Long Noncoding RNA Taurine-Upregulated Gene 1 Promotes Cell Proliferation and Invasion in Gastric Cancer via Negatively Modulating miRNA-145-5p

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Long noncoding RNA (lncRNA) taurine-upregulated gene 1 (TUG1) is involved in the development and carcinogenesis of various tumors, suggesting the diagnostic potential of TUG1 in these cancers. However, the exact role of TUG1 and its underlying mechanism in gastric cancer (GC) remain unknown. In this study, the expression of TUG1 and miR-145-5p in GC cell lines and nonmalignant gastric epithelial cell lines was detected by qRT-PCR. BGC-823 and SGC-7901 cells were transfected with si-TUG1, pcDNA 3.1-TUG1, miR-145-5p mimics, or matched controls. The biological function of TUG1 and miR-145-5p in GC cell proliferation and invasion in vitro and tumor growth in vivo was investigated by MTT assay, Transwell invasion assay, and tumor xenograft experiments. The regulating relationship between TUG1 and miR-145-5 was confirmed by luciferase reporter assay. The results showed that TUG1 was significantly overexpressed and miR-145-5p was dramatically downregulated in GC cell lines. TUG1 knockdown strikingly inhibited cell proliferation and invasion in vitro and markedly suppressed tumor growth in vivo. Furthermore, TUG1 could directly bind to miR-145-5p and repress miR-145-5p expression. TUG1 overexpression significantly relieved the inhibition on GC cell proliferation and invasion in vitro and tumor growth in vivo, mediated by miR-145-5p overexpression. In conclusion, TUG1 promotes cell proliferation and invasion in GC via negatively modulating miRNA-145-5p, which undoubtedly contributes to understanding the mechanism of GC occurrence and development.

Key words: Gastric cancer (GC); Long noncoding RNA (lncRNA); Taurine-upregulated gene 1 (TUG1); miRNA-145-5p; Proliferation; Invasion

INTRODUCTION

Gastric cancer (GC) ranks as the fourth most common type of malignancy, accounting for 700,000 deaths annually[1]. GC is the second leading cause of cancer-related deaths worldwide, with the highest incidence rates occurring in East Asia, including Japan, P.R. China, and South Korea[1,2]. Despite great improvements that have been made in treatments including chemotherapy, radiotherapy, and surgical therapy for GC in recent years, the 5-year survival rate for GC patients remains unsatisfactory[3]. More than 80% of patients with GC are diagnosed at an advanced stage. That fact, along with malignant proliferation and metastasis, leads to a poor prognosis for GC[4]. In spite of the various oncogenes and tumor suppressors that have been identified in GC tumorigenesis, few useful diagnostic biomarkers for early GC detection have been established[5]. Therefore, it is imperative that more research is devoted to developing novel prognostic biomarkers and a better understanding for the underlying mechanism of GC progression.

Recently, large-scale genome-wide sequencing projects revealed that protein-coding genes account for only about 2% of the human genome, whereas the majority of the human genome are dynamically and pervasively transcribed into noncoding RNAs (ncRNAs)[6]. ncRNAs encompass two major classes: the well-known microRNAs (miRNAs) and the recently acknowledged long noncoding RNAs (lncRNAs)[7]. miRNAs are a type of endogenous ncRNA that are 18–25 nucleotides in length and regulate tumor gene expression at the protein level via degrading target gene mRNA[8]. Accumulating evidence has demonstrated that aberrant expression of miRNAs is closely related to carcinogenesis and progression of many cancers, including GC[9–11]. miR-145-5p (guide strand from pre-miR-145), located on human chromosome 5p32, is an miRNA that has been reported to function as a

tumor suppressor in various tumors, including GC[12,13]. Additionally, it has been previously demonstrated that ectopic expression of miR-145-5p is frequently observed in many tumors such as colorectal cancer, prostate cancer, and GC[14,15]. Thus, miR-145-5p is being studied extensively for its antitumor function in the oncogenic pathway of many tumors[16].

