Effect of KLF17 overexpression on epithelial–mesenchymal transition of gastric cancer cells

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Abstract
Objective: To investigate Krüppel-like factor 17 (KLF17) expression in normal and gastric cancer tissues and cell lines.

Methods: Levels of KLF17 mRNA and protein in GES-1 normal gastric mucosal cells, and NCI-N87, SGC-7901, BGC-823 and HGC-27 gastric cancer cells were analysed by quantitative polymerase chain reaction (qPCR) and western blot. Differences in KLF17 expression between gastric cancer and adjacent tissues were analysed by qPCR and immunohistochemistry. Invasion/migration effects of KLF17 overexpression in BGC-823 and HGC-27 cells were analysed by wound-healing and Transwell chamber assays. Changes in expression of KLF17 and epithelial–mesenchymal transition (EMT)-related genes (matrix metalloproteinase [MMP]-9, vimentin and E-cadherin) were analysed in BGC-823 and HGC-27 cells before and after transfection using qPCR and western blot. Transforming growth factor (TGF)-β1, Smad family member (Smad)2/3 and phosphorylated-Smad2/3 levels in BGC-823 and HGC-27 cells were assessed by qPCR and western blot.

Results: KLF17 expression was lower in gastric cancer versus adjacent tissues, and in gastric cancer cell lines versus GES-1 normal gastric mucosal cells, and was positively correlated with degree of cancer-cell differentiation. Wound-healing and Transwell assays showed decreased migration and invasion ability of BGC-823 and HGC-27 cells transfected to overexpress KLF17. KLF17 overexpression was associated with decreased MMP-9 and vimentin in BGC-823 and HGC-27 cancer cells, and increased KLF17 and E-cadherin. KLF17 overexpression also resulted in decreased levels of TGF-β1 and p-Smad2/3 in BGC-823 and HGC-27 cancer cells.

Conclusion: KLF17 is poorly expressed in gastric cancer tissues and cell lines. KLF17 overexpression might inhibit EMT via the TGF-β/Smad pathway, thereby reducing gastric

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cancer cell invasion and migration. Therefore, KLF17 may become a novel target for treating gastric cancer.

Keywords
Gastric cancer, Krüppel-like factor 17, epithelial–mesenchymal transition, TGF-β/Smad pathway, invasion, migration

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Introduction
Cancer is the second leading cause of death worldwide, accounting for one-sixth of all reported deaths each year, and gastric cancer is the third most common cause of worldwide cancer-related death. The pathogenesis of gastric cancer remains unclear, but high recurrence and low survival rates are associated with invasion and metastasis. Epithelial–mesenchymal transition (EMT) refers to the transformation of epithelial-derived tumour cells to mesenchymal cells under pathological conditions, which plays a key role in tumour invasion and metastasis. The occurrence and development of gastric cancer has been shown to correlate with the activation of EMT. Mesenchymal cells tend to dedifferentiate, in order to obtain tumorigenic and stem cell phenotypes, causing loss of the ability to adhere to cells, and thereby inducing cell invasion and metastasis. Krüppel-like factors (KLFs), a type of zinc finger protein widely expressed in eukaryotic cells, play a role in transcriptional regulation. Comprising 17 members, KLFs have complex functions, and serve as tumour suppressor or tumour promoter genes in human tumour cells, depending on the specific tumour type. KLF17 is closely associated with the occurrence and development of a variety of epithelial tumours, participating in the regulation of tumour invasion and metastasis, and reduced KLF17 expression has been correlated with poor survival in patients with gastric cancer. In addition, KLF17 has been shown to participate in the EMT process of gastric cancer. However, the expression of KLF17 in gastric cancer cell lines and its relationship with EMT have not been fully elucidated.

The first aim of the present study was to investigate KLF17 expression in gastric cancer and adjacent normal tissues using quantitative polymerase chain reaction (qPCR) and immunohistochemistry (IHC), and KLF17 expression in normal human gastric mucosal cells and gastric cancer cell lines of varying states of differentiation using qPCR and western blots. A further aim was to use plasmid vectors to establish human gastric cancer cell lines (BGC-823 and HGC-27) that overexpressed KLF17, in order to investigate the effects of KLF17 upregulation on cell migration and invasion in vitro using wound-healing and Transwell chamber assays. In addition, differences in KLF17, matrix metalloproteinase (MMP)-9, vimentin and E-cadherin levels, before and after transfection, were investigated by qPCR and western blot. The transforming growth factor (TGF)-β/Smad family member (Smad) signalling pathway is known to be involved in regulating the EMT process of various tumour cells. Thus, in order to further determine whether KLF17 overexpression inhibits the tumour EMT process (and
consequently cancer cell invasion and metastasis) via this pathway, changes in TGF-β/Smad pathway-related proteins, TGF-β1, Smad2/3 and phosphorylated (p)-Smad2/3, were analysed by qPCR and western blot in transfected gastric cancer cells that over-expressed KLF17 and were subjected to exogenous TGF-β1 stimulating factor.

