The *lncRNA TERC* promotes gastric cancer cell proliferation, migration, and invasion by sponging *miR-423-5p* to regulate *SOX12* expression

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Background: Long non-coding RNAs (lncRNAs) play critical roles in gastric cancer (GC) initiation progression. However, the biological function of the *lncRNA* telomerase RNA component (*TERC*) remains unknown in human GC. The present study sought to determine the biological function and underlying molecular mechanism of the *lncRNA TERC* in GC progression.

Methods: The expression levels of the *lncRNA TERC* in GC tissues and cell lines were analyzed using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The effects of the *lncRNA TERC* on the proliferation, migration, and invasion of GC cells were determined using Cell Counting Kit-8 (CCK-8) and Transwell assays. Dual luciferase reporter and argonaute 2 (AGO2)-RNA immunoprecipitation (RIP) assays were used to detect the binding between the *lncRNA TERC* and microRNA-423-5p (*miR-423-5p*). Western blotting was performed to measure the expression levels of sex determining region Y-box 12 (*SOX12*), *N-cadherin*, *E-cadherin*, matrix metallopeptidase 9 (*MMP9*), and proliferating cell nuclear antigen (*PCNA*).

Results: The results demonstrated that the *lncRNA TERC* expression levels were upregulated in GC cells and tissues, while *miR-423-5p* expression levels were downregulated. The upregulation of the *lncRNA TERC* was associated with a shorter overall survival in patients with GC. The knockdown of the *lncRNA TERC* significantly reduced the proliferation, migration, and invasion of human GC cell lines HGC-27 and SNU-1 cells. Further, the *lncRNA TERC* knockdown in the HGC-27 and SNU-1 cells significantly downregulated the expression levels of *SOX12*, *N-cadherin*, *MMP9*, and *PCNA*, and upregulated the expression levels of *miR-423-5p* and *E-cadherin*. *MiR-423-5p* was also identified as a target of the *lncRNA TERC* and was found to directly bind to the *lncRNA TERC*. Additionally, *miR-423-5p* was found to directly target *SOX12* to inhibit the proliferation, migration, and invasion of the HGC-27 and SNU-1 cells.

Conclusions: In conclusion, the findings of this study suggested that the *lncRNA TERC* may regulate the *miR-423-5p*/*SOX12* signaling axis by directly sponging *miR-423-5p* and inhibiting *SOX12* expression, thereby leading to the progression of GC. These findings may reveal novel targets for future GC therapy.

Keywords: Long non-coding RNA telomerase RNA component (*lncRNA TERC*); microRNA-423-5p (*miR-423-5p*); sex determining region Y-box 12 (*SOX12*); gastric cancer (GC)

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Introduction

Gastric cancer (GC) is a common type of malignancy and the 3rd leading cause of cancer-related mortality worldwide (1). The disease is associated with a significant economic burden, especially in China (2-4). In recent decades, the incidence of GC has decreased due to the development of effective screening technologies and methods for controlling *Helicobacter pylori* infection (5,6). Surgical resection, radiotherapy, chemotherapy, and combined therapy are currently the primary treatment options available for GC, and all have been reported to significantly improve the survival of patients with GC (7-10). However, the prognosis of patients with advanced-stage GC remains unsatisfactory, and the 5-year survival rate for patients with metastatic GC is ~30% (11). At present, the underlying molecular mechanism involved in GC development and progression remains unclear. Thus, further research to extend the current understanding of the molecular mechanisms of GC progression and identify novel therapies targeting metastasis in GC urgently needs to be conducted.

Long non-coding RNAs (lncRNAs) are non-coding RNA molecules >200 nucleotides in length that have limited protein-coding potential (12). Numerous previous studies have demonstrated that lncRNAs play regulatory roles in various biological processes, including the cell cycle, cell differentiation, apoptosis, migration, invasion, and cancer progression (13-17). There is accumulating evidence that lncRNAs may also act as competing endogenous RNAs (ceRNAs) that are able to adsorb microRNAs (miRNAs/miRs), and thus influence tumorigenesis (18,19). For example, lncRNA long intergenic non-protein coding RNA 2620 (*BCRT1*) was found to promote breast cancer progression by binding with *miR-1303* (20). In bladder cancer, *lncRNA* cancer suspectibility 9 (*CASC9*) adsorbed *miR-758-3p* to induce cell proliferation and epithelial-mesenchymal transition (EMT) by regulating transforming growth factor-beta 2 (*TGF-β2*) expression (21). It has also been reported that the *lncRNA* Pvt1 oncogene (*PVT1*) promotes cell migration by sponging *miR-30a* and regulating snail family transcriptional repressor 1 expression in GC (22). Qu *et al.* (23) also demonstrated that *lncRNA HOXA cluster antisense RNA 3* promoted GC progression by sponging *miR-29a-3p*, which subsequently regulated lymphotoxin β receptor expression and activated nuclear factor kappa B signaling.

We identified that the expression level of *lncRNA* telomerase RNA component (*TERC*) was consistently significantly up-regulated in GCs in the Gene Expression Omnibus (GEO) database via through bioinformatics analysis. The *TERC* is an important RNA component of telomerase, and the *lncRNA TERC*, a non-coding RNA, provides a template sequence for telomere synthesis (24). The *lncRNA TERC* has also been reported to alleviate the progression of osteoporosis by sponging *miR-217* and upregulating RUNX family transcription factor 2 (*RUNX2*) expression (25). However, to the best of our knowledge, the biological functions of the *lncRNA TERC* in the progression of cancer, especially GC, remain largely unknown.

The current study sought to determine the expression levels of the *lncRNA TERC* in GC tissues and cell lines. In addition, the effects of the lncRNA TERC on GC cell proliferation, invasion, and migration were analyzed. We further predicted that the binding site between *lncRNA TERC* and *miR-423-5p*, and the binding site between miR-423-5p and sex determining region Y-box 12 (*SOX12*). Therefore, further mechanistic studies were performed to explore the role of the *lncRNA TERC/miR-423-5p/SOX12* signaling axis in the progression of GC. Together, the findings of the present study may provide novel insights into the potential of the *lncRNA TERC/miR-423-5p/SOX12* signaling axis as a treatment target for GC. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3545/rc).

Methods

Patient samples

A total of 20 human GC and corresponding and adjacent normal tissues were obtained from patients admitted to Shaanxi Provincial People's Hospital (Xi'an, China) between July 2021 and December 2021. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All participants provided written informed consent, and the study was approved by the Ethics Committee of Shaanxi Provincial People's Hospital (No. 2021-186).

Table 1 Primers sequences for real-time PCR and the sequences of small interfering RNA

Primer	Primer sequence
TERC	Forward: 5'-CCTGCCGCCTTCCACCGTTCATT-3'
	Reverse: 5'-GGGGACTCGCTCCGTTCCTCTTCC-3'
β-actin	Forward: 5'-CCCTGGAGAAGAGCTACGAG-3'
	Reverse: 5'-CGTACAGGTCTTTGCGGATG-3'
miR-423-5p	Loop primer: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAGTCTC-3'
	Forward primer: 5'-TGCGCTGAGGGGCAGAGAGCGAG-3'
U6	Forward: 5'-CGCTTCGGCAGCACATATAC-3'
	Reverse: 5'-AAATATGGAACGCTTCACGA-3'
si-*TERC*	5'-CCTTCCACCGTTCATTCTA-3'
si-NC	5'-UUCUCCGAACGUGUCACGUTT-3'
miR-423-5p mimic	5'-UGAGGGGCAGAGAGCGAGACUUU-3'
mimic-NC	5'-UUCUCCGAACGUGUCACGUTT-3'

PCR, polymerase chain reaction; *TERC*, telomerase RNA component; miR, microRNA; NC, negative control.

