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

Xiaoyan Huang
Shaanxi Provincial People's Hospital
Zhankui Jin
Shaanxi Provincial People's Hospital
Yangzhi He
Xi'an medical university
Xiao Liu
Xi'an medical university
Xianglong Duan
Shaanxi Provincial People's Hospital
Cuixiang Xu
Shaanxi Provincial People's Hospital
Jianhua Wang (wangjianhuaman@163.com)
Shaanxi Provincial People's Hospital

Research Article

Keywords: long non-coding RNA telomerase RNA component, microRNA-423-5p, SOX12, gastric cancer, proliferation, migration, invasion

Posted Date: March 17th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1402753/v1

Abstract

Background

Increasing evidences demonstrate long non-coding RNAs (lncRNAs) play an critical roles in gastric cancer initiation progression. However, the biological function of long non-coding RNA (lncRNA) telomerase RNA component (TERC) remains unknown in human gastric cancer. The present study aimed to determine the biological function and underlying molecular mechanism of lncRNA TERC in gastric cancer progression.

Methods

The expression levels of lncRNA TERC in gastric cancer tissues and cell lines were anaylzed using reverse transcription-quantitative PCR. The effects of lncRNA TERC on the proliferation, migration and invasion of gastric cancer cells were determined using Cell Counting Kit-8 and Transwell assays, respectively. Dual luciferase reporter and argonaute 2-RNA immunoprecipitation assays were used to detect the binding between lncRNA TERC and microRNA (miR)-423-5p. Western blotting was performed to measure the expression levels of SOX12, N-cadherin, E-cadherin, MMP9 and proliferating cell nuclear antigen (PCNA).

Results

lncRNA TERC expression levels were upregulated in gastric cancer cells and tissues, while miR-423-5p expression was downregulated. The upregulation of lncRNA TERC was associated with a shorter overall survival in patients with gastric cancer. lncRNA TERC knockdown significantly reduced the proliferation, migration and invasion of HGC-27 and SNU-1 cells. Furthermore, lncRNA TERC knockdown in 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 lncRNA TERC and was found to directly bind with lncRNA TERC. Moreover, miR-423-5p was discovered to directly target SOX12 to inhibit the proliferation, migration and invasion of HGC-27 and SNU-1 cells.

Conclusion

The findings of the current study suggested that lncRNA TERC may regulate the miR-423-5p/SOX12 signaling axis via directly sponging miR-423-5p and inhibiting SOX12 expression, thereby leading to the progression of gastric cancer. These findings may offer novel targets for future gastric cancer therapy.

Introduction

Gastric cancer is a common type of malignancy and the third 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 gastric cancer has decreased due to the development of effective screenings technologies and methods to control *Helicobacter pylori* infection [5, 6]. Surgical resection, radiotherapy, chemotherapy and combined therapy are currently the primary treatment options available for gastric cancer, and all have been reported to significantly improve the survival of patients with gastric cancer [7]. However, the prognosis of patients with advanced-stage gastric cancer remains unsatisfactory and the 5-year survival rate for patients with metastatic gastric cancer is ~ 30% [8]. At present, the underlying molecular mechanism involved in gastric cancer development and progression remains unclear, to the best of our knowledge. Thus, further research to improve the current understanding of the molecular mechanisms of gastric cancer progression and identify novel therapies targeting metastasis in gastric cancer is urgently required.

Long non-coding RNAs (lncRNAs) are non-coding RNA molecules of > 200 nucleotides in length that have a limited protein-coding potential [9]. 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 [10–12]. Accumulating evidence indicated 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 instance, lncRNA long intergenic non-protein coding RNA 2620 (BCRT1) was discovered to promote breast cancer progression via binding with miR-1303 [13]. In bladder cancer, lncRNA cancer suspectibility 9 (CASC9) adsorbed miR-758-3p to induce cell proliferation and epithelial-mesenchymal transition (EMT) by regulating TGF-β2 expression [14]. It has also been reported that lncRNA Pvt1 oncogene (PVT1) promoted cell migration via sponging miR-30a and regulating snail family transcriptional repressor 1 expression in gastric cancer [15]. Qu *et al* [16] also demonstrated that lncRNA HOXA cluster antisense RNA 3 promoted gastric cancer progression via sponging miR-29a-3p, which subsequently regulated lymphotoxin β receptor expression and activated NF-κB signaling. Telomerase RNA component (TERC) is an important RNA component of telomerase and lncRNA TERC, a non-coding RNA, provides a template sequence for telomere synthesis (24). lncRNA TERC has also been reported to alleviate the progression of osteoporosis via sponging miR-217 and upregulating RUNX family transcription factor 2 expression [17]. However, to the best of our knowledge, the biological functions of lncRNA TERC in the progression of cancer, especially gastric cancer, remain largely unknown.

The current study aimed to determine the expression levels of lncRNA TERC in gastric cancer tissues and cell lines. In addition, the effects of lncRNA TERC on gastric cancer cell proliferation, invasion and migration were analyzed. Further mechanistic studies were performed to explore the role of the lncRNA TERC/miR-423-5p/SOX12 signaling axis in the progression of gastric cancer. Together, the findings of the present study may provide a novel insight into the potential of the lncRNA TERC/miR-423-5p/SOX12 signaling axis as a treatment target for gastric cancer.

Materials And Methods

Patient samples. A total of 20 human gastric cancer and corresponding and adjacent normal tissues were obtained from patients admitted to Shaanxi Provincial People's Hospital (Xi'an, China) between July 2020 and December 2020. All participants provided written informed consent, and the study was approved by the Ethical Committee of Shaanxi Provincial People's Hospital.

*Bioinformatics analysis*Human gastric cancergene expression data were obtained from the Gene Expression Omnibus (GEO) dataset, GSE63288. Data analysis was performed using the DEGseq package of R software（1.12.0）(RStudio, Inc.). Genes with$\log_2$|Fold Change|>1and significance of P<0.05 were considered to be differentially expressed genes.The binding sites between lncRNA TERC and miR-423-5pwere predicted usingstarBase database (http://starbase.sysu.edu.cn).The binding sites betweenmiR-423-5pand SOX12 were predicted using TargetScan 7.1 database (www.targetscan.org).