Materials and methods

Tissue specimens

Tissue specimens were obtained from patients with gastric cancer, who underwent gastric cancer resection at the Department of Gastrointestinal Surgery, The Fourth Hospital of Hebei Medical University, between September 2019 and March 2020. The research protocol was approved by the Ethics Committee of The Fourth Hospital of Hebei Medical University, and prior to surgery, all patients provided written informed consent for the use of tissue samples in research. Patients with other malignant tumours, and patients receiving preoperative radiotherapy, chemotherapy and biological therapy were excluded from the present study. One piece of tissue (approximately 1.0 × 0.5 × 0.5 cm) was collected from each of cancerous and adjacent normal tissues (>3 cm from the edge of the cancerous tissue), respectively, frozen in liquid nitrogen within 15 min after surgical resection, and stored at –80°C until subsequent RNA extraction. In addition, one piece of tissue each (approximately 1.0 × 0.5 × 0.5 cm) was collected from cancerous and adjacent normal and fixed with 4% paraformaldehyde for subsequent analysis by IHC.

Cell lines and culture

Five cell lines were selected for the present study (purchased from the Cell Bank of Chinese Academy of Sciences [Shanghai, China]): GES-1 normal human gastric mucosal epithelial cells; NCI-N87 well-differentiated human gastric adenocarcinoma cells; SGC7901 moderately differentiated human gastric adenocarcinoma cells; BGC823 poorly differentiated human gastric adenocarcinoma cells; and HGC-27 undifferentiated human gastric adenocarcinoma cells. After resuscitation, cells were cultured in tissue culture flasks with RPMI-1640 medium (Gibco [Thermo Fisher Scientific], Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Biological Industries, Kibbutz Beit-Haemek, Israel), 100 U/ml penicillin and 100 mg/ml streptomycin (Solarbio Science and Technology Co., Beijing, China). Cells were incubated at 37°C with 5% CO₂, and the medium was changed every other day. Digestion and passage were performed using 0.3% pancreatin (Gibco), and cells were harvested at logarithmic growth phase for subsequent experiments.

Quantification of KLF17 mRNA in each cell line by qPCR

The GES-1, NCI-N87, SGC7901, BGC823 and HGC-27 cells were lysed with TRIzol and total RNA was isolated using a column-based total RNA extraction kit (New Cell and Molecular Biotech Co., Suzhou, China), according to the manufacturer’s instructions. The extracted RNA was then reverse transcribed to generate cDNA using a GoScript™ Reverse Transcription System (Promega; Madison, WI, USA), according to the manufacturer’s instructions. Then, qPCR was performed in a reaction volume of 10 μl, containing 5 μl of GoTaq® qPCR master mix (Promega), 1 μl of primers (Table 1) and 4 μl of cDNA, using an ABI Prism 7500 fluorescence quantitative PCR amplification instrument (BioRad, Tokyo, Japan). Each PCR reaction was initiated with one cycle of pre-denaturation at 95°C for 30 s,
followed by 40 cycles of 95°C for 20 s, 60°C for 15 s, and 72°C for 20 s. Relative target mRNA levels were calculated using the 2^{-\Delta\Delta Ct} method. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal reference gene, and the relative value of KLF17 expression was calculated.

**Determination of KLF17 protein level in cell lines by western blot**

Total proteins were extracted from the five cell lines (GES-1, NCI-N87, SGC7901, BGC823 and HGC-27), and protein concentration was determined by Bradford assay. Protein samples were then separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and electrotransferred onto a polyvinylidene fluoride membrane using a Trans-Blot® Semi-Dry Electrophoretic Transfer Cell (Bio-Rad, Hercules, CA, USA). The membrane was blocked with 5% skimmed milk powder at room temperature for 90 min, then incubated with rabbit anti-human KLF17 polyclonal primary antibody and rabbit anti-human GAPDH primary antibody as the loading control (1: 400 dilution; Abcam, Shanghai, China) overnight at 4°C. After washing, the membrane was incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG secondary antibody (Abcam) at 37°C for 1 h. After rinsing, the immunosignal was developed in a dark room and the membrane image was captured using a Labworks 4.6 system (Labworks, Novato, USA). The signal intensity (grey value) was then read with Image J software, and average absorbance of the protein bands was used to indicate the intensity of protein expression.