Bioinformatics analysis

Human GC gene expression data were obtained from the GEO data set, GSE63288. The data analysis was performed using the DEGseq package of R software (1.12.0; RStudio, Inc., Boston, MA, USA). Genes with a $\log_2|$fold change (FC)$|$ >1 and a P<0.05 were considered differentially expressed genes. The binding sites between the *lncRNA TERC* and *miR-423-5p* were predicted using the starBase database (https://starbase.sysu.edu.cn/). The binding sites between *miR-423-5p* and *SOX12* were predicted using the TargetScan 7.1 database (https://www.targetscan.org/vert_80/).

Cell lines and culture

The GES-1 human gastric mucosal epithelial cells and the NCI-N87, KATO3, Hs-746T, HGC-27, and SNU-1 human GC cell lines were obtained from Procell Life Science and Technology Co., Ltd. (Wuhan, China). The SNU-1, KATO3, and HGC-27 cell lines were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% or 20% fetal bovine serum (FBS) (Gibco; Thermo Fisher Scientific, Inc.), and 1% penicillin-streptomycin solution. The GES-1, NCI-N87, and Hs-746T cell lines were cultured in Dulbecco's Modified Eagle Medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), and 1% penicillin-streptomycin solution. All the cells were maintained at 37 ℃ in a 5% carbon dioxide humidified incubator.

Cell transfection

A total of 5×10^5 HGC-27 and SNU-1 cells/well were seeded into a 6-well plate overnight at 37 ℃. The cells were then cultured in serum-free RPMI-1640 medium for 2 h prior to transfection. The cells were subsequently transiently transfected with small interfering RNA. The sequences of small interfering RNA in *TERC* and *miR-423-5p* were listed in *Table 1*. They were transfected into cells by using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) in accordance with the manufacturer's protocol.

Cell Counting Kit-8 (CCK-8) assays

The proliferative ability of the HGC-27 and SNU-1 cells was measured using CCK-8 assays. Briefly, 5×10^3 HGC-27 and SNU-1 cells/well were seeded into a 96-well plate and incubated overnight. The cells were then transfected for a further 48 h. Following the transfection, 10 μL of CCK-8 solution was added to each well and incubated at 37 ℃ for an additional 4 h. The absorbance of each well was measured at a wavelength of 450 nm using a microplate

reader (Flexstation® 3; Molecular Devices, LLC, San Jose, CA, USA). The experiments were performed in triplicate.

Cell migration, and invasion assays

The migratory and invasive abilities of the HGC-27 and SNU-1 cells were determined using Transwell plates (24-well inserts; Corning, NY, USA). Briefly, for the migration assays, 5×10^5 HGC-27 and SNU-1 cells/well were seeded into a 6-well plate and incubated overnight. The cells were transfected for 48 h, trypsinized and resuspended in serum-free RPMI-1640 medium at a density of 3×10^5 cells/mL. A volume of 200 µL of cell suspension was added to the upper chambers of the Transwell plate, while 800 µL of growth medium (RPMI-1640 medium supplemented with 10% or 20% FBS) was added to the lower chambers. Following incubation for 24 h, the migratory cells were stained with crystal violet and counted using an inverted microscope (ECLIPSE Ts2; Nikon Corporation; magnification, ×200). The invasion assays were performed as described, with a minor alteration—the upper chamber of the Transwell plate was pre-coated with 100 µL of Matrigel (1 mg/mL; Corning, Inc.).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from the GC tissues, adjacent normal tissues, HGC-27, and SNU-1 cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA was reverse transcribed into complementary DNA (cDNA) using random primers and Hiscript Reverse Transcriptase (GeneCopoeia Company, USA) for messenger RNA quantification. For miRNA quantification, the RT step was performed using an Oligo (dT) 18/miRNA loop and Hiscript Reverse Transcriptase. The primer sequences are listed in *Table 1*. The PCR reaction conditions were as follows: 10 minutes at 95 ℃, followed by 40 cycles of 15 seconds at 95 ℃, and 60 seconds at 60 ℃. The expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (26).

Dual luciferase reporter assays

The binding relationships between the *lncRNA TERC*, *miR-423-5p*, and *SOX12* were verified using dual luciferase reporter assays. Briefly, the cDNA fragments of the *TERC* and *SOX12* containing the predicted *miR-423-5p* binding sites were inserted into the pYr-MirTarget luciferase reporter vector (Yingrun Biotechnologies Inc., China) to generate pYr-MirTarget-Homo *SOX12*-wild-type (WT) and pYr-MirTarget-Homo *TERC*-WT vectors, which are henceforth denoted as *SOX12*-WT and *TERC*-WT, respectively.

A mutant (MUT) site in the *miR-423-5p* binding site was also designed and cloned into the pYr-MirTarget luciferase reporter vector to generate pYr-MirTarget-Homo *SOX12*-MUT (*SOX12*-MUT) and pYr-MirTarget-Homo *TERC*-MUT (*TERC*-MUT) vectors. The *TERC* or *SOX12* plasmids (WT or MUT) were co-transfected with the *miR-423-5p* mimic or mimic-negative control (NC) into 293T cells. Following 48 h of transfection, a dual luciferase reporter gene assay kit (Beyotime Institute of Biotechnology, Suzhou, China) was used to determine the relative luciferase activity.

RNA immunoprecipitation (RIP) assays

A RNA-binding protein immunoprecipitation kit (MilliporeSigma, Burlington, MA, USA) was used in accordance with the manufacturer's protocol to determine the relationship between the *lncRNA TERC* and *miR-423-5p*. Anti-argonaute 2 (AGO2) (SAB4200085, MilliporeSigma) and control immunoglobulin G (IgG) (R9255, MilliporeSigma) antibodies were used to perform the RIP assays, and the expression levels of the *lncRNA TERC* and *miR-423-5p* were subsequently evaluated using qPCR.

Western blotting

The relative protein expression levels were examined using western blotting as previously described (27). Briefly, total protein was extracted from tissue samples and HGC-27 and SNU-1 cells using ristocetin-induced platelet aggregation lysis buffer (Beyotime Institute of Biotechnology) supplemented with 1% protease inhibitor cocktail (Sigma-Aldrich; Merck KGaA). Proteins were separated by sodium dodecyl-sulfate polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes (MilliporeSigma). After blocking with 5% non-fat milk for 2 h at room temperature, the membranes were incubated with the following primary antibodies at 4 ℃ overnight: anti-*N-cadherin* (cat. no. 13116, 1:1,000 dilution), anti-*E-cadherin* (cat. no. 3195, 1:1,000 dilution), anti-matrix metallopeptidase 9 (*MMP9*; cat. no. 13667, 1:1,000 dilution), anti-proliferating cell nuclear antigen (*PCNA*; cat. no. 13110, 1:1,000 dilution), and anti-*β-actin* (cat. no. 4970, 1:1,000 dilution), which were purchased

from Cell Signaling Technology, Inc. (Beverly, MA, USA). Anti-*SOX12* (cat. no. 13116, 1:1,000 dilution) was purchased from Proteintech (Chicago, IL, USA). Following the primary antibody incubation, the membranes were incubated with the appropriate secondary antibodies (Cell Signaling Technology, Inc., cat. no. 7074, 1:1,000 dilution) for 2 h at room temperature. The protein bands were visualized using an enhanced chemiluminescence (ECL) kit (Pierce; Thermo Fisher Scientific, Inc.).

Statistical analysis

All the data are presented as the mean ± standard deviation. All the experiment were replicated 3 times. The statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, Inc., San Diego, CA, USA). The statistical differences between the groups were determined using a Student's *t*-test or one-way analysis of variance followed by a Dunnett's post-hoc test. A P value <0.05 indicated a statistically significant difference.

