during G1 phase and cell cycle regulation by TGF-beta. Adv Cancer Res 1997, 71: 165–207

30 Hu ZL, Wen JF, Xiao DS, Zhen H, Fu CY. Effects of transforming growth interacting factor on biological behaviors of gastric carcinoma cells. World J Gastroenterol 2005, 11: 84–88

31 Li X, Zhang YY, Wang Q, Fu SB. Association between endogenous gene expression and growth regulation induced by TGF-β1 in human gastric cancer cells. World J Gastroenterol 2005, 11: 61–68

32 Li QL, Ito K, Sakakura C, Fukamachi H, Inoue K, Chi XZ, Lee KY *et al*. Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell 2002, 109: 113–124

33 Liotta LA. Tumor invasion and metastases – role of the extracellular matrix: Rhoads Memorial Award lecture. Cancer Res 1986, 46: 1–7

34 Jiang XN, Zhou RL. The relationship between adhesion, migration of cancer cells and metastasis. Prog Biochem Biophys 1998, 25: 404–407

35 Tokuraku M, Sato H, Murakami S, Okada Y, Watanabe Y, Seiki M. Activation of the precursor of gelatinase A/72 kDa type IV collagenase/MMP-2 in lung carcinomas correlates with the expression of membrane-type matrix metalloproteinase (MT-MMP) and with lymph node metastasis. Int J Cancer 1995, 64: 355–359

36 Davies B, Miles DW, Happerfield LC, Naylor MS, Bobrow LG, Rubens RD, Balkwill FR. Activity of type IV collagenases in benign and malignant breast disease. Br J Cancer 1993, 67: 1126–1131

37 Yoshinaga IG, Vink J, Dekker SK, Mihm MC Jr, Byers HR. Role of α3β1 and α2β1 integrins in melanoma cell migration. Melanoma Res 1993, 3: 435–441

38 Park K, Kim SJ, Bang YJ, Park JG, Kim NK, Roberts AB, Sporn MB. Genetic changes in the transforming growth factor β (TGF-β) type II receptor gene in human gastric cancer cells: Correlation with sensitivity to growth inhibition by TGF-β. Proc Natl Acad Sci USA 1994, 91: 8772–8776

39 Santibanez JF, Guerrero J, Quintanilla M, Fabra A, Martinez J. Transforming growth factor-β1 modulates matrix metalloproteinase-9 production through the Ras/MAPK signaling pathway in transformed keratinocytes. Biochem Biophys Res Commun 2002, 296: 267–273

40 Kim ES, Kim MS, Moon A. TGF-beta-induced upregulation of MMP-2 and MMP-9 depends on p38 MAPK, but not ERK signaling in MCF10A human breast epithelial cells. Int J Oncol 2004, 25: 1375–1382

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