**Quantification of KLF17 mRNA in gastric cancer and adjacent tissues by qPCR**

Frozen gastric cancer and adjacent normal tissue specimens were ground using a homogenizer, and tissue was processed for qPCR as described above. Briefly, after cell lysis using TRIzol, column-based total RNA extraction, and reverse transcription, the synthesized cDNA underwent subsequent qPCR amplification as described above using the KLF17 and GAPDH primers stated in Table 1. Relative target mRNA levels were calculated using the 2^{-\Delta\Delta Ct} method.

**Detection of KLF17 protein in gastric cancer and adjacent tissues by IHC**

Paraformaldehyde-fixed gastric cancer and adjacent normal tissue specimens were dehydrated and embedded in paraffin using standard techniques, then sliced into 5-μm-thick sections. After deparaffinization, antigen retrieval was performed using a microwave oven, and sections were incubated with rabbit anti-human KLF17 polyclonal primary antibody (1: 5000 dilution; Abcam, Shanghai, China) overnight at 4°C. After washing, sections were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Abcam) at 37°C for 1 h. After rinsing, the immunosignal was developed

Table 1. Primer sequences used in quantitative polymerase chain reaction to analyse Krüppel-like factor 17 (KLF17) mRNA levels in gastric cancer cell lines, and in human gastric cancer and normal adjacent tissues.

| Target gene | Primer sequence (5'-3') |
|-------------|------------------------|
| KLF17       | Upstream 5'-AGCTGAGTCCCAGTCATTCG-3'  
              Downstream 5'-TCCTGAGGCGGATCTTTT-3' |
| GAPDH       | Upstream 5'-TGACGAGGACTCAGTGGG-3'  
              Downstream 5'-GCTTCACCACCTTCTTGATGTC-3' |

GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
Immunoway Biotechnology, Newark, DE, USA) overnight at 4°C. After washing three times with phosphate buffered saline (PBS), sections were incubated with HRP-goat anti-rabbit IgG secondary antibody (1: 5000 dilution; Immunoway Biotechnology) at 37°C for 30 min. Sections were again washed three times with PBS, and the immunosignal was developed using a DAB colour kit (MXB Biotech Co., Fuzhou, China). Images were acquired using an Olympus CKX-31 optical microscope (Tokyo, Japan) and immunostaining was quantified using a scoring system based on the % of positively stained nuclei (0–10%, 0 points; 11–25%, 1 point; 26–50%, 2 points; >50%, 3 points) and scoring criteria for staining intensity (no staining, 0; mild staining, 1++; moderate staining, 2++; severe staining, 3+). Samples with a combined score of ≥2 were classified as positive for KLF17 protein, and the positive staining rate (%) for gastric cancer versus adjacent tissues was calculated.

**Cell transfection**

The KLF17 plasmid (General Biosystems; Durham, NC, USA) was extracted and purified using an Endotoxin-Free Plasmid Megaprep Kit (TIANGEN Biotech Co., Beijing, China). BGC-823 and HGC-27 cells were harvested while in logarithmic growth phase, seeded into a six-well plate, and cultured to 70–80% confluence for subsequent transfection using Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer’s instructions. The constructed G0162329-1 COA of the KLF17 full-length plasmid was transfected into the above two cell lines, and after 48 h, KLF17 mRNA and protein levels were verified by qPCR and western blot. Each cell line was divided into three groups for subsequent experiments: non-transfected group (blank group), empty plasmid transfected group (control group), and KLF17 plasmid transfected group (transfection group).

**Wound-healing assay**

The BGC-823 and HGC-27 cells (each divided into blank, control and transfection groups) were seeded at 1 × 10^4 cells/well into six-well plates and routinely cultured in RMPI 1640 medium containing 5% FBS until cell confluence. Then, a sterile 1000-μl pipette tip was used to create a straight scratch in the cell monolayer, and the scraped cells were washed with medium, followed by incubation at 37°C/5% CO₂ for 24 h. The conditions of the two cell lines in the blank, control and transfection groups were observed under an inverted phase-contrast microscope (Nikon, Tokyo, Japan). Cell migration activity at 24 h was evaluated by counting the number of migrated cells in five randomly selected fields of view for each well under 200 × magnification.