Results

LncRNA TERC expression levels are upregulated in GC tissues and cell lines and associated with a poor prognosis

The present study first identified differentially expressed lncRNAs in GC using the GSE63288 data set from the GEO database. Volcano plot (see *Figure 1A*) and heat map (see *Figure 1B*) showed differentially expressed lncRNAs identified from the GEO database. The identified upregulated lncRNAs that were thought to play important roles in GC progression were subsequently further analyzed. Among the lncRNAs, the expression levels of the *lncRNA TERC* were found to be consistently significantly upregulated in GC in the GEO database. Thus, GC and adjacent normal tissues (n=20) were collected and the expression levels of the *lncRNA TERC* were determined using RT-qPCR. As *Figure 1C* shows, *lncRNA TERC* expression was significantly more upregulated in GC tissues than adjacent normal tissues. Additionally, the expression levels of the *lncRNA TERC* in human gastric mucosal epithelial cells and 5 GC cell lines were analyzed. Compared to the GES-1 cells, the expression levels of the *lncRNA TERC* were significantly upregulated in the SNU-1, HGC-27, and KATO3 cells (see *Figure 1D*). Additionally, as *Figure 1E* shows, higher *lncRNA TERC* expression levels were found to be associated with shorter overall survival in patients with GC. Collectively, these results suggested that *lncRNA TERC* expression may be upregulated in GC tissues and poor survival outcomes may be associated with *lncRNA TERC* expression levels in GC.

Knockdown of the lncRNA TERC inhibits GC cell proliferation, migration, and invasion

To explore the biological function of the *lncRNA TERC* in GC, the effects of the *lncRNA TERC* on the proliferation, migration, and invasion of the HGC-27 and SNU-1 cells were investigated. The si-*TERC* was used to knockdown the expression levels of the *lncRNA TERC* in the HGC-27 and SNU-1 cells, and the interference efficiency of the *lncRNA TERC* was detected using RT-qPCR. Compared to the si-NC group, the expression levels of the *lncRNA TERC* were significantly downregulated in the HGC-27 and SNU-1 cells in the si-*TERC* group (see *Figure 2A*). The *lncRNA TERC* knockdown also significantly reduced the proliferation of the HGC-27 and SNU-1 cells (see *Figure 2B*). Moreover, the migratory and invasive abilities of the HGC-27 and SNU-1 cells were significantly more reduced in the si-*TERC* group than the si-NC group (see *Figure 2C-2F*). EMT plays an important role in cancer invasiveness and metastasis (28,29). Thus, the effect of the *lncRNA TERC* on EMT-related markers, such as *N-cadherin* and *E-cadherin*, was also analyzed using western blotting. As *Figure 3A-3C* show, the expression levels of *N-cadherin* were significantly more downregulated in the si-*TERC* group, while the expression levels of *E-cadherin* were more upregulated compared to those in the si-NC group. Additionally, the protein expression levels of *MMP9* and *PCNA*, which are closely associated with tumor metastasis and cell proliferation, were investigated (30,31). The results revealed that the *lncRNA TERC* knockdown significantly downregulated the protein expression levels of *N-cadherin*, *MMP9* and *PCNA* in the HGC-27 and SNU-1 cells (see *Figure 3A,3D,3E*), while significantly upregulated the levels of *E-cadherin* (see *Figure 3B*). These data indicated that the *lncRNA TERC* may regulate the proliferation, migration, and invasion of GC cells by regulating EMT and the protein expression levels of *MMP9* and *PCNA*.

LncRNA TERC functions as a molecular sponge for miR-423-5p in GC

There is increasing evidence that the *lncRNA TERC* acts as a ceRNA to regulate the biological function of miRNAs (20-23). Using the starBase database (https://starbase.sysu.

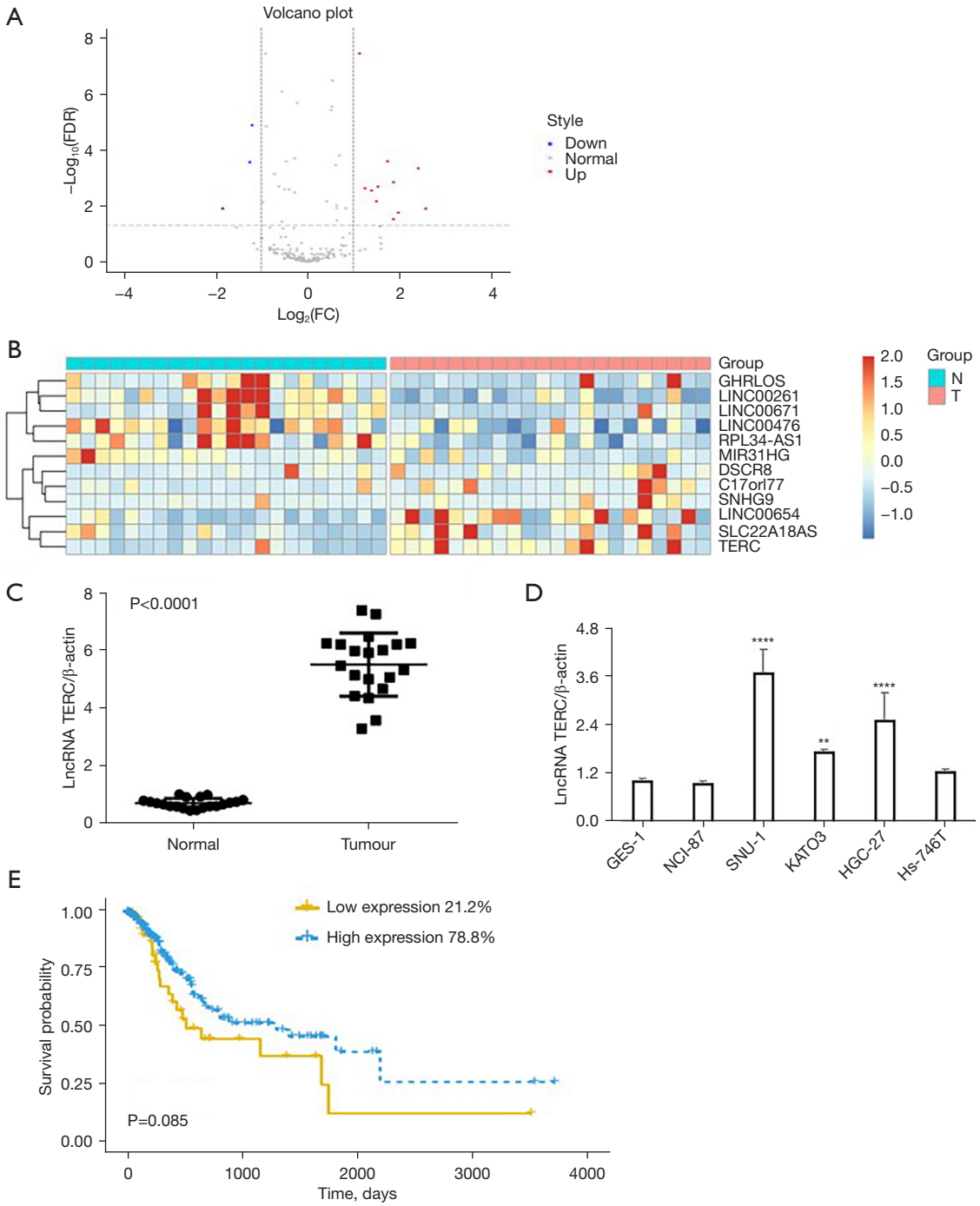

Figure 1 *LncRNA TERC* expression profiles and their association with prognosis in GC. (A) Volcano plots of the differentially expressed lncRNAs. (B) Heat maps of differentially expressed lncRNAs in GC tissues compared to adjacent normal tissues. The data were obtained from the GSE63288 GEO data set. (C) The expression levels of the *lncRNA TERC* in the GC and adjacent normal tissues (n=20) were determined using RT-qPCR. (D) The expression levels of the *lncRNA TERC* in the human gastric mucosal epithelial cells and 5 GC cell lines were determined using RT-qPCR. (E) The association between the *lncRNA TERC* and the overall survival of patients with GC was determined using a Kaplan-Meier analysis. *LncRNA TERC*-low expression (n=58) or *lncRNA TERC*-high expression (n=320). **P<0.01; ****P<0.0001. FDR, false discovery rate; FC, fold change; N, normal; T, tumor; *lncRNA*, long non-coding RNA; *TERC*, telomerase RNA component; GC, gastric cancer; GEO, Gene Expression Omnibus; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

