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Regular paper

Krüppel-like factor 1 serves as a facilitator in gastric cancer progression *via* activating the Wnt/β-catenin pathway

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Krüppel-like factor 1 (KLF1) is a transcription factor that exhibits promoting effect in cervical cancer, but its correlation with gastric cancer (GC) has not been reported yet. In this study, we explored the role and potential mechanism of KLF1 in GC progression by using a series of experimental methods including RT-qPCR, Western blot, CCK-8 assay, EdU staining, and cell cycle analysis. KLF1 was found to be elevated in GC tissues (n=415) compared with the normal tissues by applying UALCAN to analyze datasets from The Cancer Genome Atlas (TCGA). The upregulation of KLF1 was also validated in GC cell lines. Functional studies proved that RNA interference-mediated silencing of KLF1 inhibited GC cell growth, as evidenced by the decreased cell viability, DNA synthesis, and arrested cell cycle in G1 phase. Moreover, KLF1 knockdown exerted the inhibitory effects on cell migration and invasion as well as the epithelial-mesenchymal transition (EMT) in GC cells. Conversely, overexpression of KLF1 had the opposite effects on GC progression. Furthermore, we proved that the activation of Wnt/β-catenin pathway was markedly inhibited by KLF1 knockdown and promoted by KLF1 overexpression. The blockade of Wnt/β-catenin pathway rescued the effects of KLF1 overexpression. These results suggested that KLF1 promoted the growth, migration, invasion, and EMT process in GC cells, and this promotion was achieved by activating the Wnt/β-catenin pathway. This work will be helpful for searching the potential therapeutic targets for treatment of GC.

Keywords: Epithelial-to-mesenchymal transition, gastric cancer, Krüppel-like factor 1, the Wnt/β-catenin pathway

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Abbreviations: CCK-8, Cell counting kit-8; EdU, 5-Ethynyl-2′-deoxyuridine EMT, Epithelial-mesenchymal transition; GC, Gastric cancer; KLF1, Krüppel-like factor 1; TCGA, The Cancer Genome Atlas

INTRODUCTION

Gastric cancer (GC) is one of the most common malignant tumors of human digestive system. It ranks fifth for incidence and fourth for mortality globally and is responsible for over one million new cases in 2020 and an estimated 769,000 deaths (Lin *et al.*, 2009). Despite the great advance that has been made in the diagnosis and treatment of GC, the five-year survival rate of patients with advanced GC remains unfavorable (Joshi & Badgwell, 2021; Katai *et al.*, 2018). Therefore, searching for potential targets for the precision treatment in GC is needed.

Krüppel-like factor (KLF) is a representative of zinc finger-containing transcription factor family (McConnell & Yang, 2010), which regulates multiple biological processes such as proliferation, differentiation, development, and programmed cell death. Additionally, the functions of KLFs have been reported in different types of cancer, including breast cancer (Kim & Singh, 2019; Lu *et al.*, 2014), hepatocellular carcinoma (Kremer-Tal *et al.*, 2007), colorectal cancer (Kim *et al.*, 2016), and cervical cancer (Marrero-Rodríguez *et al.*, 2014; Zhu *et al.*, 2018). Moreover, KLFs have been reported to play different roles in GC progression. For example, KLF7 and KLF8 were highly expressed in GC and were associated with poor prognosis (Jiang *et al.*, 2017; Liu *et al.*, 2014; Mao *et al.*, 2019; Wang *et al.*, 2013), while KLF2, KLF4, and KLF17 were down-regulated in GC tissues and exhibited anti-tumor effects (Chen *et al.*, 2020; Peng *et al.*, 2014; Wang *et al.*, 2017; Zhang *et al.*, 2019). As for KLF1, it is important in differentiation and maturation of red blood cells (Gnanapragasam *et al.*, 2016; Yang *et al.*, 2017) and is involved in the regulation of cell cycle (Tallack *et al.*, 2009; Vinjamur *et al.*, 2014). Furthermore, a recent study reported that KLF1 was up-regulated in cervical cancer cells, and KLF1 knockdown inhibited the proliferation, migration, and invasion of cervical cancer cells (Zhu *et al.*, 2018). However, whether KLF1 participates in GC has not been reported yet.

Epithelial-mesenchymal transition (EMT) is a cellular process, by which epithelial cells lose their polarity and tight junction and acquire migratory and invasive phenotypes (Kalluri & Weinberg, 2009). EMT plays a pivotal role in tumorigenesis, and it is characterized by down-regulation of epithelial markers like E-cadherin and up-regulation of mesenchymal markers, including Vimentin and fibronectin (Kalluri & Weinberg, 2009). Moreover, numerous studies have revealed the regulation on EMT by KLFs in cancers, such as KLF4 (Tiwari *et al.*, 2013; Tseng *et al.*, 2016) and KLF8 (Liu *et al.*, 2014). In detail, KLF4 was reported to reverse or suppress EMT process and metastasis in GC and could serve as a novel prognostic indicator of GC (Kong *et al.*, 2018; Zhang *et al.*, 2019). However, the correlation between KLF1 and EMT in GC has not been reported yet.

Therefore, in the present study, we explored the role of KLF1 in growth, migration, and invasion in GC cells and we also investigated its regulation on EMT process and the Wnt/β-catenin signaling pathway. This work could be helpful for searching the potential targets for treatment of GC.