**Transwell invasion assay**

Cell invasion assays were performed with Corning Costar 24-well Transwell® inserts (Corning Life Sciences, Tewksbury, MA, USA). Upper chambers were each coated with 100μl of Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) and exposed to ultraviolet radiation for 2 h for Matrigel polymerization. BGC-823 and HGC-27 cells (from the blank, control and transfection groups) were seeded into each upper chamber of the 24-transwell plate at 2 × 10^4 cells in a 200μl volume, and RPMI 1640 culture medium containing 10% FBS was added to the lower chamber. Cells were incubated for 24 h, then the Matrigel and cells remaining on the upper surface of the chamber were wiped off using a cotton swab and the membrane was fixed with methanol for 10 min. After crystal violet staining, the number of cells on the
underside of the insert was counted in five random visual fields using an optical microscope (Olympus, Tokyo, Japan) under 200× magnification. Effects on cell invasion were evaluated as the mean ± SD number of cells per field of view of triplicate experiments.

**Analysis of KLF17, MMP-9, vimentin and E-cadherin in response to KLF17 overexpression**

Quantitative PCR and western blots were used to detect changes in KLF17, MMP-9, vimentin and E-cadherin expression at the mRNA and protein level, respectively, in the blank, control, and transfection groups, for gastric cancer BGC-823 and HGC-27 cell lines. The qPCR experiment was performed as described above, except using the primer sequences presented in Table 2. Relative target mRNA levels were calculated using the 2^{ΔΔCt} method.

Western blot assays were performed as described above, except membranes were incubated with the following primary antibodies: rabbit anti-human KLF17 polyclonal; rabbit anti-human MMP-9 polyclonal; rabbit anti-human vimentin polyclonal; rabbit anti-human E-cadherin polyclonal; and rabbit anti-human GAPDH primary antibody as the loading control (1:5000 dilution; all Immunoway Biotechnology).

**Analysis of TGF-β1, Smad2/3 and p-Smad2/3 in response to KLF17 overexpression**

The BGC-823 and HGC-27 cells that had been harvested in log growth phase and seeded at 5×10^4 into T-25 tissue culture flasks with RPMI 1640 and 10% FBS, were divided into four groups: blank group; TGF group (cells incubated with 10 ng/ml TGF-β1 stimulating factor [R&D Systems; Shanghai, China] for 12 h at 37°C); transfection group (transfected cells overexpressing KLF17); and treatment group (transfected cells overexpressing KLF17 incubated with 10 ng/ml TGF-β1 stimulating factor, as above). Levels of TGF-β1, Smad2/3 and p-Smad2/3 mRNA and protein were then analysed by qPCR and western blot, as described above, except using the qPCR primer sequences presented in Table 3, and the following primary antibodies for the western blot assay: rabbit anti-human TGF-β1 polyclonal primary antibody, rabbit anti-human Smad2/3 polyclonal primary antibody, rabbit anti-human p-Smad2/3 polyclonal primary antibody, and rabbit anti-human GAPDH primary antibody as the loading control.

### Table 2. Primer sequences used in quantitative polymerase chain reaction to analyse matrix metalloproteinase (MMP)-9, vimentin, and E-cadherin mRNA levels in BGC-823 and HGC-27 cells before or after transfection to overexpress Krüppel-like factor 17 (KLF17).

| Target gene | Primer sequence (5′–3′) |
|-------------|-------------------------|
| MMP-9 | Upstream 5′-AGAACCAATCTCAACCGACAGG-3′  
Downstream 5′-CGACTCTCCACCGCATCCT-3′ |
| Vimentin | Upstream 5′-CACCTCTAAAGCCCATCACCACTAA-3′  
Downstream 5′-TCAAGGTCAGACGTGCCAGA-3′ |
| E-cadherin | Upstream 5′-GAGTCACAAGGACCTCCAAGCTAA-3′  
Downstream 5′-AGTGACCCACCTCTACTGACAGCAGA-3′ |
| GAPDH | Upstream 5′-CGCTGAGTACGTGGTGGATC-3′  
Downstream 5′-GCTGATGATCTTGAGGCTGTTGTC-3′ |

GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
Statistical analyses

Data were analysed using SPSS software, version 19.0 (IBM, Armonk, NY, USA). Measurement data are presented as mean ± SD, and were statistically analysed using Student’s t-test. Categorical data were analysed by $\chi^2$-test. A two-tailed $P$-value of <0.05 was considered statistically significant.