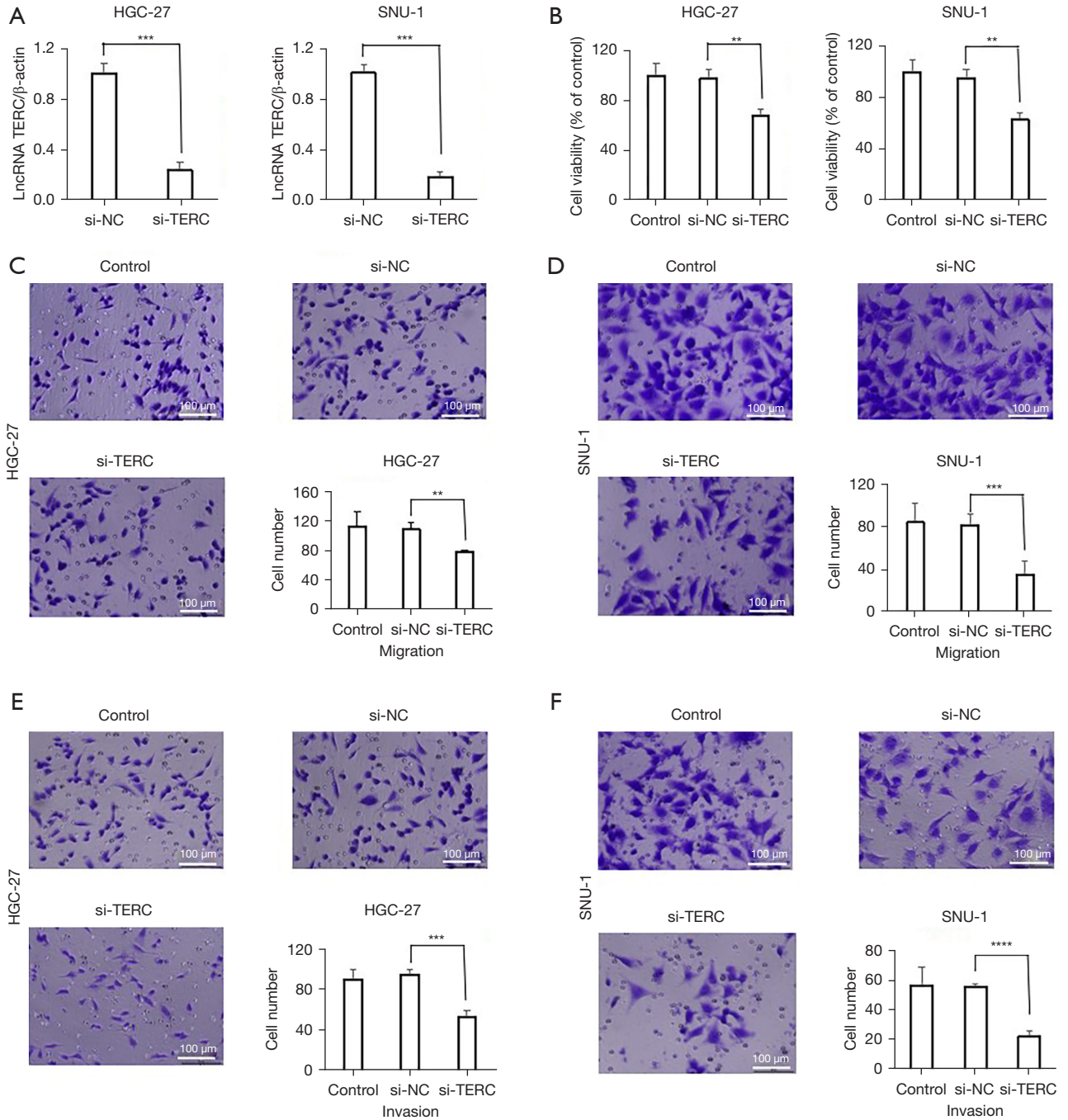

Figure 2 Effects of the *lncRNA TERC* on HGC-27 and SNU-1 GC cell proliferation, migration, and invasion. The HGC-27 and SNU-1 cells were transfected with si-NC or si-*TERC* for 48 h. (A) The interference efficiency of the si-*TERC* in the HGC-27 and SNU-1 cells was determined using RT-qPCR. (B) Cell proliferation was determined using CCK-8 assays. (C,D) The migration and (E,F) invasion of the HGC-27 and SNU-1 cells were detected using Transwell assays. Only use 1% crystal violet staining solution for staining. **P<0.01; ***P<0.001; ****P<0.0001. LncRNA, long non-coding RNA; *TERC*, telomerase RNA component; si, small interfering RNA; NC, negative control; GC, gastric cancer; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; CCK-8, Cell Counting Kit-8.