MATERIALS AND METHODS

Cell culture and treatment

Human gastric mucosa epithelial cell line (GES-1) was purchased from Fenghui Biotechnology Co., Ltd. (Changsha, China). GC cell line SNU-1 was purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China). GC cell lines NCI-N87, AGS, and HGC-27 were purchased from iCell bioscience Co., Ltd. (Shanghai, China). All the cells used in present study were stored in liquid nitrogen and were resuscitated in an incubator at 37°C with 5% CO_2. When the cells grew to a density of about 90%, 0.25% trypsin was added to digest the cells. After the cells became round, complete culture medium was added to terminate the reaction. Cells were counted using trypan blue dye. The cells were grouped according to the experimental plan and cultured in an incubator at 37°C and 5% CO_2 until the cells adhered to the wall. GES-1 cells were cultured in DMEM medium (Servicebio, Wuhan, China) with 10% fetal bovine serum (FBS; Tianhang, Huzhou, China). SNU-1, NCI-N87, and HGC-27 cells were cultured in RPMI-1640 medium (Solarbio, Beijing, China) with 10% FBS. AGS cells were cultured in Ham's F-12 medium (Procell) with 10% FBS. Cells were cultured at 37°C in an atmosphere containing 5% CO_2. Cell transfection was then performed. AGS cells were transfected with KLF1 overexpressing plasmids (OE-KLF1; 2.5 μg per well) or empty vectors (2.5 μg per well). NCI-N87 cells were transfected with specific siRNAs against KLF1 (si-KLF1; 75 pmol per well) or negative control siRNAs (si-NC; 75 pmol per well). Lipofectamine 3000 (Invitrogen, CA, USA) was utilized to perform cell transfection. After cell transfection for 24 h, cells were incubated with the Wnt/β-catenin pathway inhibitor ICG-001 (6 μM, dissolved in DMSO; Yuanye, Shanghai, China) or DMSO (vehicle) for 24 h.

Cell counting kit-8 (CCK-8) assay

Cells were seeded in 96-well plates at a density of 3×10^3 cells per well. After cell transfection for 0, 6, 24, 48, or 72 h or ICG-001 treatment for 24 h, cells were incubated with CCK-8 reagent (10 μl per well; Beyotime, Shanghai, China) at 37°C for 2 h, and the absorbance at the wavelength of 450 nm was determined using a microplate reader (BioTek, VT, USA).

Cell cycle analysis

The cell cycle was analyzed using a Cell Cycle Analysis Kit (Beyotime) following the manufacturer's protocol. Briefly, cells were seeded in 6-well plates at a density of 4×10^5 per well. Cells were collected after transfection for 24 h. After being fixed in 70% ethanol at 4°C for 12 h, cells were incubated with Propidium iodide (PI; 25 μl) and RNase A (10 μl) at 37°C for 30 min in the darkroom. A NovoCyte flow cytometer was used to analyze the cells (ACEA Biosciences, CA, USA). The percentages of the cells in different cell cycle phases were calculated.

EdU assay

Cell proliferation was assessed by EdU assay. In brief, the transfected NCI-N87 or AGS cells were incubated with 5-Ethynyl-2'-deoxyuridine (EdU; 10 μM; KeyGEN, Nanjing, China) for 2 h. Cells were then fixed with 4% paraformaldehyde for 15 min and treated with 0.5% Triton X-100 for 20 min at room temperature. After being treated with Click-iT reaction solution for 30 min in the darkroom at room temperature, cells were subsequently counterstained with DAPI (Beyotime). EdU-positive cells were visualized and calculated using a fluorescence microscope (magnification 400×; Olympus, Tokyo, Japan).

Cell migration

Cell migration was tested using the wound-healing assay. Transfected cells were maintained until confluence was reached. Next, cells were incubated in serum-free medium containing 1 μg/ml mitomycin C (Sigma, MO, USA) for 1 h. Afterwards, 200-μl pipette tip was used to scratch the cells. Migration photos were captured at 0 and 24 h after scratching using a microscope (magnification 100×; Olympus).

Cell invasion

Cell invasion was tested using transwell chambers (Corning, NY, USA). The transfected cells (3×10^4 cells

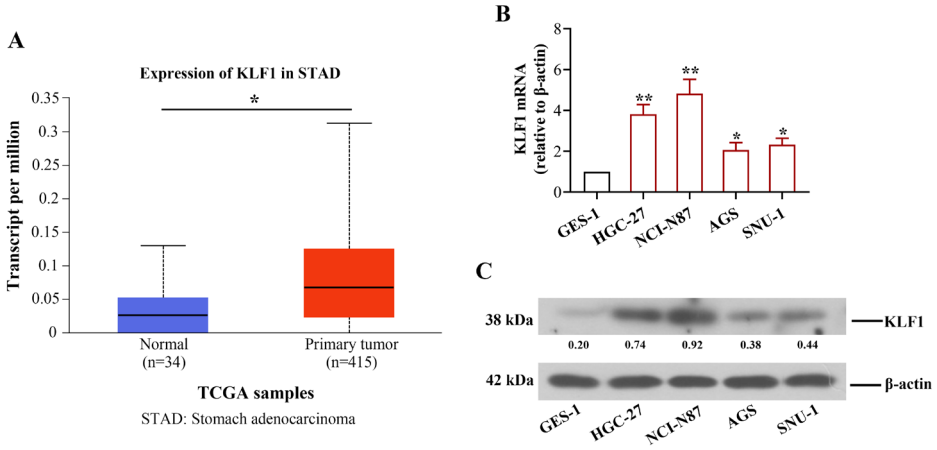

Figure 1. KLF1 was elevated in human gastric cancer tissues and cell lines.
(**A**) The expression of KLF1 in stomach adenocarcinoma tissues (n=415) and normal tissues (n=34) was analyzed using TCGA RNA-seq datasets via UALCAN. (**B, C**) Analysis of KLF1 level in human gastric mucosa epithelial cells GES-1 and gastric cancer cell lines (HGC-27, NCI-N87, AGS, and SNU-1) by RT-qPCR and Western blot. One-way ANOVA with Tukey's multiple comparisons test. Each experiment was conducted at least three times. *p<0.05 and **p<0.01, compared to GES-1.