Cell lines and culture. The human gastric mucosal epithelial cells GES-1 and the human gastric cancer cell lines, NCI-N87,KATO3, Hs-746T, HGC-27 and SNU-1, were obtained from Procell Life Sciecne & Technology Co., Ltd. SNU-1, KATO3 and HGC-27 cell lines were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.)supplemented with 10 or 20% FBS(Gibco; Thermo Fisher Scientific, Inc.), respectively, and 1% penicillin-streptomycin solution. . GES-1,NCI-N87 and Hs-746T cell lines were cultured in DMEM medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% FBS(Gibco; Thermo Fisher Scientific, Inc.), respectively, and 1% penicillin-streptomycin solution. Alll cells were maintained at 37˚C in a 5% CO_2 humidified incubator.

Cell transfection. A total of $5x10^5$HGC-27 and SNU-1 cells/well were seeded into a 6-well plate overnight at 37˚C. Then, cells were cultured in serum-free RPMI-1640medium for 2 h prior to transfection. Cells were subsequently transiently transfected with small interfering RNA (siRNA/si)-TERC (5'-CCTTCCACCGTTCATTCTA-3'), si-negative control (NC, 5'-UUCUCCGAACGUGUCACGUTT-3') (both Guangzhou Ribobio Co., Ltd.), miR-423-5p mimic (5'-UGAGGGGCAGAGAGCGAGACUUU-3')or mimic-NC (5'-UUCUCCGAACGUGUCACGUTT-3') (both Shanghai GenePharma Co., Ltd.) using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

Cell Counting Kit-8 (CCK-8) assay. The proliferative ability of HGC-27 and SNU-1 cells was measured using a CCK-8 assay. Briefly, $5x10^3$HGC-27 and SNU-1 cells/well were seeded into a 96-well plate and incubated overnight. Cells were then transfected for a further 48 h. Following the transfection, 10 µl CCK-8 solution was added to each well and incubated at 37˚C for an additional 4 h. The absorbance of each well was measured at a wavelength of 450 nm using amicroplatereader (Flexstation® 3; Molecular Devices, LLC).The experiments were performed in triplicate.

Cell migration and invasion assays. The migratory and invasive abilities of HGC-27 and SNU-1 cells were determined using Transwell plates (24-well inserts; Corning, Inc.). Briefly, for the migration assay, $5x10^5$HGC-27 and SNU-1 cells/well were seeded into a 6-well plate and incubated overnight. Cells were transfected for 48 h, trypsinized and resuspended in serum-free RPMI-1640 medium at a density of $3x10^5$cells/ml.A volume of 200 µl cell suspension was added intothe upper chambers of the Tranwell

plate, while 800 µl growth medium (RPMI-1640 mediumsupplemented with 10 or 20% FBS) was added into 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, x200). The invasion assay was performed as described, with a minor alteration in that the upper chamber of the Transwell plate was precoated with 100 µl Matrigel (1 mg/ml; Corning, Inc.).

Reverse transcription-quantitative PCR(RT-qPCR). Total RNA was extracted fromgastric cancer tissues, adjacent normal tissues, HGC-27 and SNU-1 cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA was reverse transcribed into cDNA using random primers and Hiscript Reverse Transcriptase(GeneCopoeia Company, USA) for mRNA quantification. For miRNA quantification, the reverse transcription step was performed using an Oligo (dT) 18/miRNA loop and Hiscript Reverse Transcriptase. Primer sequences were listed inTable 1.The PCR reaction conditions were as follows: 10 minute at 95°C, and then 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C.Expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (26).

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

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-NC into 293T cells.Following 48 h of transfection,aDual Luciferase Reporter Gene assay kit(Beyotime Institute of Biotechnology) was used to determine the relative luciferase activity.

RNA immunoprecipitation (RIP) assay. An RNA-Binding Protein Immunoprecipitation kit (MilliporeSigma) was used, according to the manufacturer's protocol, to determine the relationship between lncRNA TERC and miR-423-5p. Anti-argonaute 2 (AGO2)(SAB4200085, MilliporeSigma) and control IgG (R9255, MilliporeSigma) antibodies were used to performed the RIP assay, and the expression levels of lncRNA TERC and miR-423-5p were subsequently evaluated using RT-qPCR.

Western blotting. Relative protein expression levels were examined using western blotting as previously described[18]. Briefly, total protein was extracted from tissue samples and HGC-27 and SNU-1 cells using RIPA lysis buffer (Beyotime Institute of Biotechnology) supplemented with 1% protease inhibitor cocktail (Sigma-Aldrich; Merck KGaA). Proteins were separated via SDS-PAGE and then transferred to nitrocellulose membranes (MilliporeSigma). After blocking with 5% non-fat milk for 2 hat room temperature,the membranes were incubated with the following primary antibodies at 4˚C overnight: Anti-

N-cadherin (cat. no.13116,1:1000 dilution), anti-E-cadherin(cat. no.3195,1:1000 dilution), anti-MMP9 (cat. no.13667,1:1000dilution), anti-proliferating cell nuclear antigen (PCNA) (cat. no.13110,1:1000dilution), and anti-β-actin (cat. no.4970,1:1000 dilution) were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). Anti-SOX12 (cat. no.13116,1:1000dilution)was purchased fromProteintech (Chicago, USA).Following the primary antibody incubation, the membranes were incubated withthe appropriate secondary antibodies(Cell Signaling Technology, Inc., cat. no.7074,1:1000dilution) for 2 hat room temperature. Protein bands were visualized using an ECL kit (Pierce; Thermo Fisher Scientific, Inc.).

Statistical analysis. All data are presented as the mean ± SD. Statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, Inc.). Statistical differences between groups were determined using a Student's t-test or one-way ANOVA followed by a Dunnett's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

lncRNA TERC expression levels are upregulated in gastric cancer tissues and cell lines, and associated with a poor prognosis. The present study first identified differentially expressed lncRNAs in gastric cancer using the GSE63288 dataset from the GEO database. Volcano plots (Fig. 1A) and heat maps (Fig. 1B) showed differentially expressed lncRNAs identified from the GEO database. The identified upregulated lncRNAs that were considered to play an important role in gastric cancer progression were subsequently analyzed in further detail. Among the lncRNAs, the expression levels of lncRNA TERC were found to be consistently significantly upregulated in gastric cancer in GEO database. Thus, gastric cancer and adjacent normal tissues (n = 20) were collected and the expression levels of lncRNA TERC were determined using RT-qPCR. As shown in Fig. 1C, lncRNA TERC expression was revealed to be significantly upregulated in gastric cancer tissues compared with that in adjacent normal tissues. Moreover, the expression levels of lncRNA TERC in human gastric mucosal epithelial cells and five gastric cancer cell lines were analyzed. Compared with the GES-1 cells, the expression levels of lncRNA TERC were significantly upregulated in SNU-1, HGC-27 and KATO3 cells (Fig. 1D). In addition, as shown in Fig. 1E, a shorter overall survival in patients with gastric cancer was found to be associated with higher lncRNA TERC expression levels. Collectively, these results suggested that lncRNA TERC expression may be upregulated in gastric cancer tissues and poor survival outcomes may be associated with lncRNA TERC expression levels in gastric cancer.