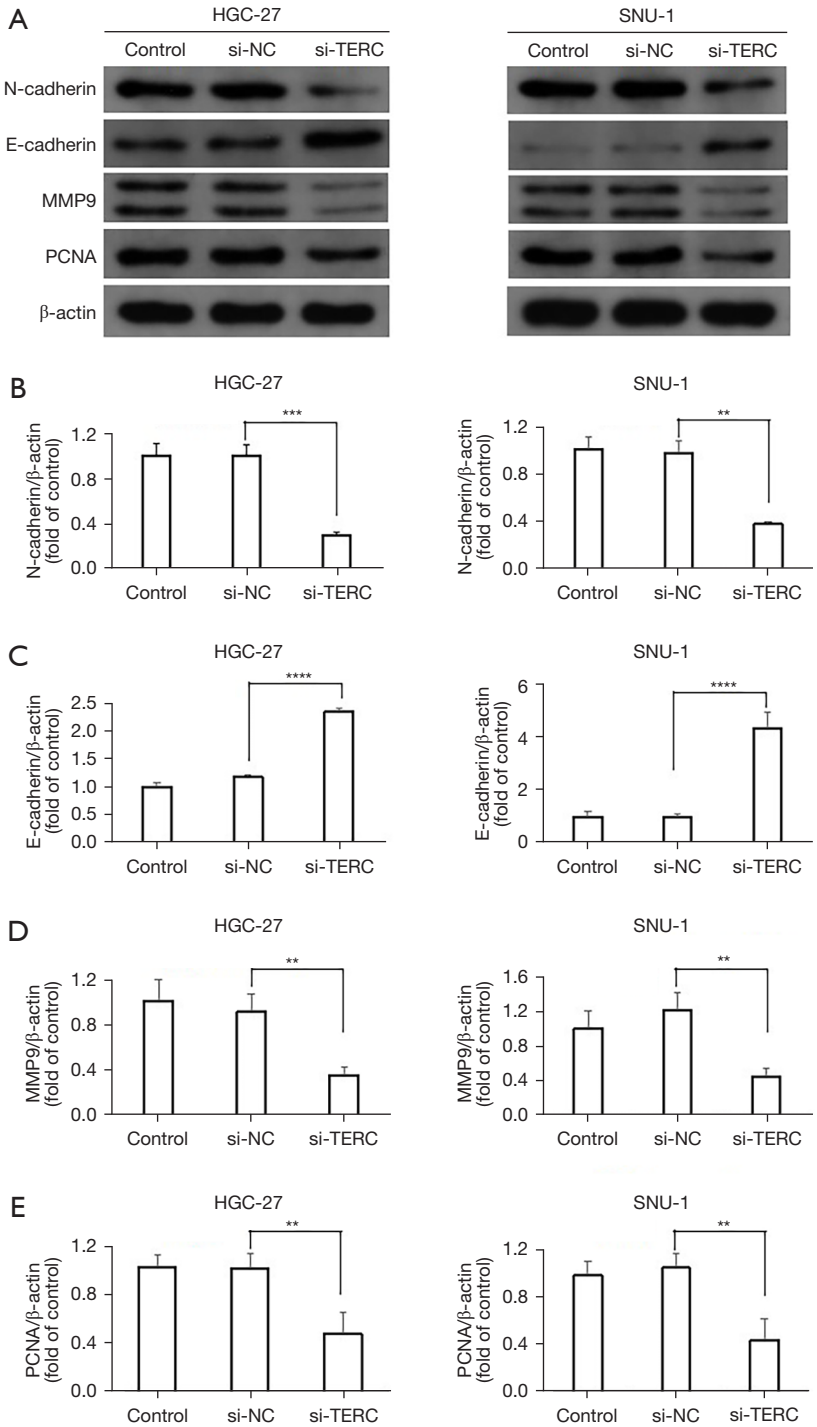

Figure 3 Effects of the *lncRNA TERC* on the expression levels of *N-cadherin*, *E-cadherin*, *MMP9*, and *PCNA* proteins in the HGC-27 and SNU-1 GC cells. The HGC-27 and SNU-1 cells were transfected with si-NC or si-*TERC* for 48 h. (A) The expression levels of *N-cadherin*, *E-cadherin*, *MMP9*, and *PCNA* proteins were detected using western blotting. *β-actin* served as the loading control. The semi-quantification of (B) *N-cadherin*, (C) *E-cadherin*, (D) *MMP9* and (E) *PCNA* protein expression levels in the HGC-27 and SNU-1 cells. **P<0.01; ***P<0.001; ****P<0.0001. *MMP9*, matrix metallopeptidase 9; *PCNA*, proliferating cell nuclear antigen; si, small interfering RNA; NC, negative control; *TERC*, telomerase RNA component; lncRNA, long non-coding RNA; GC, gastric cancer.

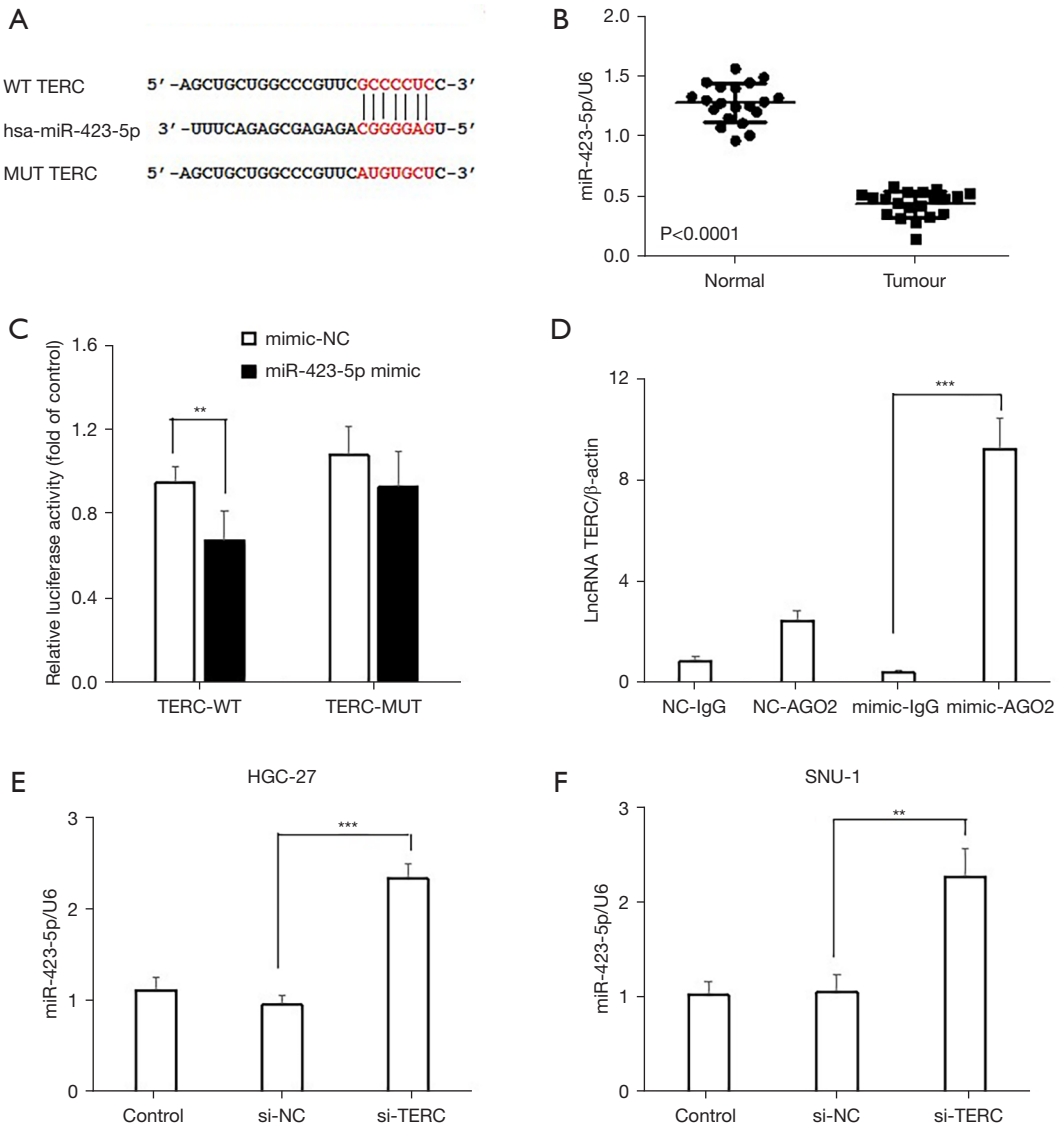

Figure 4 The *lncRNA TERC* acted as a sponge and targeted *miR-423-5p* in the HGC-27 and SNU-1 GC cells. (A) Potential binding sites between the *lncRNA TERC* and *miR-423-5p* were predicted using starBase (https://starbase.sysu.edu.cn/). (B) The expression levels of *miR-423-5p* in the GC and adjacent normal tissues (n=20) were analyzed using RT-qPCR. (C) The relative luciferase activities of cells transfected with TERC-WT or *TERC*-MUT luciferase reporter vectors and *miR-423-5p* mimic or mimic-NC were determined using dual luciferase reporter assays in the 293T cells. (D) Following the transfection of the *miR-423-5p* mimic or mimic-NC into the HGC-27 cells for 48 h, a RIP assay was performed and the expression levels of the *lncRNA TERC* were determined using RT-qPCR. (E,F) After transfection of si-NC or si-*TERC* into the HGC-27 and SNU-1 cells for 48 h, the expression levels of *miR-423-5p* were determined using RT-qPCR. **P<0.01; ***P<0.001. WT, wild-type; *TERC*, telomerase RNA component; miR, microRNA; MUT, mutant; si, small interfering RNA; NC, negative control; lncRNA, long non-coding RNA; GC, gastric cancer; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; RIP, RNA immunoprecipitation.