Results

KLF17 mRNA levels in normal and gastric cancer cell lines

Expression of KLF17 mRNA, determined by qPCR, was highest in GES-1 normal gastric mucosal cells (1.00 ± 0.00), and sequentially decreased in the gastric cancer cell lines, according to reducing levels of differentiation: NCI-N87 (0.81 ± 0.13), SGC7901 (0.67 ± 0.13), BGC823 (0.63 ± 0.18), and HGC-27 (0.39 ± 0.04) (all $P < 0.05$ versus GES-1 cells; Figure 1). These results suggest that KLF17 expression was positively correlated with degree of differentiation.

KLF17 protein levels in normal and gastric cancer cell lines

Western blot results showed that relative KLF17 protein levels were highest in GES-1 cells (1.07 ± 0.03), and decreased with reduced degrees of differentiation in the gastric cancer cell lines, as follows: NCI-N87 (1.02 ± 0.02), SGC7901 (0.67 ± 0.01), BGC823 (0.62 ± 0.02), and HGC-27 (0.42 ± 0.01) (all $P < 0.05$ versus GES-1 cells; Figure 2). These results suggest that KLF17 expression was positively correlated with degree of differentiation.

KLF17 mRNA levels in gastric cancer and adjacent normal tissues

The study included matched gastric cancer and adjacent normal tissue samples from a total of 50 patients, characterised as follows: 23 patients aged <55 years and 27 aged ≥55 years; 38 male and 12 female patients; and 14 patients with tumour size <5cm and 36 with tumour size ≥5cm. Gastric cancer type comprised the following Borrmann’s classifications: Type I ($n = 3$); Type II ($n = 38$); Type III ($n = 9$); and Type IV ($n = 0$). The qPCR results revealed that in the 50 paired tissues, relative KLF17 mRNA levels were significantly lower in

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Table 3. Primer sequences used in quantitative polymerase chain reaction to analyse transforming growth factor (TGF)-β1, Smad family member (Smad)2 and Smad3 mRNA levels in BGC-823 and HGC-27 cells before or after transfection to overexpress Krüppel-like factor 17 (KLF17) and with or without treatment with TGF-β1 stimulating factor.

| Target gene | Primer sequence (5’–3’) |
|-------------|-------------------------|
| TGF-β1      | Upstream 5’-AACCCACAACGAAATCTATGAC-3’ Downstream 5’-GCTGAGGTATCGCCAAGGAT-3’ |
| Smad2       | Upstream 5’-AAATGCCACGGTGAATT-3’ Downstream 5’-AGACTGAGCAGAAGATG-3’ |
| Smad3       | Upstream 5’-AGGAGAAATGGTGCGAGA-3’ Downstream 5’-CCACAGGCGGCAGTAGAT-3’ |
| GAPDH       | Upstream 5’-GACCTGACCTGCCGTCTAG-3’ Downstream 5’-AGGAGTGGGTGTCGCT-3’ |

GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
gastric cancer tissues (2.50 ± 2.23) than in adjacent normal tissues (11.01 ± 4.75; P < 0.05; Figure 3).

**Immunohistochemical staining of KLF17 protein in gastric cancer and adjacent normal tissues**

Expression of KLF17 at the protein level was analysed by IHC in gastric cancer and corresponding adjacent tissues from 50 patients. The rate of KLF17-positive expression was found to be significantly lower in gastric cancer tissues (16 out of 50 patients [positive expression rate, 32%]), compared with the corresponding adjacent tissues (41 out of 50 patients [positive expression rate, 82%]; χ² value = 25.499; P < 0.05; Figure 4).

**Effect of KLF17 overexpression on migration of BGC-823 and HGC-27 cells**

The wound-healing assay revealed inhibition of migration activity in BGC-823 and HGC-27 cells transfected to overexpress KLF17. Compared with the blank and control groups, the migration ability of cells in the transfection group was significantly decreased (P < 0.05; Figure 5 and 6).
Effect of KLF17 overexpression on invasion of BGC-823 and HGC-27 cells

The Transwell assay revealed that KLF17 overexpression inhibited the invasion activity of transfected BGC-823 and HGC-27 cells. Compared with the blank and control groups, the invasion ability of cells in the transfection group was significantly decreased ($P < 0.05$; Figure 7).
Figure 5. Effect of Krüppel-like factor 17 (KLF17) overexpression on the migration ability of BGC-823 cells. Left, representative phase-contrast photomicrographs (original magnification, ×200); right, mean ± SD of triplicate experiments ($P < 0.05$ versus blank group and control group; Student’s t-test).