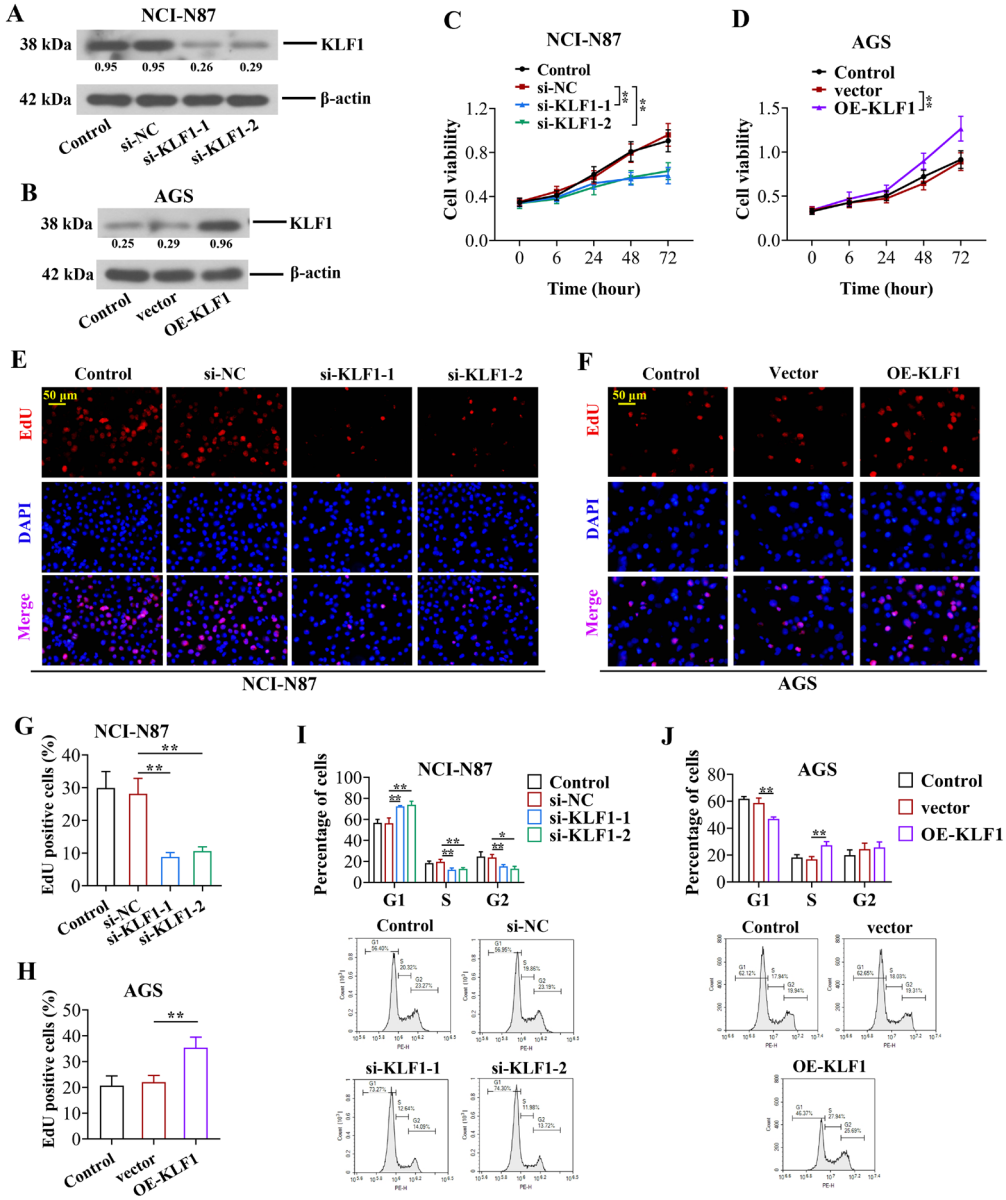

Figure 2. KLF1 promoted growth of gastric cancer cells.
(**A, B**) Western blot was used to determine the efficiencies of cell transfection in NCI-N87 and AGS cells. (**C, D**) The CCK-8 assay was performed to assess the cell viability at indicated time points. Two-way ANOVA with Tukey's multiple comparisons test. (**E, F**) The EdU staining assay was conducted to measure the cell proliferation in NCI-N87 and AGS cells (magnification 400×), (**G, H**) and the proportion of EdU-positive cells was calculated. One-way ANOVA with Tukey's multiple comparisons test. (**I, J**) Flow cytometry was used to analyze the cell cycle. One-way ANOVA with Tukey's multiple comparisons test. Each experiment was conducted at least three times. *p<0.05 and **p<0.01.

per well) were seeded in the upper chamber pre-coated with Matrigel (Corning) and were maintained in serum-free medium. The medium (800 μl) with 10% FBS was placed in the bottom chamber. After 24 h, cells on the bottom chamber were fixed in 4% paraformaldehyde (Aladdin, Shanghai, China) and dyed using 0.5% crystal violet (Amresco, Shanghai, China). Finally, cells were visualized and counted in five randomly selected fields using a microscope (magnification 200×; Olympus).

Immunofluorescence staining

Cells were fixed with 4% paraformaldehyde (Sinopharm, Shanghai, China) for 15 min and washed in PBS three times for 5 min. Permeabilization was performed using 0.4% Triton X-100 (Beyotime) for 10 min at room temperature. Immunofluorescence staining was performed using following antibodies: E-cadherin (1:100; A3044, Abclonal, Wuhan, China), Vimentin (1:100; A19607, Abclonal), β-catenin (1:100; A19657, Abclonal), Cy3-labeled goat anti rabbit IgG (1:200; A0516, Beyotime). Finally, the cells were counterstained with DAPI (Beyotime) and imaged by a fluorescence microscope (magnification 400×; Olympus).