edu.cn/), *miR-423-5p* was identified as a potential target of the *lncRNA TERC* (see *Figure 4A*). To further examine the binding relationship between the *lncRNA TERC* and *miR-423-5p*, the expression levels of *miR-423-5p* in GC tissues and adjacent normal tissues (n=20) were analyzed using RT-qPCR. The results revealed that *miR-423-5p* was

significantly more downregulated in the GC tissues than the normal tissues (see *Figure 4B*). To verify the binding relationship between the *lncRNA TERC* and *miR-423-5p*, a dual luciferase reporter assay was performed. The results showed that the overexpression of *miR-423-5p* significantly decreased the relative luciferase activity of the *TERC*-WT group, while the relative luciferase activity was unaltered in the *TERC*-MUT group (see *Figure 4C*). Moreover, the RIP analysis demonstrated that the anti-AGO2 antibody could pulldown the *lncRNA TERC* (see *Figure 4D*), and revealed the enrichment of *lncRNA TERC*, *miR-423-5p*, and *SOX12* in IgG or AGO2 pulled-down RNA products in HGC-27 and SNU-1 cells (see Figure S1). The RIP analysis also revealed that the overexpression of *miR-423-5p* led to the substantial upregulation of *lncRNA TERC* expression in the RIP-AGO2 group compared to the RIP-IgG + *miR-423-5p* mimic or RIP-AGO2 + mimic-NC groups (see *Figure 4D*). Additionally, *lncRNA TERC* knockdown significantly upregulated the expression levels of *miR-423-5p* in the HGC-27 and SNU-1 cells (see *Figure 4E,4F*). These results suggested that *miR-423-5p* may be a direct target of the *lncRNA TERC*, and *lncRNA TERC* expression may be negatively associated with *miR-423-5p* expression.

Overexpression of miR-423-5p inhibits GC cell proliferation, migration, and invasion

To further confirm the biological function of *miR-423-5p* in GC, the effects of *miR-423-5p* on the proliferation, migration, and invasion of the HGC-27 and SNU-1 cells were determined. As *Figure 5A,5B* show, the cell viability in *miR-423-5p* mimic group was significantly reduced compared to mimic-NC group. The migratory and invasive abilities of the HGC-27 and SNU-1 cells were also examined using Transwell assays. The results revealed that the migration of the HGC-27 and SNU-1 cells was significantly impaired in the *miR-423-5p* mimic group compared to the mimic-NC group (see *Figure 5C,5D*). And the invasion was also significantly impaired in the *miR-423-5p* mimic group compared to the mimic-NC group in the HGC-27 and SNU-1 cells (see *Figure 5E,5F*). These results indicated that *miR-423-5p* may play crucial roles in the proliferation, migration, and invasion of GC cells.

SOX12 is a downstream target of miR-423-5p and is negatively regulated by miR-423-5p in GC

Using the online software TargetScan Human 7.1, *SOX12* was identified as a candidate target gene of *miR-423-5p* (see *Figure 6A*). To further validate the binding association between *miR-423-5p* and *SOX12*, the expression levels of *SOX12* in GC tissues and adjacent normal tissues (n=20) were first analyzed using western blotting and RT-qPCR. As *Figure 6B,6C* show, *SOX12* expression levels were significantly more upregulated in the GC tissues than the adjacent normal tissues. Subsequently, *SOX12*-WT or *SOX12*-MUT luciferase reporter vectors were constructed, and a dual luciferase reporter assay was performed. The results revealed that the overexpression of *miR-423-5p* significantly attenuated the relative luciferase activity in the *SOX12*-WT group, while the relative luciferase activity was unaltered in the *SOX12*-MUT group (see *Figure 6D*). Further, the effect of *miR-423-5p* on *SOX12* expression was examined in the HGC-27 and SNU-1 cells using western blotting. The protein expression level in *miR-423-5p* mimic group was significantly reduced compared to mimic-NC group in the HGC-27 and SNU-1 cells (see *Figure 6E,6F*). These results indicated that *miR-423-5p* may negatively regulate *SOX12* expression in GC cells.

Discussion

The results of the present study revealed that *lncRNA TERC* expression was more upregulated in GC tissues and cell lines than adjacent normal tissues and gastric mucosal epithelial cells. High *lncRNA TERC* expression was also found to be associated with the poor prognosis of patients with GC. In addition, the *lncRNA TERC* knockdown significantly inhibited the proliferation, migration, and invasion of the HGC-27 and SNU-1 GC cell lines. The functional mechanistic studies further revealed that the *lncRNA TERC* regulated the expression of the SOX12 protein via *miR-423-5p* in GC. These data suggested that the *lncRNA TERC* may function as an oncogene in GC.

An increasing number of studies have suggested that lncRNAs are closely associated with the occurrence and progression of various types of cancer (32,33). For example, it was previously reported that *lncRNA PVT1* was involved in the pathogenesis of human colorectal cancer (34), and *lncRNA* small nucleolar RNA host gene 3 (*SNHG3*) induced proliferation, migration, invasion and EMT in bladder cancer cells (35). Huang *et al.* (36) reported that *lncRNA AK023391* promoted cell proliferation and invasion by targeting the phosphatidylinositol-3-kinase and protein kinase B signaling pathway in GC. Wu *et al.* (37) demonstrated that *lncRNA SNHG11* promoted