Knockdown of lncRNA TERC inhibits gastric cancer cell proliferation, migration and invasion. To explore the biological function of lncRNA TERC in gastric cancer, the effects of lncRNA TERC on the proliferation, migration and invasion of HGC-27 and SNU-1 cells were investigated. si-TERC was used to knock down the expression levels of lncRNA TERC in HGC-27 and SNU-1 cells, and the interference efficiency of lncRNA TERC was detected using RT-qPCR. Compared with the si-NC group, the expression levels of lncRNA TERC were significantly downregulated in HGC-27 and SNU-1 cells in the si-TERC group (Fig. 2A and B). lncRNA TERC knockdown also significantly reduced the proliferation of HGC-27 and SNU-1 cells (Fig. 2A and B). Moreover, the migratory and invasive abilities of HGC-27 and SNU-1 cells were

significantly reduced in the si-TERC group compared with those in the si-NC group (Fig. 2C-F). EMT plays an important role in cancer invasiveness and metastasis (28, 29). Thus, the effect of lncRNA TERC on EMT-related markers, such as N-cadherin and E-cadherin, was also analyzed using western blotting. As shown in Fig. 3A-C, the expression levels of N-cadherin were significantly downregulated in the si-TERC group, while the expression levels of E-cadherin were upregulated, compared with those in the si-NC group. In addition, 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 lncRNA TERC knockdown significantly downregulated the protein expression levels of MMP9 and PCNA in HGC-27 and SNU-1 cells (Fig. 3A, D and E). These data indicated that lncRNA TERC may regulate the proliferation, migration and invasion of gastric cancer cells via regulating EMT and the protein expression levels of MMP9 and PCNA.

lncRNA TERC functions as a molecular sponge for miR-423-5p in gastric cancer. Increasing evidence has demonstrated that lncRNA TERC acts as a ceRNA to regulate the biological function of miRNAs (20–23). Using the starBase database (http://starbase.sysu.edu.cn), miR-423-5p was identified as a potential target of lncRNA TERC (Fig. 4A). To further determine the binding relationship between lncRNA TERC and miR-423-5p, the expression levels of miR-423-5p in gastric cancer tissues and adjacent normal tissues (n = 20) were analyzed using RT-qPCR. The results revealed that miR-423-5p was significantly downregulated in the gastric cancer tissues compared with that in adjacent normal tissues (Fig. 4B). To verify the binding relationship between 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 (Fig. 4C). Moreover, RIP analysis demonstrated that the anti-AGO2 antibody could pull down lncRNA TERC (Fig. 4D). RIP analysis also found that the overexpression of miR-423-5p led to the substantial upregulation of lncRNA TERC expression in the RIP-AGO2 group compared with the RIP-IgG + miR-423-5p mimic or RIP-AGO2 + mimic-NC groups (Fig. 4D). In addition, lncRNA TERC knockdown significantly upregulated the expression levels of miR-423-5p in HGC-27 and SNU-1 cells (Fig. 4E and F). These results suggested that miR-423-5p may be a direct target of lncRNA TERC, and lncRNA TERC expression may be negatively associated with miR-423-5p expression.

Overexpression of miR-423-5p inhibits gastric cancer cell proliferation, migration and invasion. To further confirm the biological function of miR-423-5p in gastric cancer, the effects of miR-423-5p on the proliferation, migration and invasion of HGC-27 and SNU-1 cells were determined. As shown in Fig. 5A and B, the overexpression of miR-423-5p significantly reduced cell proliferation compared with that in the the mimic-NC group. The migratory and invasive abilities of HGC-27 and SNU-1 cells were also determined using Transwell assays. The results revealed that the migration of HGC-27 and SNU-1 cells was significantly impaired in the miR-423-5p mimic group compared with the mimic-NC group (Fig. 5C and D). Moreover, the overexpression of miR-423-5p significantly inhibited the invasion of HGC-27 and SNU-1 cells (Fig. 5E and F). These results indicated that miR-423-5p may play crucial roles in the proliferation, migration and invasion of gastric cancer cells.

SOX12 is a downstream target of miR-423-5p and is negatively regulated by miR-423-5p in gastric cancer. Using the online software TargetScan Human 7.1, SOX12 was identified as a candidate target gene of miR-423-5p (Fig. 6A). To further validate the binding association between miR-423-5p and SOX12, the expression levels of SOX12 in gastric cancer tissues and adjacent normal tissues (n = 20) were first analyzed using western blotting. As shown in Fig. 6B and C, SOX12 expression levels were significantly upregulated in the gastric cancer tissues compared with those in 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 markedly attenuated the relative luciferase activity in the SOX12-WT group, while the relative luciferase activity was unaltered in the SOX12-MUT group (Fig. 6D). Furthermore, the effect of miR-423-5p on SOX12 expression was examined in HGC-27 and SNU-1 cells using western blotting. The analysis demonstrated that the overexpression of miR-423-5p notably downregulated the expression levels of SOX12 in HGC-27 and SNU-1 cells compared with those in the mimic-NC group (Fig. 6D and F). These results indiated that miR-423-5p may negatively regulate SOX12 expression in gastric cancer cells.

Discussion

The results of the present study revealed that lncRNA TERC expression was upregulated in gastric cancer tissues and cell lines compared with that in 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 gastric cancer. In addition, lncRNA TERC knockdown significantly inhibited the proliferation, migration and invasion of the gastric cancer cell lines, HGC-27 and SNU-1. Functional mechanistic studies further revealed that lncRNA TERC regulated the expression of the SOX12 protein via miR-423-5p in gastric cancer. These data suggested that lncRNA TERC may function as an oncogene in gastric cancer.