Figure 6. Effect of Krüppel-like factor 17 (KLF17) overexpression on the migration ability of HGC-27 cells. Left, representative phase-contrast photomicrographs (original magnification, ×200); right, mean ± SD of triplicate experiments ($P < 0.05$ versus blank group and control group; Student’s t-test).

Figure 7. Effect of Krüppel-like factor 17 (KLF17) overexpression on the invasion ability of BGC-823 and HGC-27 cells. Left, representative photomicrographs showing crystal violet-stained cells (original magnification, ×200); right, mean ± SD of triplicate experiments ($P < 0.05$ versus blank group and control group; Student’s t-test).
Effect of KLF17 overexpression on EMT-related mRNA levels in BGC-823 and HGC-27 cells

Analysis by qPCR showed that KLF17 mRNA levels were increased in KLF17-transfected BGC-823 and HGC-27 cells compared with the blank and control groups ($P < 0.05$), indicating successful transfection in both cell lines. Compared with the blank and control groups, the KLF17-transfected cells displayed decreased levels of MMP-9 and vimentin mRNA, and increased levels of E-cadherin mRNA ($P < 0.05$; Figure 8).

Effect of KLF17 overexpression on EMT-related protein levels in BGC-823 and HGC-27 cells

Western blot analysis showed that KLF17 protein levels were increased in KLF17-transfected BGC-823 and HGC-27 cells compared with the blank and control groups ($P < 0.05$), suggesting successful transfection. Compared with the blank and control groups, the KLF17-transfected cells showed decreased levels of MMP-9 and vimentin, and increased levels of E-cadherin ($P < 0.05$; Figure 9 and 10).

Changes in TGF-β1, Smad2 and Smad3 mRNA levels in BGC-823 and HGC-27 cells

Analysis of BGC-823 and HGC-27 cells by qPCR showed that levels of TGF-β1, Smad2 and Smad3 mRNA were significantly higher in cells treated with TGF-β1 stimulating factor (TGF group) compared with those in the blank group ($P < 0.05$), while levels of TGF-β1, Smad2 and Smad3 mRNA were significantly lower in cells overexpressing KLF17 (transfected group) compared with the blank group ($P < 0.05$; Figure 11). The above indexes were all significantly decreased in the treatment group (transfected with KLF17 and treated with TGF-β1 stimulator) versus the TGF group ($P < 0.05$), and were all significantly increased in the treatment group (transfection plus TGF-β1 stimulator) versus the transfection group ($P < 0.05$; Figure 11).

Changes in TGF-β1, Smad2/3 and p-Smad2/3 protein levels in BGC-823 and HGC-27 cells

Western blot analysis of BGC-823 and HGC-27 cells showed that levels of TGF-β1 and p-Smad2/3 proteins were significantly higher in cells treated with...
TGF-β1 stimulating factor (TGF group) than those in the blank group ($P < 0.05$), whereas levels of TGF-β1 and p-Smad2/3 proteins were significantly lower in cells overexpressing KLF17 (transfected group) versus the blank group ($P < 0.05$). Levels of TGF-β1 and p-Smad2/3 protein were significantly lower in the treatment group versus the transfection group ($P < 0.05$). There were no statistically significant differences in Smad2/3 protein levels between the four groups in either cell line (Figure 12 and 13).

**Discussion**

Krüppel-like factor (KLF) 17 is a member of the Sp/KLF zinc finger protein family, and is a transcription factor with multiple
KLF17 is widely present in human tissues and cells that are closely correlated to a variety of tumour diseases, and is thought to affect the occurrence and development of tumours. Numerous studies have revealed that KLF17 is poorly expressed in cancer tissues and highly expressed in normal tissues, playing a role as a tumour suppressor gene in various cancers, including lung cancer, breast cancer, liver cancer, oesophageal cancer, and rectal cancer. However, few studies have been conducted to determine the relationship between KLF17 and gastric cancer. In the present study, qPCR and western blot were used to analyse KLF17 mRNA and protein levels in gastric cancer cell lines. KLF17 expression was found to be highest in the GES-1 normal gastric mucosal cell line, and decreased in
the NCI-N87, SGC-7901, BGC-823 and HGC-27 gastric cancer cell lines with decreasing differentiation levels. Therefore, KLF17 appears to act as a tumour suppressor gene in gastric cancer cells, and its expression level is correlated to the degree of differentiation.