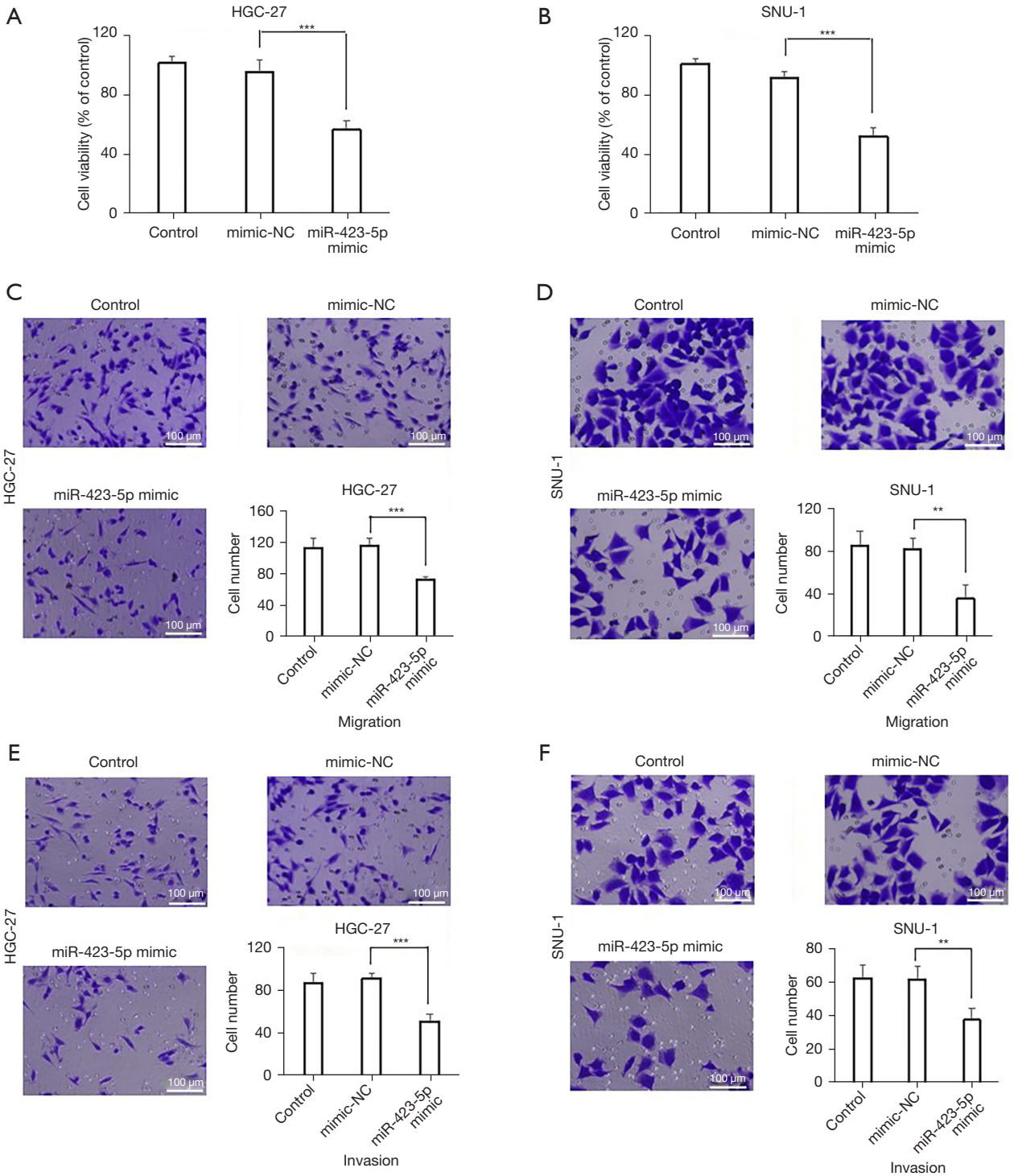

Figure 5 Effects of *miR-423-5p* on HGC-27 and SNU-1 GC cell proliferation, migration, and invasion. The HGC-27 and SNU-1 cells were transfected with *miR-423-5p* mimic or mimic-NC for 48 h. (A,B) The proliferation of the HGC-27 and SNU-1 cells was determined using CCK-8 assays. (C,D) The migration and (E,F) invasion of the HGC-27 and SNU-1 cells were detected using Transwell assays. Only use 1% crystal violet staining solution for staining. **P<0.01; ***P<0.001. NC, negative control; miR, microRNA; GC, gastric cancer; CCK-8, Cell Counting Kit-8.

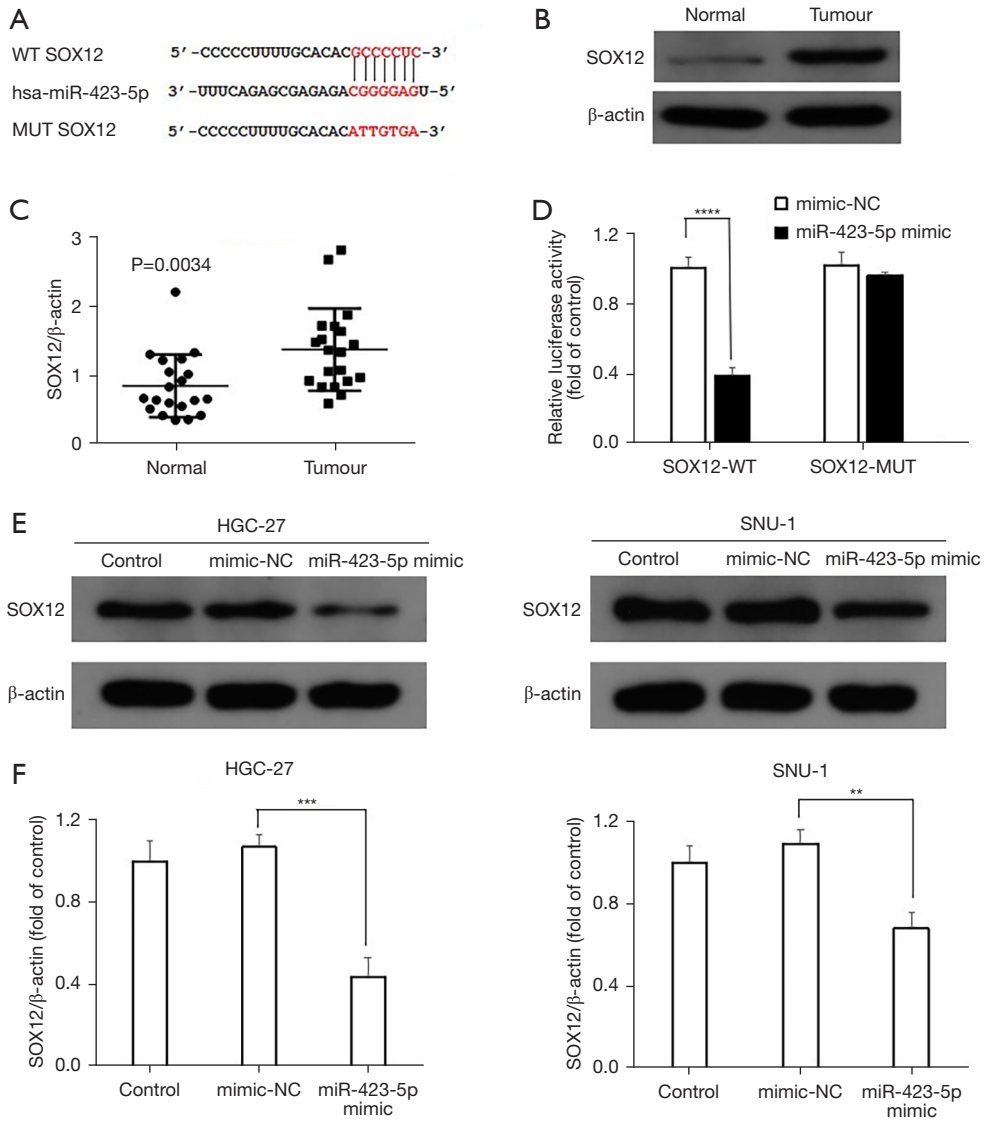

Figure 6 *MiR-423-5p* negatively modulated SOX12 expression in the HGC-27 and SNU-1 GC cell lines. (A) Binding sites between *miR-423-5p* and *SOX12* were predicted using starBase (https://starbase.sysu.edu.cn/). (B) The expression levels of SOX12 protein in GC and adjacent normal tissues were detected using western blotting. (C) The expression levels of SOX12 protein in GC and adjacent normal tissues (n=20) were detected using RT-qPCR. (D) The relative luciferase activities of 293T cells co-transfected with the *SOX12*-WT and *SOX12*-MUT luciferase reporter vectors and *miR-423-5p* mimic or mimic-NC were determined using dual luciferase reporter assays. (E) After the transfection of the *miR-423-5p* mimic or mimic-NC into HGC-27 and SNU-1 cells for 48 h, the expression levels of SOX12 protein were detected using western blotting. β-actin served as the loading control. (F) Semi-quantification of SOX12 protein expression in the HGC-27 and SNU-1 cells. **P<0.01; ***P<0.001; ****P<0.0001. WT, wild-type; *SOX12*, sex determining region Y-box 12; miR, microRNA; MUT, mutant; NC, negative control; GC, gastric cancer.

cell proliferation, migration, invasion, and EMT in GC. Consistent with these findings, the results of the present study showed that *lncRNA TERC* expression was more upregulated in GC tissues and the HGC-27 and SNU-1 cell lines than the adjacent normal tissues and the GES-1 gastric mucosal epithelial cells.

Upregulated *lncRNA TERC* expression was also found to be associated with the poor prognosis of patients with

GC. Further, the results of the current study demonstrated that the knockdown of the *lncRNA TERC* significantly increased the proliferation, migration, and invasion of the HGC-27 and SNU-1 GC cell lines. Numerous previous studies have reported that *PCNA*, *EMT*, and *MMP9* play important roles in the proliferation, migration, and invasion of cancer cells (38-43). Notably, in the present study, the results demonstrated that the *lncRNA TERC* knockdown significantly upregulated the expression levels of the EMT-related marker, *E-cadherin*, and downregulated the expression levels of *PCNA*, *MMP9*, and *N-cadherin* in the HGC-27 and SNU-1 GC cell lines. These results indicated that the *lncRNA TERC* may play a key role in GC progression.