Reverse transcription quantitative real-time PCR (RT-qPCR)

Total RNA was extracted from cells using TRIpure reagent (BioTeke, Beijing, China). BeyoRT II M-MLV

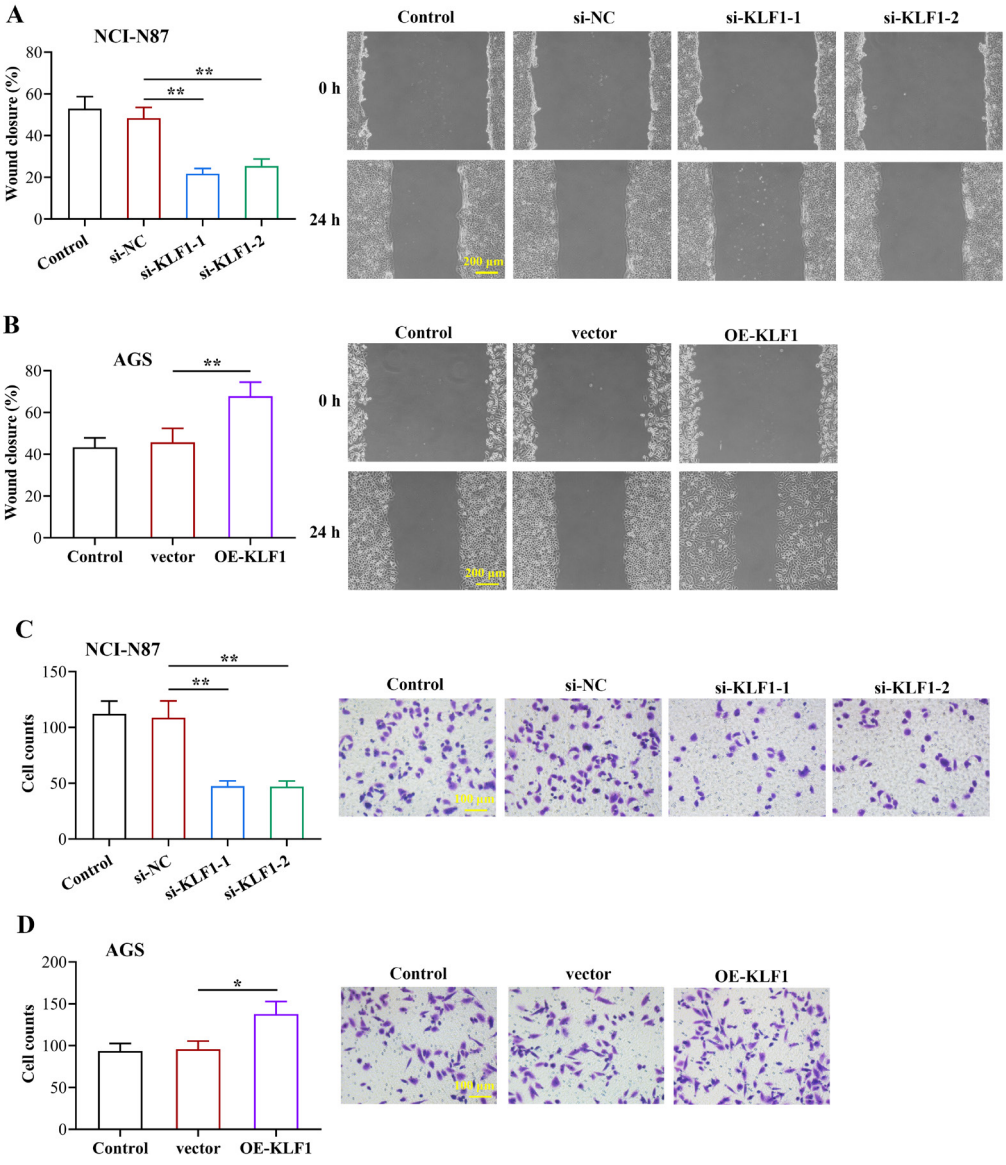

Figure 3. KLF1 promoted migration and invasion of gastric cancer cells.
(A, B) The wound-healing assay was used to test the migration capacity of NCI-N87 and AGS cells (magnification 100×). **(C, D)** The transwell assay was used to test the invasion capacity of NCI-N87 and AGS cells (magnification 200×). Each experiment was conducted at least three times. *$p<0.05$ and **$p<0.01$. One-way ANOVA with Tukey's multiple comparisons test.

reverse transcriptase (Beyotime) was used to synthesize cDNA. Real-time PCR was carried out using 2×Taq PCR MasterMix and SYBR Green (Solarbio) on a fluorescent quantitative PCR instrument (Exicycler 96; Bioneer, Daejeon, Korea). The mRNA levels were quantified using the $2^{-\Delta\Delta Ct}$ method. β-actin was used as the internal control. The following primer sequences were used for the qPCR: KLF1: 5'-CCCCTCCTTC-CTGAGTTGTTTG-3' (forward) and 5'-GCAGGCG-TATGGCTTCTCCC-3' (reverse); β-actin: 5'-CACTGT-GCCCATCTACGAGG-3' (forward) and 5'-TAATGT-CACGCACGATTTCC-3' (reverse).

Western blot

Total proteins were lysed from cells using PMSF and cell lysis buffer for Western blot and IP (Beyotime). Proteins were separated using SDS-PAGE and transferred onto PVDF membranes (Millipore, MA, USA). Primary antibodies against KLF1 (A10581, 1:1000), Wnt1 (A2475, 1:2000), β-catenin (A19657, 1:1000), c-Myc (A19032, 1:1000), cyclin D1 (A19038, 1:1000), E-cadherin (A3044, 1:1000), and Vimentin (A19607, 1:1000) were purchased from ABclonal Technology (Wuhan, China). Primary antibody against β-actin (sc-47778, 1:1000) was purchased from Santa Cruz Biotechnology (CA, USA). Primary antibody against Histone H3 (AM8433, 1:2000) was purchased from Abcepta Biotech (Suzhou, China). β-actin served as the cytoplasmic control, and Histone H3 served as the nuclear control. Horseradish peroxidase-labeled secondary antibodies goat anti-rabbit IgG (A0208, 1:5000) and goat anti-mouse IgG (A0216, 1:5000) were obtained from Beyotime Biotechnology.