An increasing number of studies have suggested that lncRNAs are closely associated with the occurrence and progression of a variety of types of cancer [19, 20]. For example, it was previously reported that lncRNA PVT1 was involved in the pathogenesis of human colorectal cancer [21] and lncRNA small nucleolar RNA host gene 3 induced proliferation, migration, invasion and EMT in bladder cancer cells [22]. Huang *et al* [23] reported that lncRNA AK023391 promoted cell proliferation and invasion via targeting the PI3K/Akt signaling pathway in gastric cancer. Wu *et al* [24] demonstrated that lncRNA small nucleolar RNA host gene 11 promoted cell proliferation, migration, invasion and EMT in gastric cancer. Consistent with these findings, the results of the present study showed that lncRNA TERC expression was upregulated in gastric cancer tissues and cell lines, HGC-27 and SNU-1, compared with that in adjacent normal tissues and the gastric mucosal epithelial cells, GES-1. Upregulated lncRNA TERC expression was also found to be associated with the poor prognosis of patients with gastric cancer. Furthermore, the results of the current study demonstrated that the knockdown of lncRNA TERC markedly increased the proliferation, migration and invasion of the gastric cancer cell lines, HGC-27 and SNU-1. Numerous previous studies have reported that PCNA, EMT and MMP9 played an important role in the proliferation, migration and invasion of cancer cells [25–27]. Notably, in the present study, the results demonstrated that 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 gastric cancer cell lines, HGC-27 and SNU-1. These results indicated that lncRNA TERC may play a key role in gastric cancer progression.

miRNAs are a class of non-coding RNAs of 18–25 nucleotides in length, which have been found to be involved in the regulation of tumorigenesis and cancer progression [28]. It was previously shown that miR-3622a increased the proliferation and invasion of bladder cancer cells by decreasing ceramine synthase 2 expression [29]. Furthermore, miR-325-3p was discovered to promote breast cancer cell proliferation, invasion and EMT via targeting S100 calcium binding protein A2 [30]. MR-200a/205 has been reported to involve in the the EMT process of gastric cancer cells [31]. MicroRNA-216a inhibits the metastasis of gastric cancer cells via regulating EMT process through targeting JAK2/STAT3 siganling pathway [32]Tang *et al* [33] also reported that the overexpression of miR-423-5p significantly inhibited the proliferation, colony formation and invasion of ovarian cancer A2780s (also kown 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 downregulated in the gastric cancer tissues compared with those in adjacent normal tissues. The overexpression of miR-423-5p also significantly reduced the proliferation, migration and invasion of the gastric cancer cell lines, HGC-27 and SNU-1. These data suggested that miR-423-5p may play a significant role in the regulation of gastric cancer progression.

Increasing evidence has suggested that lncRNAs act as endogenous sponges to modulate miRNA expression and biological function (20–23). For example, lncRNA BCRT1 promoted breast cancer progression by sponging miR-1303, thereby modulating the expression of polypyrimidine tract binding protein 3 [13]. It was also reported that lncRNA CASC9 induced bladder cancer cell proliferation and EMT by sponging miR7583p, thereby upregulating TGFβ2 expression [14]. Du *et al* [34] 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* [35] also demonstrated that lncRNA PVT1 acted as a ceRNA to sponge miR-423-5p and promote thyroid cancer cell proliferation and invasion by upregulating p21 (RAC1) activated kinase 3 expression.

In the present study, online prediction tool analysis identified target binding sites between lncRNA TERC and miR-423-5p. Thus, the binding relationship between 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 assay also demonstrated that the overexpression of miR-423-5p significantly upregulated lncRNA TERC expression in the RIP-AGO2 group compared with the RIP-IgG + miR-423-5p mimic or RIP-AGO2 + mimic-NC groups. In addition, lncRNA TERC knockdown significantly upregulated miR-423-5p expression in HGC-27 and SNU-1 cells. These results suggested that lncRNA TERC may promote gastric cancer 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 [36–38]. 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 via regulating asparagine synthesis in colorectal cancer [39]. Another study also found that miR-370 inhibited cell proliferation, migration and invasion via downregulating SOX12 expression in bladder cancer [40]. Ge *et al* [41] reported that lncRNA long intergenic non-protein coding RNA 2908 promoted cell proliferation via regulating the miR-663a/SOX12 signaling axis in pancreatic cancer. Du *et al* [42] also found that SOX12 promoted cell migration, invasion and metastasis by upregulating MMP7 and insulin-like growth factor 1 expression in gastric cancer. The results of the present study revealed that SOX12 was upregulated in gastric cancer tissues compared with matched adjacent normal tissues. 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, a dual luciferase reporter assay was performed in 293T cells. The results illustrated that the overexpression of miR-423-5p markedly attenuated the relative luciferase activity in the SOX12-WT group, while the relative luciferase activity was unaltered in the SOX12-MUT group. Furthermore, the overexpression of miR-423-5p notably downregulated the expression levels of SOX12 in HGC-27 and SNU-1 cells. These data indicated that miR-423-5p may inhibit gastric cancer progression by downregulating SOX12 expression.

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

Declarations

Acknowledgements

Not applicable.

Funding

This work was financially supported by Technology Talent Support Program of Shaanxi Provincial People's Hosptal(2021JY-48), National Natural Science Foundation of China (81760441) , Innovation Capability Support Program of Shaanxi Province (2019GHJD-14), Province Key R&D Program of Shaanxi Province (2021ZDLSF01-07).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

XYH, CXX and JHW conducted the experiments and analyzed the data. CXX, XYH, and JHW made substantial contributions to the design of the present study and prepared the manuscript ZKL, YZH, XL and XLD *perform*ed the western blotting and analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All participants provided written informed consents, and the study was approved by the Ethical Committee of Shaanxi Provincial People's Hospital.

Conflict of interests

The authors declare that there are no conflict of interests.