In the present study, the two cell lines with lowest KLF17 expression levels, BGC-823 and HGC-27, were selected for cell transfection to overexpress KLF17. The constructed G0162329-1 COA of the KLF17 full-length plasmid was transfected into the above two cell lines, and levels of KLF17 mRNA and protein after 48 h were found to be upregulated in the transfection group compared with the blank and control groups, indicating successful transfection and overexpression of KLF17. Wound-healing and Transwell assays revealed that KLF17 overexpression inhibited the migration and invasion abilities of cells. Several studies have reported that amongst the matrix metalloproteinases, MMP-9 is the most closely related to degradation of the extracellular matrix, playing a key role in gastric cancer invasion and metastasis.\(^\text{22}\)

In the present study, overexpression of KLF17 led to downregulated expression of MMP-9, suggesting an inhibitory role for KLF17 in the occurrence and development of gastric cancer.

Gastric cancer cells induce EMT through complex molecular mechanisms, in order to affect cell migration and invasion, and the transformation of epithelial cells to mesenchymal cells in EMT can be reflected by the related protein markers.\(^\text{23}\) In the process of neoplastic EMT, tumour cells acquire migration and invasion abilities, leading to downregulated expression of the epithelial cell marker E-cadherin, and upregulated expression of the mesenchymal cell marker vimentin.\(^\text{24}\) In the present study, KLF17 expression was upregulated by transfection in two gastric cancer cell lines, and this KLF17 overexpression was associated with upregulated expression of E-cadherin and downregulated expression of vimentin, which may be associated with the suppression of tumour invasion and metastasis by inhibiting the EMT process.

When stimulated by different signal molecules in the tumour microenvironment,
such as hypoxia, inflammatory factors and growth factors, gastric cancer cells activate related signal pathways and stimulate downstream transcription factors to participate in the EMT process, thereby affecting biological characteristics, such as migration and invasion. The most well-known pathway related to the EMT process is the TGF-β/Smad signalling pathway. TGF-β is a cytokine with multiple biological activities that can regulate cell proliferation, differentiation, migration and apoptosis, and plays a dual role in the occurrence and development of tumours. In the precancerous stage, TGF-β may suppress cell proliferation and induce apoptosis, thereby inhibiting the occurrence of tumours. Conversely, in advanced tumours, TGF-β may promote the invasion and metastasis of tumour cells by regulating the immune system and tumour microenvironment. TGF-β binds to a TGF-β receptor (TGF-βR), such as TGF-βR1 and TGF-βR2, and phosphorylates the downstream signal transduction molecules Smad2 and Smad3. Smad2, Smad3 and Smad4 are combined to form a trimeric complex, and enter the nucleus to promote the EMT process of tumour cells. Overexpression of KLF17 suppresses the migration and invasion of gastric cancer cells by inhibiting the EMT process. Its correlation to the TGF-β/Smad signalling pathway was verified through the experiments conducted in the present study, whereby exogenous TGF-β1 factors were introduced to increase the activation level of the TGF-β/Smad signalling pathway, and the effect of KLF17 overexpression on TGF-β/Smad pathway-related factors was observed. The results indicated that, in BGC-823 and HGC-27 gastric cancer cell lines, KLF17 overexpression inhibited levels of TGF-β1, Smad2 and Smad3 mRNA, and TGF-β1 and p-Smad2/3 protein. These findings suggest that KLF17 overexpression might suppress the EMT process by inhibiting activation of the TGF-β/Smad signalling pathway, thereby inhibiting the invasion and metastasis of gastric cancer cells.

In conclusion, the present study revealed that KLF17 may be a tumour suppressor in gastric cancer. KLF17 overexpression might inhibit the EMT process by suppressing the activation of the TGF-β/Smad signalling pathway, thereby inhibiting the invasion and metastasis of gastric cancer cells. Thus, further studies should investigate KLF17 as a potential novel target for the treatment of gastric cancer.

**Declaration of conflicting interest**

The authors declare that there is no conflict of interest.

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