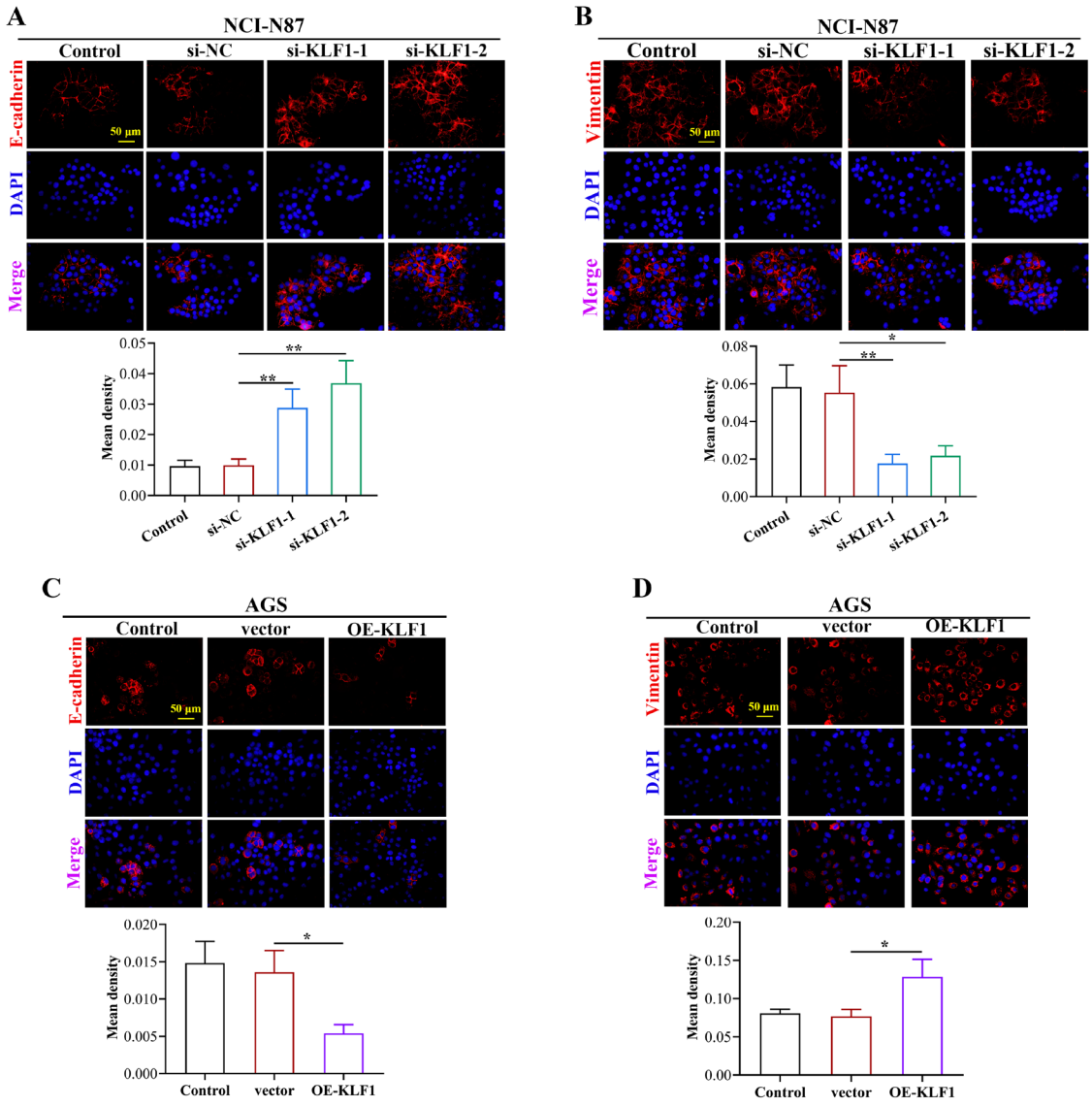

Figure 4. KLF1 promoted EMT process in gastric cancer cells.
Immunofluorescence staining was used to detect the epithelial marker E-cadherin (**A, C**) and the mesenchymal marker Vimentin (**B, D**) in NCI-N87 and AGS cells (magnification 400×), and the mean density of fluorescence was calculated. Each experiment was conducted at least three times. *p<0.05 and **p<0.01. One-way ANOVA with Tukey's multiple comparisons test.

Statistical analysis

Each experiment was conducted using three replicates. Data were determined as mean ±standard deviation (S.D.) and were analyzed via GraphPad Prism 8.0 software (CA, USA). One-way ANOVA, two-way ANOVA, and Tukey's multiple comparisons test were used to test the differences among different groups. p<0.05 indicated the statistical significance.

RESULTS

1. KLF1 was up-regulated in GC tissues and cell lines

As shown in Fig. 1A, the expression level of KLF1 in stomach adenocarcinoma tissues (n=415) was significantly higher than that in normal samples (n=34) by analyzing TCGA datasets via UALCAN website. Furthermore, the increased expression of KLF1 was observed in GC cell lines (HGC-27 (281%), NCI-N87 (382%), AGS (106%), and SNU-1 (132%)) as compared to human gastric mucosa epithelial cells GES-1 at both mRNA and protein levels (Fig. 1B, C). These results showed that KLF1 was significantly up-regulated in GC tissues and cells.