MiRNAs are a class of non-coding RNAs, 18–25 nucleotides in length, which have been found to be involved in the regulation of tumorigenesis and cancer progression (44). It was previously reported that *miR-3622a* increased the proliferation and invasion of bladder cancer cells by decreasing *ceramine synthase 2* expression (45). Further, *miR-325-3p* was discovered to promote breast cancer cell proliferation, invasion, and EMT by targeting S100 calcium binding protein A2 (46). The *miR-200a/205* has been reported to be involved in the EMT process in GC cells (47). *MiR-216a* inhibits the metastasis of GC cells by regulating the EMT process by targeting the JAK2/STAT3 signaling pathway (48). Tang *et al.* (49) also reported that the overexpression of *miR-423-5p* significantly inhibited the proliferation, colony formation, and invasion of the ovarian cancer A2780s (also known as A2780) and A2780cp (cisplatin resistant) cell lines. The findings of the present study revealed that the expression levels of *miR-423-5p* were significantly more downregulated in the GC tissues than the adjacent normal tissues. The overexpression of *miR-423-5p* also significantly reduced the proliferation, migration, and invasion of the HGC-27 and SNU-1 GC cell lines. These data suggested that *miR-423-5p* may play a significant role in the regulation of GC progression.

There is increasing evidence that lncRNAs act as endogenous sponges to modulate miRNA expression and biological functions (20-23). For example, *lncRNA BCRT1* promoted breast cancer progression by sponging *miR-1303*, thereby modulating the expression of polypyrimidine tract binding protein 3 (20). It was also reported that *lncRNA CASC9* induced bladder cancer cell proliferation and EMT by sponging *miR-758-3p*, thereby upregulating *TGF-β2* expression (21). Du *et al.* (50) found that *lncRNA long intergenic non-protein coding RNA 319* acted as the sponge for *miR-423-5p*, which subsequently upregulated nucleus accumbens associated 1 expression and promoted the proliferation, migration, and invasion of ovarian cancer cells. Lin *et al.* (51) also demonstrated that *lncRNA PVT1* acted as a ceRNA to sponge *miR-423-5p* and promoted thyroid cancer cell proliferation and invasion by upregulating p21 (*RAC1*) activated kinase 3 expression.

In the present study, an online prediction tool analysis identified target binding sites between the *lncRNA TERC* and *miR-423-5p*. The binding relationship between the *lncRNA TERC* and *miR-423-5p* was further determined using dual luciferase reporter and RIP assays. The results revealed that *miR-423-5p* significantly reduced the relative luciferase activity in the *TERC*-WT group, while the relative luciferase activity was unaltered in the *TERC*-MUT group. The results of the RIP assays also demonstrated that the overexpression of *miR-423-5p* significantly upregulated *lncRNA TERC* expression in the RIP-AGO2 group compared to the RIP-IgG + *miR-423-5p* mimic or RIP-AGO2 + mimic-NC groups. Additionally, the *lncRNA TERC* knockdown significantly upregulated *miR-423-5p* expression in the HGC-27 and SNU-1 cells. These results suggested that the lncRNA TERC may promote GC progression by sponging *miR-423-5p*.

The SOX transcription factor family comprises 20 members in vertebrates, which play an important role in cell differentiation, tumorigenesis, and embryonic development (52-54). *SOX12* is a member of the SOXC family and has been reported to promote multiple malignant processes in various types of cancer (55-58). For example, it was previously demonstrated that *SOX12* mediated cell proliferation and metastasis by regulating asparagine synthesis in colorectal cancer (55). Another study also found that *miR-370* inhibited cell proliferation, migration, and invasion by downregulating *SOX12* expression in bladder cancer (56). Ge *et al.* (59) reported that *lncRNA long intergenic non-protein coding RNA 2908* promoted cell proliferation by regulating the *miR-663a/SOX12* signaling axis in pancreatic cancer. Du *et al.* (60) also found that *SOX12* promoted cell migration, invasion, and metastasis by upregulating *MMP7* and *insulin-like growth factor 1* expression in GC.

The results of the present study revealed that *SOX12* was more upregulated in GC tissues than matched adjacent normal tissues. An online prediction tool analysis also identified target sites between *miR-423-5p* and *SOX12*. To further determine the binding relationship between *miR-423-5p* and *SOX12*, dual luciferase reporter assays were

performed in the 293T cells. The results illustrated that the overexpression of *miR-423-5p* significantly attenuated the relative luciferase activity in the *SOX12*-WT group, while the relative luciferase activity was unaltered in the *SOX12*-MUT group. Further, the overexpression of *miR-423-5p* significantly downregulated the expression levels of *SOX12* in the HGC-27 and SNU-1 cells. These data indicated that *miR-423-5p* may inhibit GC progression by downregulating *SOX12* expression. In our next experiment, we will further confirm the associations between EMT relative proteins (such as *N-cadherin*, *E-cadherin*, *MMP9*, and *PCNA*) and *miR-423-5p* and *SOX12*, to further verify whether overexpression of *miR-423-5p* and knockdown of *SOX12* can significantly upregulate the expression level of E-cadherin and downregulate the expression levels of *PCNA*, *MMP9* and *N-cadherin* in HGC-27 and SNU-1 GC cell lines.

In conclusion, the findings of the current study indicated that *lncRNA TERC* expression may be significantly upregulated in GC tissues and cells and closely associated with a poor prognosis in patients with GC. The *lncRNA TERC* was discovered to promote the proliferation, invasion, and migration of GC cells. Further mechanistic studies revealed that the *lncRNA TERC* promoted cell proliferation, invasion, and migration by acting as a natural sponge of *miR-335-5p* and affecting *SOX12* expression. Thus, these findings suggested that the *lncRNA TERC* may act as an oncogene in GC, and it may represent a promising prognostic biomarker and novel therapeutic target for the disease.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-3545/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-3545/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3545/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All participants provided written informed consent, and the study was approved by the Ethics Committee of Shaanxi Provincial People's Hospital (No. 2021-186).

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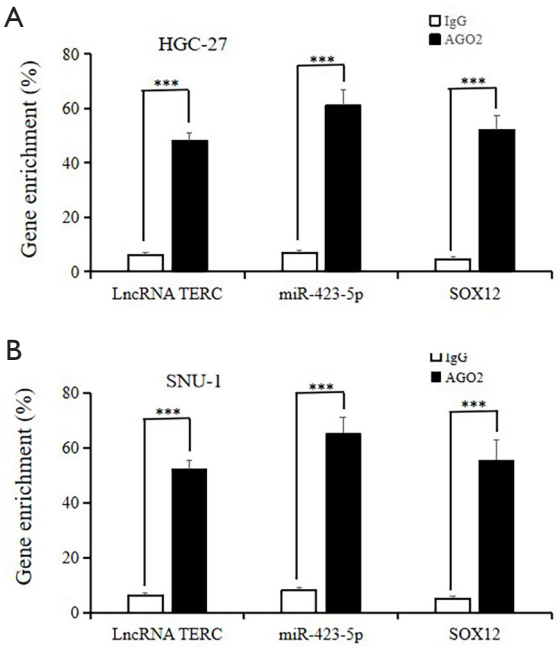

Figure S1 Gene enrichment in RIP aasay. (A,B) RIP assay was performed to assess the enrichment of *lncRNA TERC*, *miR-423-5p*, and *SOX12* in IgG or AGO2 pulled-down RNA products in HGC-27 and SNU-1 cells. ***P<0.001. LncRNA, long non-coding RNA; *TERC*, telomerase RNA component; miR, microRNA; *SOX12*, sex determining region Y-box 12; IgG, immunoglobulin G; AGO2, argonaute 2; RIP, RNA immunoprecipitation.

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