lncRNAs are ncRNAs that are more than 200 nucleotides in length with limited or no protein-coding capacity and are generally expressed in a disease-, cell type-, or developmental stage-specific pattern[17]. lncRNAs have been shown to function as oncogenes or tumor suppressors by inhibiting or promoting the transcription of target genes[18]. Previous studies have indicated that lncRNAs are closely related to physiological and pathological processes, including cellular development and differentiation[19]. Over the past several years, dysregulation of lncRNAs has been implicated as a novel therapeutic biomarker in numerous cancers, including GC[20]. Taurine-upregulated gene 1 (TUG1), a 1.7-kb lncRNA, was initially identified in a genomic screen for genes that play a vital role in retinal development[21]. It is well documented that the expression of TUG1 is upregulated in lung cancer[22] and colorectal cancer[23], as well as in GC[24]. Moreover, many studies have reported that altered expression of TUG1 is involved in the development and carcinogenesis of various tumors, suggesting a diagnostic potential for TUG1 in these cancers[25,26]. Interestingly, a previous study reported that there existed a reciprocal repression between TUG1 and miR-145, and high levels of TUG1 promoted cell invasion and radioresistance in human bladder cancer cells by inhibiting miR-145 expression[6]. However, the relationship between TUG1 and miR-145-5p in GC is still largely unknown.

In the present study, we investigated the expression of TUG1 and miR-145-5p in GC cells. The biological function of TUG1 and miR-145-5p in vitro and in vivo as well as their interaction in GC were further explored.

MATERIALS AND METHODS

Cell Lines and Culture

The human GC cell lines (BGC-823 and SGC-7901) were obtained from the Cell Bank of the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, P.R. China). Nonmalignant gastric epithelial cell line GES-1 was purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). All cell lines used in the present study were maintained in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Gibco), 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) in a humidified incubator containing 5% CO_2 at 37°C, and the medium was refreshed every 2 days.

Cell Transfection

The full-length sequence of the human TUG1 gene (NC_000022.11, in the NCBI) was synthesized and subcloned into pcDNA 3.1 vector (pcDNA 3.1-TUG1; GenePharma, Shanghai, P.R. China). siRNA-targeting TUG1 (si-TUG1), scrambled negative control (si-NC), miR-145-5p mimics (miR-145-5p), and miRNA negative control (miR-NC) were all purchased from Invitrogen. GC cells BGC-823 and SGC-7901 were seeded into six-well plates the day prior to transfection. Cell transfections with si-TUG, si-NC, miR-145-5p, miR-NC, miR-145-5p mimics + pcDNA 3.1-TUG1 (miR-145-5p + TUG1), or miR-145-5p mimics + pcDNA 3.1 (miR-145-5p + NC) were performed with Lipofectamine 2000 reagents (Invitrogen) before cells were grown to 70% confluency. Transfected cells were collected for subsequent study 48 h after transfection.

Quantitative Real-Time PCR (qRT-PCR)

The expression of TUG1 and miR-145-5p in BGC-823 and SGC-7901 cells was evaluated by quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was isolated from cultured cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. For qRT-PCR analysis, isolated RNA was reversely transcribed to complementary DNA (cDNA) by using the PrimeScript™ One Step RT-PCR Kit (Takara, Dalian, P.R. China). RT-PCR analysis for TUG1 and miR-145-5p was performed with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on the StepOnePlus Real-Time PCR System (Applied Biosystems). The specific primers sequences were as follows: TUG1, 5′-TAG CAG TTC CCC AAT CCT TG-3′ (forward) and 5′-CAC AAA TTC CCA TCA TTC CC-3′ (reverse); glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5′-GTC AAC GGA TTT GGT CTG TAT T-3′ (forward) and 5′-AGT CTT CTG GGT GGC AGT GAT-3′ (reverse). The PCR conditions for TUG1 and miR-145-5p were performed as follows: 95°C for 10 min; 40 cycles at 95°C for 15 s, 60°C for 30 s, and 70°C for 30 s. The expression of TUG1 and miR-145-5p was quantified in relation to the control values and normalized to GAPDH using the $2^{-\Delta\Delta CT}$ methods.

Cell Proliferation Assay

Cell proliferation was measured using the MTT kit (Sigma-Aldrich, St. Louis, MO, USA). The transfected BGC-823 and SGC-7901 cells (3,000 cells/well, six repeated wells) were seeded into 96-well plates and cultured for 24, 48, 72, and 96 h at 37°C and 5% CO_2. Subsequently, 20 μl of MTT solution (5 mg/ml; Sigma-Aldrich) was added into each well. Following an additional 4 h of incubation, the supernatant was discarded, and 150 μl of DMSO was supplemented to dissolve

formazan crystals for 10 min. Absorbance value at a wavelength of 490 nm was detected on an ELISA reader (Molecular Devices, Sunnyvale, CA, USA). All experiments were repeated three times.