2. KLF1 promoted GC cell growth

To determine the role of KLF1 in GC progression, NCI-N87 cells with the highest KLF1 expression were transfected with the si-KLF1 or negative control, and AGS cells with the lowest KLF1 expression were transfected with KLF1 overexpressing plasmids or empty vectors. Western blot was performed to measure the transfection efficiency (Fig. 2A, B). The

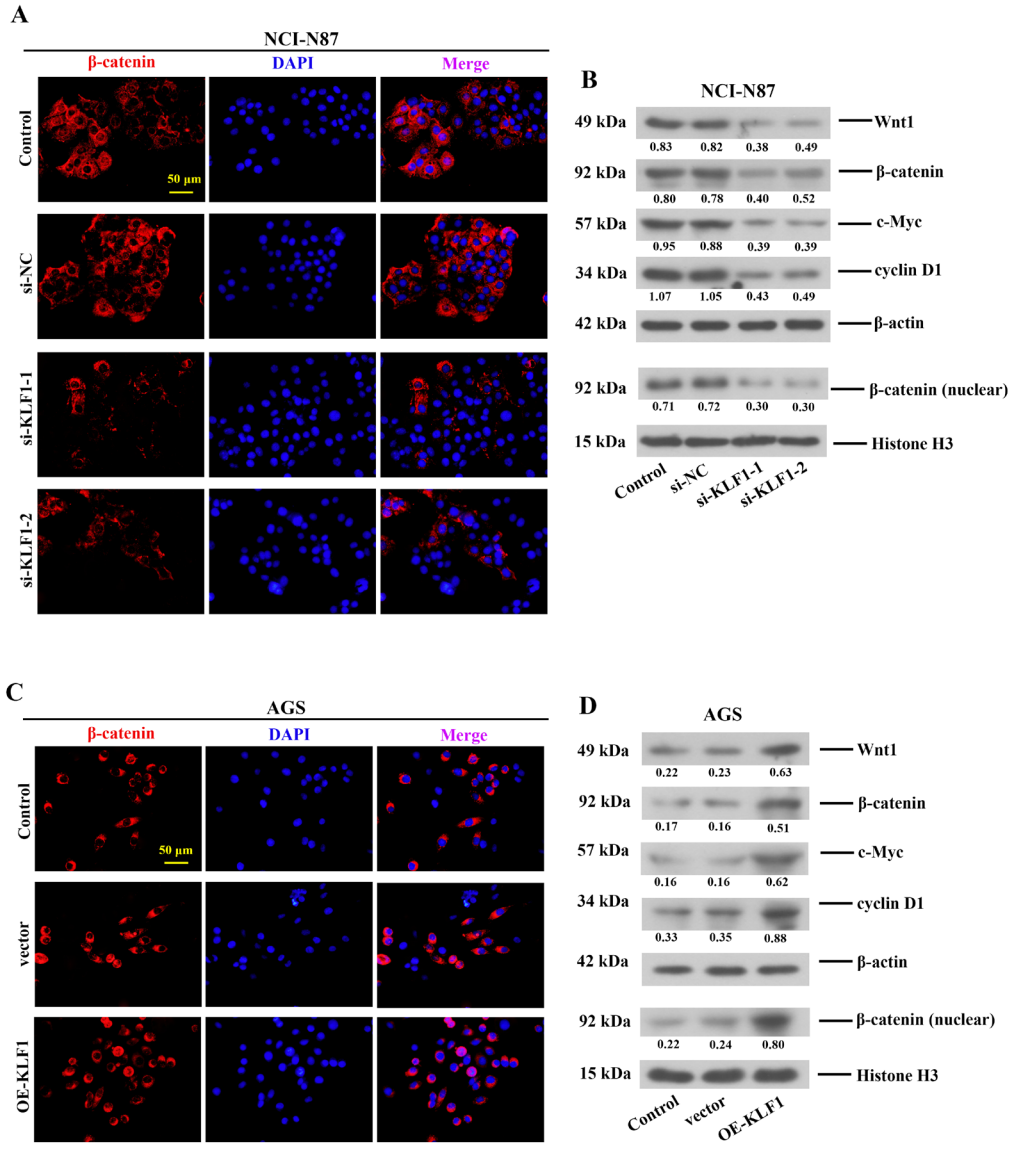

Figure 5. KLF1 contributed to activation of Wnt/β-catenin pathway in gastric cancer cells.
(**A, C**) The immunofluorescence staining was performed to detect the β-catenin in NCI-N87 and AGS cells (magnification 400×). (**B, D**) Western blot analysis was conducted to measure the expression levels of Wnt1, β-catenin and the downstream targets c-Myc and cyclin D1 in NCI-N87 and AGS cells.

CCK-8 assay suggested that knockdown of KLF1 inhibited the cell viability, and overexpression of KLF1 enhanced the cell viability (Fig. 2C, D). The EdU staining assay validated that the proliferation of GC cells was promoted by KLF1 up-regulation and inhibited by KLF1 down-regulation, as evidenced by the reduced proportion of EdU-positive cells in NCI-N87 cells (–69% and –62%; Fig. 2E, G) and the increased proportion of EdU-positive cells in AGS cells (61%; Fig. 2F, H). Furthermore, by performing cell cycle analysis, we found that KLF1 knockdown significantly increased the percentage of cells in G1 phase (29% and 31%) whereas decreased the percentage of cells in S phase (-38% and -35%; Fig. 2I), and overexpression of KLF1 exhibited the opposite effects (Fig. 2J). These findings indicated that KLF1 promoted GC cells growth.

3. KLF1 promoted migration and invasion of GC cells.

In addition, wound-healing assay showed that the migration capacity was reduced in KLF1 silenced cells (–55% and –48%) and increased in KLF1 overexpressing cells (48%; Fig. 3A, B). Consistently, transwell assay verified that down-regulation of KLF1 markedly decreased the cell invasion (–56% and –57%), whereas up-regulation of KLF1 enhanced the cell invasion (44%; Fig. 3C, D). These results suggested that KLF1 promoted the migration and invasion of GC cells.