References

1. Dai Q, Zhang T, Pan J, Li C: **LncRNA UCA1 promotes cisplatin resistance in gastric cancer via recruiting EZH2 and activating PI3K/AKT pathway**. *Journal of Cancer*2020, **11**(13):3882-3892.
2. Hu Y, Hu D, Li W, Yu X: **Neoadjuvant chemotherapy brings more survival benefits than postoperative chemotherapy for resectable gastric cancer: a Meta-analysis of randomized controlled trials**. *Journal of BUON : official journal of the Balkan Union of Oncology*2019, **24**(1):201-214.
3. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J: **Cancer statistics in China, 2015**. *CA: a cancer journal for clinicians*2016, **66**(2):115-132.
4. Van Cutsem E, Sagaert X, Topal B, Haustermans K, Prenen H: **Gastric cancer**. *Lancet (London, England)*2016, **388**(10060):2654-2664.
5. Choi KS, Suh M: **Screening for gastric cancer: the usefulness of endoscopy**. *Clinical endoscopy*2014, **47**(6):490-496.
6. Hashim D, Boffetta P, La Vecchia C, Rota M, Bertuccio P, Malvezzi M, Negri E: **The global decrease in cancer mortality: trends and disparities**. *Annals of oncology : official journal of the European Society for Medical Oncology*2016, **27**(5):926-933.
7. Ren N, Jiang T, Wang C, Xie S, Xing Y, Piao D, Zhang T, Zhu Y: **LncRNA ADAMTS9-AS2 inhibits gastric cancer (GC) development and sensitizes chemoresistant GC cells to cisplatin by regulating miR-223-3p/NLRP3 axis**. *Aging*2020, **12**(11):11025-11041.
8. Yamashita K, Sakuramoto S, Nemoto M, Shibata T, Mieno H, Katada N, Kikuchi S, Watanabe M: **Trend in gastric cancer: 35 years of surgical experience in Japan**. *World journal of gastroenterology*2011, **17**(29):3390-3397.

9. Martens-Uzunova ES, Böttcher R, Croce CM, Jenster G, Visakorpi T, Calin GA: **Long noncoding RNA in prostate, bladder, and kidney cancer**. *European urology*2014, **65**(6):1140-1151.
10. Sun M, Nie FQ, Wang ZX, De W: **Involvement of lncRNA dysregulation in gastric cancer**. *Histology and histopathology*2016, **31**(1):33-39.
11. Zhang Y, Su X, Kong Z, Fu F, Zhang P, Wang D, Wu H, Wan X: **An androgen reduced transcript of LncRNA GAS5 promoted prostate cancer proliferation**. 2017, **12**(8):e0182305.
12. Liu HT, Fang L, Cheng YX, Sun Q: **LncRNA PVT1 regulates prostate cancer cell growth by inducing the methylation of miR-146a**. *Cancer medicine*2016, **5**(12):3512-3519.
13. Liang Y, Song X, Li Y, Chen B, Zhao W, Wang L, Zhang H, Liu Y, Han D, Zhang N*et al*: **LncRNA BCRT1 promotes breast cancer progression by targeting miR-1303/PTBP3 axis**. 2020, **19**(1):85.
14. Zhang Z, Chen F, Zhan H, Chen L, Deng Q, Xiong T, Li Y, Ye J: **lncRNA CASC9 sponges miR-758-3p to promote proliferation and EMT in bladder cancer by upregulating TGF-β2**. *Oncology reports*2021, **45**(1):265-277.
15. Wang L, Xiao B, Yu T, Gong L, Wang Y, Zhang X, Zou Q: **lncRNA PVT1 promotes the migration of gastric cancer by functioning as ceRNA of miR-30a and regulating Snail**. 2021, **236**(1):536-548.
16. Qu F, Zhu B, Hu YL, Mao QS, Feng Y: **LncRNA HOXA-AS3 promotes gastric cancer progression by regulating miR-29a-3p/LTβR and activating NF-κB signaling**. 2021, **21**(1):118.
17. Gao GC, Yang DW, Liu W: **LncRNA TERC alleviates the progression of osteoporosis by absorbing miRNA-217 to upregulate RUNX2**. *European review for medical and pharmacological sciences*2020, **24**(2):526-534.
18. Kang X, Wang H, Li Y, Xiao Y, Zhao L, Zhang T, Zhou S, Zhou X, Li Y, Shou Z*et al*: **Alantolactone induces apoptosis through ROS-mediated AKT pathway and inhibition of PINK1-mediated mitophagy in human HepG2 cells**. *Artificial cells, nanomedicine, and biotechnology*2019, **47**(1):1961-1970.
19. Jiang MC, Ni JJ, Cui WY, Wang BY, Zhuo W: **Emerging roles of lncRNA in cancer and therapeutic opportunities**. *American journal of cancer research*2019, **9**(7):1354-1366.
20. Peng WX, Koirala P, Mo YY: **LncRNA-mediated regulation of cell signaling in cancer**. *Oncogene*2017, **36**(41):5661-5667.
21. Wu H, Wei M, Jiang X, Tan J, Xu W, Fan X, Zhang R, Ding C, Zhao F, Shao X*et al*: **lncRNA PVT1 Promotes Tumorigenesis of Colorectal Cancer by Stabilizing miR-16-5p and Interacting with the VEGFA/VEGFR1/AKT Axis**. *Molecular therapy Nucleic acids*2020, **20**:438-450.
22. Dai G, Huang C, Yang J, Jin L, Fu K, Yuan F, Zhu J, Xue B: **LncRNA SNHG3 promotes bladder cancer proliferation and metastasis through miR-515-5p/GINS2 axis**. *Journal of cellular and molecular medicine*2020, **24**(16):9231-9243.
23. Huang Y, Zhang J, Hou L, Wang G, Liu H, Zhang R, Chen X, Zhu J: **LncRNA AK023391 promotes tumorigenesis and invasion of gastric cancer through activation of the PI3K/Akt signaling pathway**. *Journal of experimental & clinical cancer research : CR*2017, **36**(1):194.