Transwell Invasion Assay

Transwell chambers (8-μm pore size; BD Biosciences, San Jose, CA, USA) were used to investigate cell invasiveness. Briefly, approximately 5×10^4 transfected GC cells were resuspended in 200 μl of serum-free media and then inoculated into the upper chambers of an insert coated with 50 μl of Matrigel. RPMI-1640 medium (600 μl) with 10% FBS as a chemoattractant was added to the bottom chambers of the Transwell plates. Following incubation for 48 h at 37°C and 5% CO_2, the cells on the upper chambers were scraped with a cotton swab. Subsequently, the cells attached to the bottom surface of the membrane were fixed with methanol for 10 min and stained with 0.1% crystal violet for 20 min, imaged, and counted using an IX71 inverted microscope (Olympus, Tokyo, Japan). Experiments were performed independently in triplicate.

Luciferase Reporter Assay

On the basis of the prediction, TUG1 fragments containing the predicted miR-145-5p binding sites were amplified by PCR from human genomic DNA and cloned into the downstream of the luciferase gene of the pGL3 vector (Promega Corporation, Madison, WI, USA) to form pGL3-TUG1-WT. The sequence of putative miR-145-5p binding sites in the TUG1 gene was replaced as indicated to construct mutant pGL3-TUG1-MUT. For the luciferase reporter assay, BGC-823 and SGC-7901 cells were inoculated into 12-well plates and incubated for 48 h. Then the cells were cotransfected with 300 ng of pGL3-TUG1-WT (WT) or pGL3-TUG1-MUT (MUT) and 50 nM of miR-145-5p or miR-NC using Lipofectamine 2000 (Invitrogen). Cells were collected and lysed 48 h after transfection, and luciferase activity was analyzed with a Dual-Luciferase Reporter Assay System (Beyotime Institute of Biotechnology, Haimen, P.R. China). Firefly luciferase activity of each sample was normalized to that of *Renilla* luciferase.

Tumor Xenograft Experiments

The animal experiments were approved by the Institutional Animal Care and Use Committee of The First Affiliated Hospital of Zhengzhou University. All 6- to 7-week-old female BALB/c nude mice used were purchased from the Experimental Animal Centre of Zhengzhou University and maintained under pathogen-free conditions. Briefly, SGC-7901 cells were transfected with si-TUG1, si-NC, miR-145-5p+TUG1, or miR-145-5p+NC by Lipofectamine 2000 (Invitrogen). Subsequently, stably transfected SGC-7901 cells (1×10^6) were suspended in 0.2 ml of Matrigel Matrix (BD Biosciences) and subcutaneously inoculated into either side of the posterior flank of mice (five mice per group). Four days after injection, the tumor growth was monitored via tumor volume, which was measured every 4 days for at least 24 days. Tumor volume was calculated according to the equation: volume $= 0.5 \times W^2 \times L$ (W, width; L, length). After 24 days of tumor growth, the mice were sacrificed, and tumors were removed, measured, and weighed. Additionally, resected tumor tissues were used to perform immunostaining analysis of Ki-67 protein expression.

Immunohistochemical Staining

GC tumors from mice were immunostained for Ki-67 with two-step immunohistochemical staining using streptavidin–peroxidase (SP) and diaminobenzidine (DAB). Fresh gastric tumor biopsies collected from nude mice were fixed immediatelsy with 4% paraformaldehyde (Sigma-Aldrich, Irvine, Ayrshire, UK) for 30 min at 30°C. The biopsies were then embedded in paraffin, sectioned (4 μm thick) onto slides, and rehydrated in xylene. Antigen retrieval was performed in 20 mmol/L sodium citrate-repairing solution (pH 6.0) at 95°C for 15 min. The tissue sections were then incubated in 3% H_2O_2 solution for 10 min to block endogenous peroxidase activity and incubated with 1:50 diluted Ki-67 antibody (Boster Biological Technology, Ltd., Wuhan, P.R. China) at 4°C overnight. PBS solution as a substitute for primary antibody was used as the negative control. After rinsing three times with PBS, the tissue sections were further incubated with the appropriate secondary antibodies at 37°C for 30 min followed by exposure to SP for another 30 min. Subsequently, the tissue section was stained with DAB (Tiangen Biotechnology, Beijing, P.R. China), counterstained with hematoxylin, dehydrated, and sealed.