4. KLF1 promoted EMT process in GC cells

Considering the important role of EMT process in progression of GC (Kozak *et al.*, 2020), we explored the effects of KLF1 on EMT. Immunofluorescence staining assay showed that silencing of KLF1 significantly enhanced E-cadherin (epithelial marker, 209% and 276%)

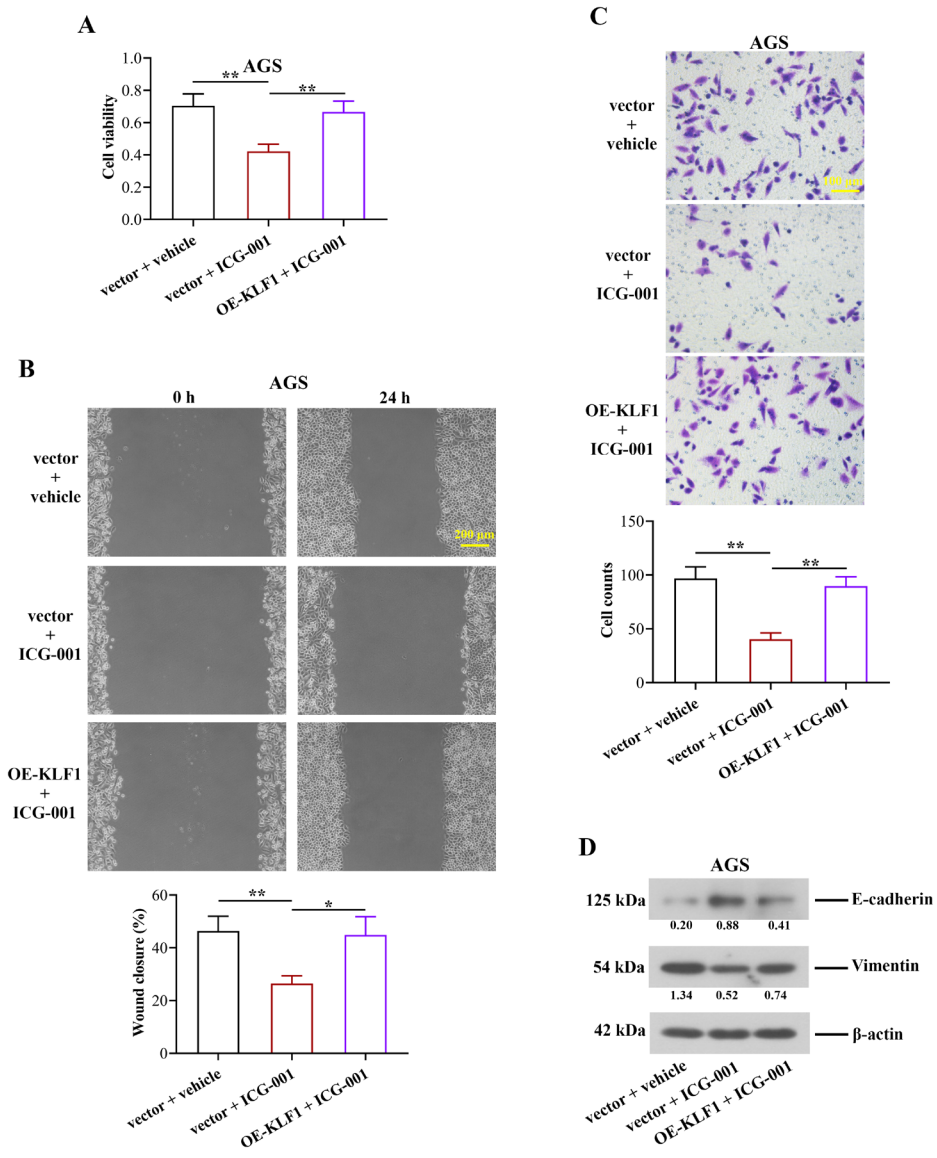

Figure 6. KLF1 overexpression rescued the inhibitory effects of Wnt/β-catenin pathway inhibitor ICG-001 on gastric cancer cells. After incubated with Wnt/β-catenin pathway inhibitor ICG-001 (6 μM) or DMSO vehicle for 24 h, (**A**) the viability of cells was assessed by CCK-8 assay. (**B, C**) The cell migration and invasion were measured using wound-healing assay (magnification 100×) and transwell assay (magnification 200×), respectively. (**D**) The expression levels of E-cadherin and Vimentin were evaluated using Western blot analysis. Each experiment was conducted at least three times. *$p<0.05$ and **$p<0.01$. One-way ANOVA with Tukey's multiple comparisons test.

expression and reduced Vimentin (mesenchymal marker, –66% and -63%) expression (Fig. 4A, B), whereas overexpression of KLF1 displayed converse effects (Fig. 4C, D). These results suggested that KLF1 promoted EMT process in GC cells.

5. KLF1 contributed to activation of Wnt/β-catenin pathway

As the Wnt/β-catenin pathway regulates many cell functions and is closely linked to tumorigenesis and disease progression (Flanagan *et al.*, 2017; Flanagan *et al.*, 2019), we further investigated the effects of KLF1 on this pathway. As shown in Fig. 5A, knockdown of KLF1 dramatically decreased β-catenin expression in NCI-N87 cells. Western blot analysis further validated that knockdown of KLF1 inhibited the activation of Wnt/β-catenin signaling pathway, as evidenced by the decreased expression levels of Wnt1 (–55% and –39%), cytoplasmic (–50% and –39%) and nuclear (–57% and –60%) β-catenin, and downstream targets c-Myc (–61%) and cyclin D1 (–50% and –44%; Fig. 5B). Conversely, up-regulation of KLF1 promoted the activation of Wnt/β-catenin pathway (Fig. 5C, D). These findings implied the important role of KLF1 in the activation of Wnt/β-catenin pathway in GC.