24. Wu Q, Ma J, Wei J, Meng W, Wang Y, Shi M: **lncRNA SNHG11 Promotes Gastric Cancer Progression by Activating the Wnt/β-Catenin Pathway and Oncogenic Autophagy**. *Molecular therapy : the journal of the American Society of Gene Therapy*2021, **29**(3):1258-1278.
25. Saitoh M: **Involvement of partial EMT in cancer progression**. *Journal of biochemistry*2018, **164**(4):257-264.
26. Duan Y, Fang Z, Shi Z, Zhang L: **Knockdown of lncRNA CCEPR suppresses colorectal cancer progression**. *Experimental and therapeutic medicine*2019, **18**(5):3534-3542.
27. Wang F, Zhu W, Yang R, Xie W, Wang D: **LncRNA ZEB2-AS1 contributes to the tumorigenesis of gastric cancer via activating the Wnt/β-catenin pathway**. *Molecular and cellular biochemistry*2019, **456**(1-2):73-83.
28. Lin S, Gregory RI: **MicroRNA biogenesis pathways in cancer**. *Nature reviews Cancer*2015, **15**(6):321-333.
29. Fu S, Luan T, Jiang C, Huang Y, Li N, Wang H, Wang J: **miR-3622a promotes proliferation and invasion of bladder cancer cells by downregulating LASS2**. *Gene*2019, **701**:23-31.
30. Wang H, Hu X, Yang F, Xiao H: **miR-325-3p promotes the proliferation, invasion and EMT of breast cancer cells by directly targeting S100A2**. *Oncology research*2021.
31. Mirzaei S, Baghaei K, Parivar K, Hashemi M, Asadzadeh Aghdaei H: **The expression level changes of microRNAs 200a/205 in the development of invasive properties in gastric cancer cells through epithelial-mesenchymal transition**. *European journal of pharmacology*2019, **857**:172426.
32. Tao Y, Yang S, Wu Y, Fang X, Wang Y, Song Y, Han T: **MicroRNA-216a inhibits the metastasis of gastric cancer cells by targeting JAK2/STAT3-mediated EMT process**. *Oncotarget*2017, **8**(51):88870-88881.
33. Tang X, Zeng X, Huang Y, Chen S, Lin F, Yang G, Yang N: **miR-423-5p serves as a diagnostic indicator and inhibits the proliferation and invasion of ovarian cancer**. *Experimental and therapeutic medicine*2018, **15**(6):4723-4730.
34. Du W, Feng Z, Sun Q: **LncRNA LINC00319 accelerates ovarian cancer progression through miR-423-5p/NACC1 pathway**. *Biochemical and biophysical research communications*2018, **507**(1-4):198-202.
35. Lin QY, Qi QL, Hou S, Chen Z, Zhang L, Zhao HG, Lin CH: **LncRNA PVT1 Acts as a Tumor Promoter in Thyroid Cancer and Promotes Tumor Progression by Mediating miR-423-5p-PAK3**. *Cancer management and research*2020, **12**:13403-13413.
36. Castillo SD, Sanchez-Cespedes M: **The SOX family of genes in cancer development: biological relevance and opportunities for therapy**. *Expert opinion on therapeutic targets*2012, **16**(9):903-919.
37. She ZY, Yang WX: **SOX family transcription factors involved in diverse cellular events during development**. *European journal of cell biology*2015, **94**(12):547-563.
38. Hou L, Srivastava Y, Jauch R: **Molecular basis for the genome engagement by Sox proteins**. *Seminars in cell & developmental biology*2017, **63**:2-12.

39. Du F, Chen J, Liu H, Cai Y, Cao T, Han W, Yi X, Qian M, Tian D, Nie Y*et al*: **SOX12 promotes colorectal cancer cell proliferation and metastasis by regulating asparagine synthesis**. *Cell death & disease*2019, **10**(3):239.
40. Wang Y, Ma DL, Yu CH, Sha KF, Zhao MJ, Liu TJ: **MicroRNA-370 suppresses SOX12 transcription and acts as a tumor suppressor in bladder cancer**. *European review for medical and pharmacological sciences*2020, **24**(5):2303-2312.
41. Ge JN, Yan D, Ge CL, Wei MJ: **LncRNA C9orf139 can regulate the growth of pancreatic cancer by mediating the miR-663a/Sox12 axis**. *World journal of gastrointestinal oncology*2020, **12**(11):1272-1287.
42. Du F, Feng W, Chen S, Wu S, Cao T, Yuan T, Tian D, Nie Y, Wu K, Fan D*et al*: **Sex determining region Y-box 12 (SOX12) promotes gastric cancer metastasis by upregulating MMP7 and IGF1**. *Cancer letters*2019, **452**:103-118.