Statistical Analysis

Experimental data were expressed as the mean±standard error of the mean (SEM) from at least three independent experiments. Significant differences were calculated by the Student's *t*-test between two groups or one-way multivariate analysis of variance (ANOVA) among three or more groups. Statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA). Differences were considered statistically significant at $p < 0.05$.

RESULTS

TUG1 Knockdown Inhibits Proliferation and Invasion of GC Cells In Vitro

To investigate the biological function of TUG1 in GC carcinogenesis, an analysis of TUG1 expression in GC cell lines (BGC-823 and SGC-7901) and nonmalignant

gastric epithelial cell line (GES-1) was first carried out by qRT-PCR. The results showed that TUG1 was significantly upregulated in BGC-823 and SGC-7901 cells compared with GES-1 cells (Fig. 1A). Thus, we tried to reduce the expression of TUG1 to confirm its role in GC progression. siRNA-mediated TUG knockdown

was then performed in BGC-823 and SGC-7901 cells. Forty-eight hours after transfection, the efficiency of TUG1 knockdown (about 80%) was subsequently verified by qRT-PCR (Fig. 1B). MTT assay was used to examine the effect of TUG1 knockdown on GC cell viability, and the results indicated that TUG1 knockdown
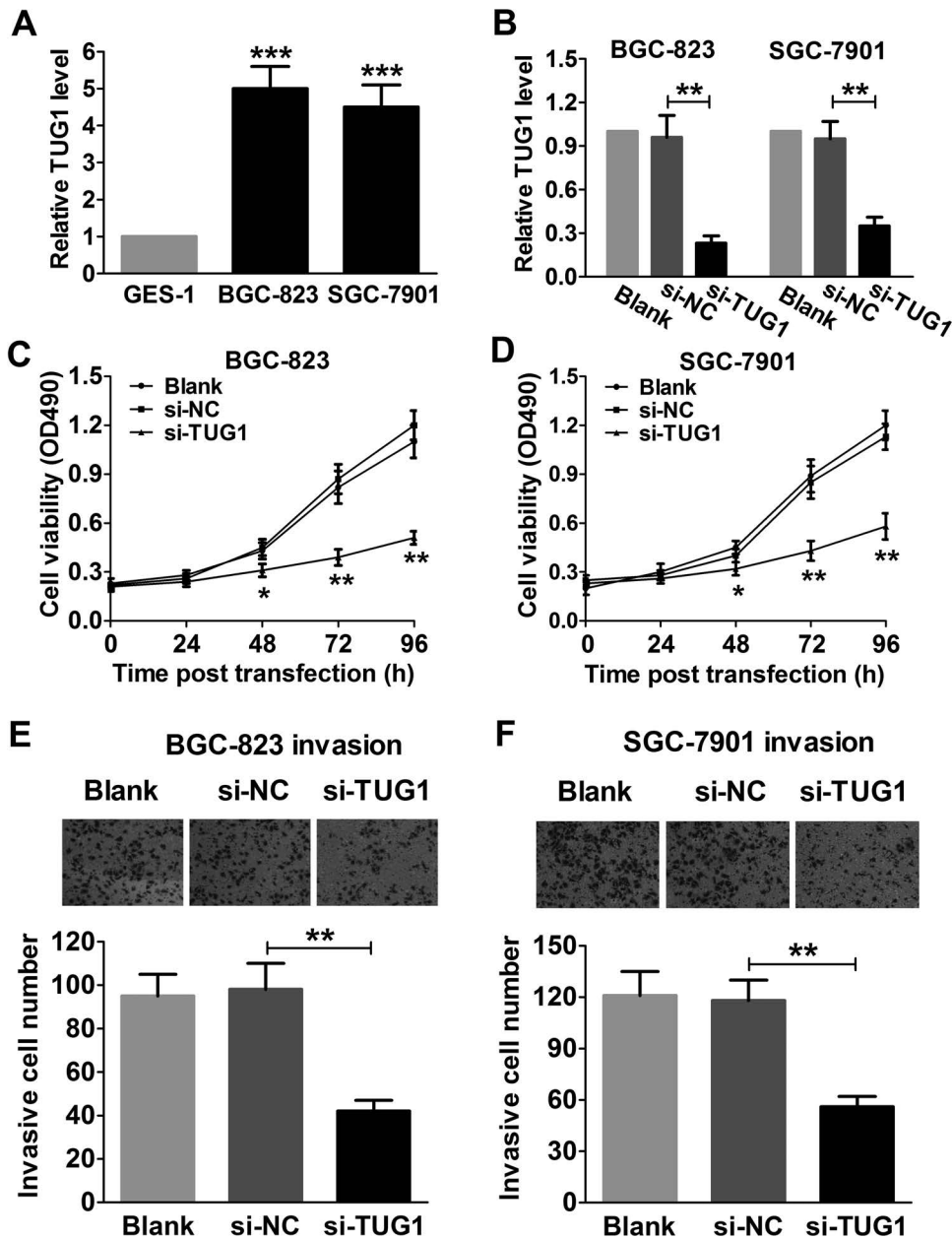