6. Overexpression of KLF1 rescued the inhibitory effects of Wnt/β-catenin pathway inhibitor ICG-001 on GC cells

To explore whether KLF1 exhibits these promoting effects on GC cells *via* the activation of Wnt/β-catenin pathway, the inhibitor of the Wnt/β-catenin pathway, ICG-001 (Liu *et al.*, 2017) was added into the culture

medium. As shown in Fig. 6A, the cell viability was markedly reduced by ICG-001 treatment (-160%) but rescued by KLF1 overexpression (58%). Wound-healing assay and transwell assay also suggested that the inhibitory effects of ICG-001 on the migration and invasion capability of AGS cells were significantly rescued by KLF1 up-regulation (Fig. 6B, C). Consistently, Western blot analysis showed that ICG-001 significantly inhibited the EMT process, as evidenced by the increased E-cadherin (313%) and the decreased Vimentin (–63%), while overexpression of KLF1 reversed these changes (Fig. 6D). Based on these results, we speculated that KLF1 might exhibit its promoting role in GC cells through activating the Wnt/β-catenin pathway.

DISCUSSION

In the present study, we revealed the role of KLF1 in GC cells for the first time. We found that KLF1 was up-regulated in GC tissues and cell lines. Additionally, KLF1 promoted growth, migration, invasion, and EMT in GC cells and these promoting effects might be mediated by the Wnt/β-catenin signaling pathway.

Uncontrolled cell proliferation is a major feature of cancer cells. The cell proliferation rate is considered as an important factor in judging the malignancy of GC cells and is helpful in determining the treatment and evaluating the prognosis of patients (Ohyama *et al.*, 1992). Alterations in the cell cycle also have a significant impact on the gastric carcinogenesis process. It has been reported that the DNA synthesis time is significantly prolonged in the tumors with a high S-phase fraction (Ohyama *et al.*, 1992). Performing immunohistochemistry on cyclin D1, an important regulator in the progression from G1 phase to S phase, can also serve as a good standard method for differentiating early stage GC from hyperplastic polyp patients (Gao *et al.*, 2004). According to a recent study, leukemia inhibitory factor (LIF) inhibited the proliferation of GC cells by arresting the cell cycle at the G1 phase and decreasing the protein level of cyclin D1 (Xu *et al.*, 2019). In addition, a series of studies reveal the pivotal role of KLF1 in the cell cycle of red blood cells but its function in cancer cells is unknown (Gnanapragasam *et al.*, 2016; Tallack *et al.*, 2009). In this work, we found that KLF1 was elevated in GC tissues and cell lines. Knockdown of KLF1 inhibited proliferation and DNA synthesis and induced a G1 phase arrest of GC cells, which was consistent with previous studies. Our findings indicate the involvement of KLF1 in GC cell growth.

EMT plays a role in the malignant transformation and affects the aggressiveness of GC (Kozak *et al.*, 2020). Alteration of the expression of EMT-related molecules is a common event in GC. For example, E-cadherin (an epithelial marker taking part mainly in cell-cell adhesion maintenance) was described to be down-regulated in GC and acted as a tumor suppressor (Di Bartolomeo *et al.*, 2016; van Roy & Berx, 2008). Moreover, emerging evidence demonstrated that the EMT process was regulated by KLFs in GC. For instance, KLF4 increased E-cadherin and decreased Vimentin through suppressing the expression of Serine/Threonine Kinase 33, a pro-mesenchymal marker, thus acting as a tumor suppressor in GC (Kong *et al.*, 2018). On the contrary, overexpression of KLF8 promoted GC cell metastasis, increased the mesenchymal markers, and decreased the epithelial markers (Liu *et al.*, 2014). In the present work, KLF1 was a positive regulator of migration and invasion of GC cells, and it also promoted EMT process, as it reduced E-cadherin and increased Vimentin. These findings suggest that KLF1 probably promotes GC development through regulating EMT. Furthermore, we additionally compared the relevant indicators of the control group (without exogenous modifications) in NCI-N87 cells with higher KLF1 endogenous expression and ASG cells with lower KLF1 endogenous expression. The results showed that the proliferation, migration, and invasion abilities of the control group in NCI-N87 cells were higher than those in AGS cells. The results indicate that GC cells with higher KLF1 endogenous expression have a stronger malignant phenotype compared to cells with lower KLF1 expression, which is similar to the results obtained with overexpression of KLF1 in ASG cells and silencing of this target in NC-N87 cells. Altogether, the results demonstrate that KLF1 acts as a tumor promoter in GC progression.

Wnt signaling regulates many cell functions and its dysregulation is correlated with EMT process in many types of cancer, including the GC (Li *et al.*, 2018; Lu *et al.*, 2019). The stabilization and accumulation of β-catenin are in response to activated Wnt signaling, and the accumulated β-catenin is then translocated into the nucleus and induces downstream target gene transcription such as c-Myc and cyclin D1 (Acebron & Niehrs, 2016; MacDonald *et al.*, 2009; Soucek *et al.*, 2008). Most GC patients display deregulated Wnt/β-catenin signaling (Cristescu *et al.*, 2015; Wang *et al.*, 2014), and inhibition of Wnt signaling is considered as a potential strategy for treating GC (Flanagan *et al.*, 2017). According to a clinical study, relapse-free survival and overall survival were significantly worse for patients with abnormal β-catenin expression (Di Bartolomeo *et al.*, 2016). In the present study, we determined that KLF1 contributed to the activation of Wnt/β-catenin pathway, and KLF1 overexpression significantly impaired the inhibitory effects of the Wnt/β-catenin inhibitor ICG-001 on GC cells. Collectively, these results partially explain the potential mechanism underlying the KLF1-mediated promoting effects on GC. Besides, KLF1 was reported to promote proliferation and invasion *via* the PI3K/AKT signaling pathway in cervical cancer cells (Zhu *et al.*, 2018). To explore the role of other pathways in the functions of KLF1 in GC, more experimental studies are required.

CONCLUSIONS

Our work demonstrated that KLF1 promoted the growth, migration, invasion, and EMT process in GC cells, and this promoting effect might be achieved by activating the Wnt/β-catenin pathway. This study provided new evidence for understanding the pathogenesis of GC and was helpful for searching the therapeutic targets for GC treatment.

Acknowledgements

None.

Conflict of interest

The authors declare no conflict of interest in this article.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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