Figures

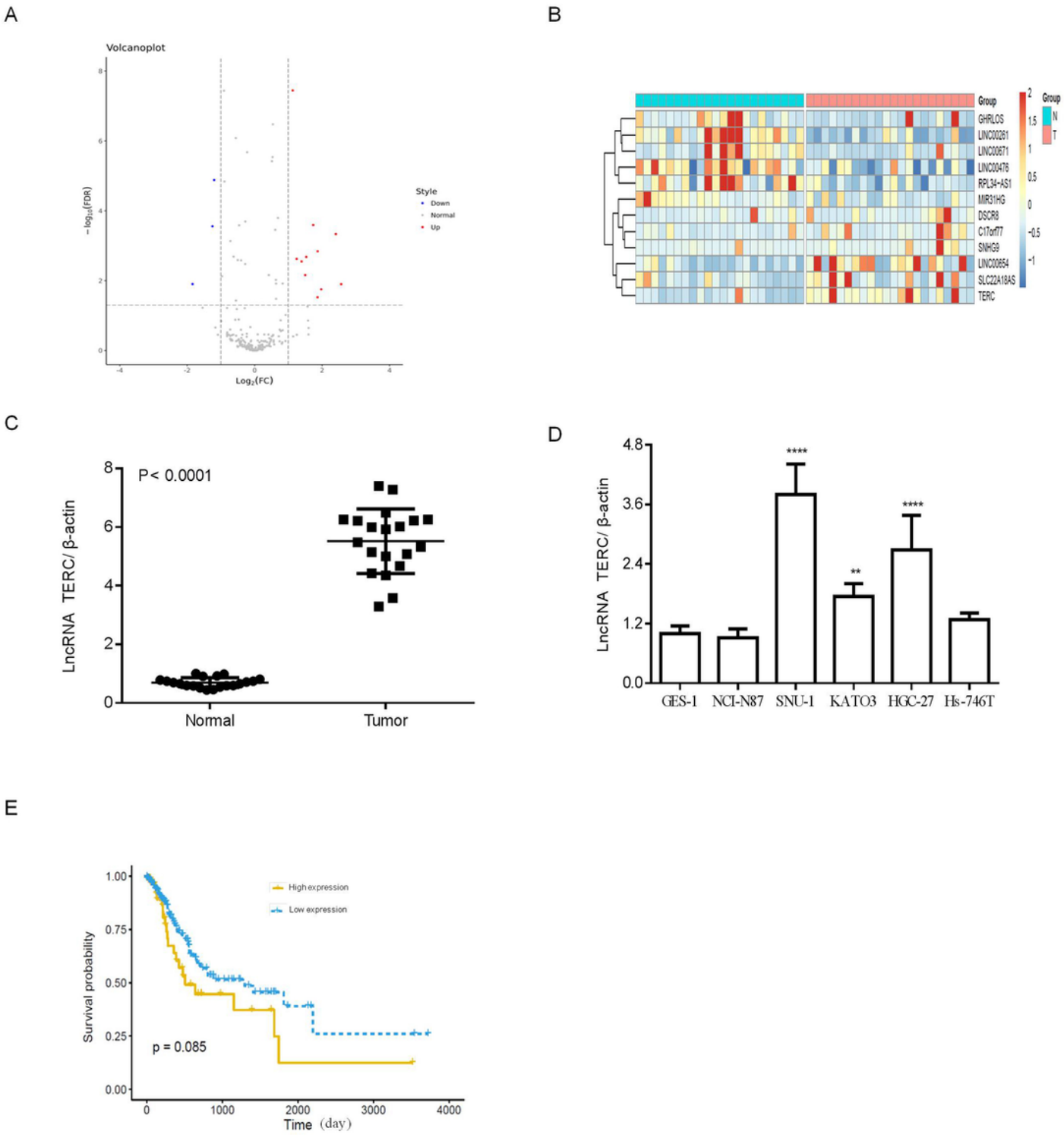

Figure 1

lncRNA TERC expression profiles and association with prognosis in gastric cancer. (A) Volcano plots of the differentially expressed lncRNAs. (B) Heat maps of differentially expressed lncRNAs in gastric cancer tissues compared with adjacent normal tissues. Data were obtained from the GSE63288 Gene Expression Omnibus dataset. (C) Expression levels of lncRNA TERC in gastric cancer and adjacent normal tissues (n=20) were determined using RT-qPCR. (D) Expression levels of lncRNA TERC in human gastric mucosal

epithelial cells and five gastric cancer cell lines were determined using RT-qPCR. (E) Association between lncRNA TERC and overall survival of patients with gastric cancer was determined using Kaplan-Meier analysis. lncRNA TERC-low expression (n = 58) or lncRNA TERC-high expression (n = 320). **P<0.01, ****P<0.0001. lncRNA, long non-coding RNA; TERC, telomerase RNA component; RT-qPCR, reverse transcription-quantitative PCR.

Figure 2

Effects of lncRNA TERC on HGC-27 and SNU-1 gastric cancer cell proliferation, migration and invasion. HGC-27 and SNU-1 cells were transfected with si-NC or si-TERC for 48 h. (A) Interference efficiency of si-TERC in HGC-27 and SNU-1 cells was determined using reverse transcription-quantitative PCR. (B) Cell proliferation was determined using a Cell Counting Kit-8 assay. (C and D) Migration and (E and F) invasion of HGC-27 and SNU-1 cells were detected using Transwell assays. **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.

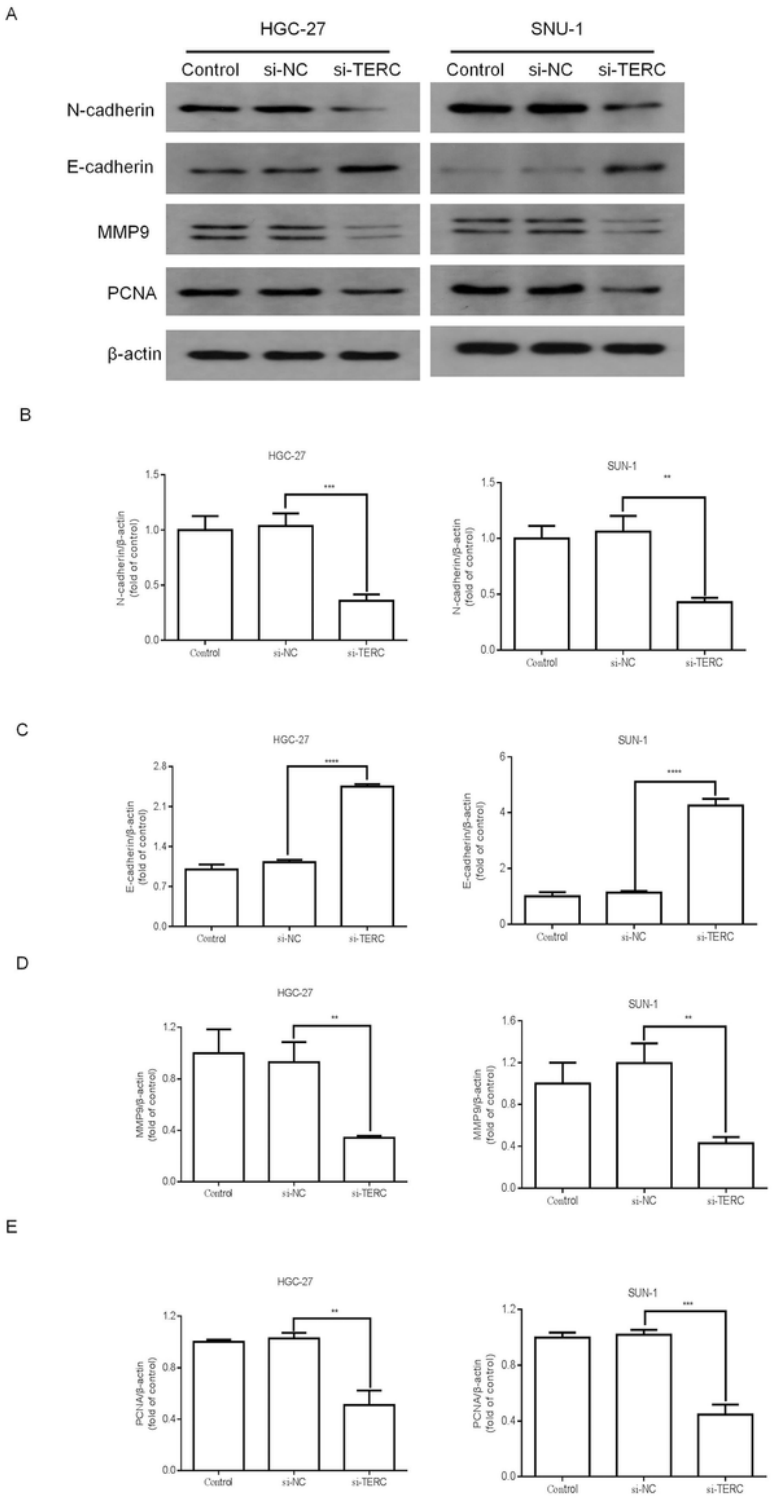

Figure 3

Effects of long non-coding RNA TERC on the expression levels of N-cadherin, E-cadherin, MMP9 and PCNA proteins in gastric cancer cells, HGC-27 and SNU-1. HGC-27 and SNU-1 cells were transfected with si-NC or si-TERC for 48 h. (A) Expression levels of N-cadherin, E-cadherin, MMP9 and PCNA proteins were detected using western blotting. β-actin served as the loading control. Semi-quantification of (B) N-cadherin, (C) E-cadherin, (D) MMP9 and (E) PCNA protein expression levels in HGC-27 and SNU-1 cells.

**P<0.01, ***P<0.001, ****P<0.0001. TERC, telomerase RNA component; si, small interfering RNA; NC, negative control; PCNA, proliferating cell nuclear antigen.

Figure 4

lncRNA TERC acts as a sponge and targets miR-423-5p in gastric cancer cells, HGC-27 and SNU-1. (A) Potential binding sites between lncRNA TERC and miR-423-5p were predicted using starBase (http://starbase.sysu.edu.cn). (B) Expression levels of miR-423-5p in gastric cancer and adjacent normal tissues (n=20) were analyzed using RT-qPCR. (C) 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 a dual luciferase reporter assay in 293T cells. (D) Following transfection of the miR-423-5p mimic or mimic-NC into HGC-27 cells for 48 h, an RNA immunprecpitation assay was performed and the expression levels of lncRNA TERC were determined using RT-qPCR. (E and F) After transfection of si-NC or si-TERC into 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. lncRNA, long non-coding RNA; TERC, telomerase RNA component; miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; WT, wild-type; MUT, mutant; NC, negative control; si, small interfering RNA.

Figure 5

Effects of miR-423-5p on HGC-27 and SNU-1 gastric cancer cell proliferation, migration and invasion. HGC-27 and SNU-1 cells were transfected with miR-423-5p mimic or mimic-NC for 48 h. (A) Proliferation of HGC-27 and SNU-1 cells was determined using a Cell Counting Kit-8 assay. (C and D) Migration and (E and F) invasion of HGC-27 and SNU-1 cells were detected using Transwell assays. **P<0.01, ***P<0.001. miR, microRNA; NC, negative control.

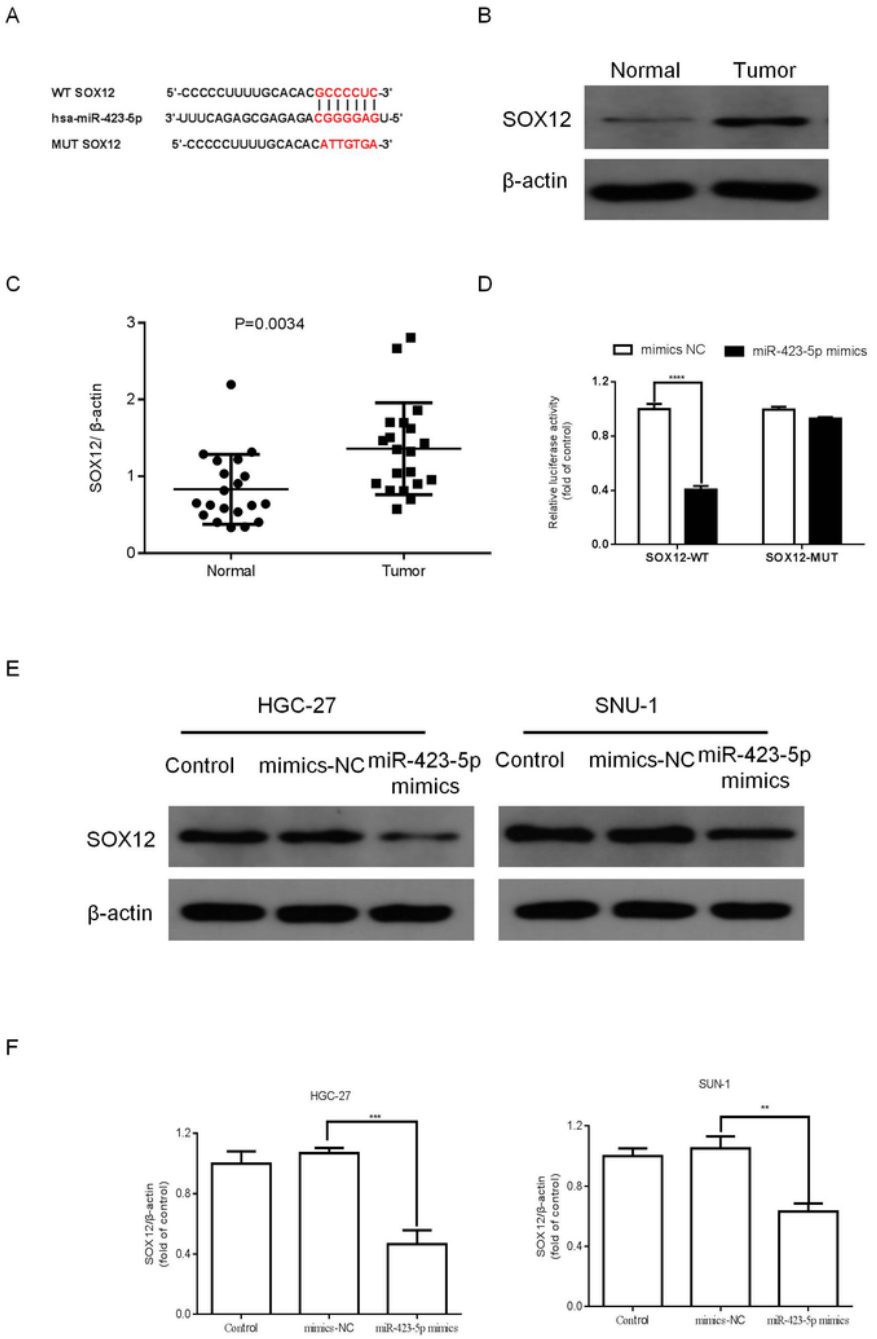

Figure 6

miR-423-5p negatively modulates SOX12 expression in gastric cancer cell lines, HGC-27 and SNU-1. (A) Binding sites between miR-423-5p and SOX12 were predicted using starBase (http://starbase.sysu.edu.cn). (B and C) Expression levels of SOX12 protein in gastric cancer and adjacent normal tissues (n=20) were detected using western blotting. (D) 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 a dual luciferase reporter assay. (E) After 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 HGC-27 and SNU-1 cells. $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$. miR, microRNA; WT, wild-type; MUT, mutant; NC, negative control.