Figure 1. Effect of TUG1 knockdown on GC cell proliferation and invasion in vitro. (A) qRT-PCR was performed to evaluate the expression of TUG1 in GC cell lines (BGC-823 and SGC-7901) and nonmalignant gastric epithelial cell line (GES-1). GAPDH was considered as the endogenous control. (B) The expression of TUG1 was analyzed in BGC-823 and SGC-7901 cells transfected with si-TUG1 or si-NC. GAPDH was considered as the endogenous control. Cell viability in BGC-823 (C) and SGC-7901 (D) cells transfected with si-TUG1 or si-NC was assessed by MTT assay at 24, 48, 72, and 96 h. Cell invasiveness in BGC-823 (E) and SGC-7901 (F) cells transfected with si-TUG1 or si-NC was detected by Transwell invasion assay. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

dramatically inhibited cell viability at 48, 72, and 96 h compared with that of si-NC cell groups in vitro in both GC cell lines BGC-823 (Fig. 1C) and SGC-7901 (Fig. 1D). To explore the effect of TUG1 knockdown on GC cell invasiveness, a Transwell cell invasion assay was performed on GC cells transfected with si-TUG1 or si-NC. It has been shown that cell invasiveness was remarkably inhibited by TUG1 knockdown in BGC-823 (Fig. 1E) and SGC-7901 (Fig. 1F) cells in vitro. Therefore, these results revealed that TUG1 might play an oncogenic role in regulating GC progression.

TUG1 Knockdown Suppressed Tumor Growth In Vivo

To confirm the role of TUG1 in in vivo growth of GC cells, si-TUG1- or si-NC-transfected SGC-7901 cells were intravenously injected into nude mice and a xenograft model was constructed. Twenty-four days after incubation, tumors developed from si-TUG1-transfected SGC-7901 cells grew slower than those derived from si-NC-transfected SGC-7901 cells (Fig. 2A). Also, TUG1 knockdown significantly suppressed tumor growth at the indicated time during the whole tumor growth period (Fig. 2B). In addition, the average tumor weight in the si-TUG1 group was obviously smaller compared with that in the si-NC group (Fig. 2C). Additionally, the expression of TUG1 in excised tumor samples was determined by qRT-PCR, and it has been shown that the TUG1 expression in tumors derived from si-TUG1-transfected SGC-7901 cells was strikingly reduced compared with that in tumors derived from si-NC-transfected SGC-7901 cells (Fig. 2D). Furthermore, immunohistochemical analysis of proliferating cell nuclear antigen (Ki-67) protein expression suggested that the positive rate of Ki-67 expression in tumors derived from si-TUG1-transfected SGC-7901 cells was markedly declined compared with that in tumors derived from si-NC-transfected SGC-7901 cells. These data indicated that TUG1 knockdown suppressed GC cell growth in vivo.

TUG1 Binds to miR-145-5p and Inhibits miR-145-5p Expression in GC Cells

Recently, mounting evidence has demonstrated that lncRNAs may serve as competitive endogenous RNAs (ceRNA) to sponge miRNAs and have an inhibitory effect on miRNA expression and activity[27]. A previous study has demonstrated that there existed a reciprocal repression between TUG1 and miR-145 in human bladder cancer cells[9]. To examine whether TUG1 has a similar regulatory role on miR-145-5p in GC, bioinformatics analysis by online softwares including TargetScan (http://www.targetscan.org) and miRanda (www.microrna.org) was performed to predict potential miRNAs that can be regulated by TUG1. Interestingly, TUG1 was found to bind to miR-145-5p (Fig. 3A). In addition, a significant decrease in the miR-145-5p expression of BGC-823 and SGC-7901 was observed in comparison with that in GES-1 cells (Fig. 3B). Luciferase activity assay was performed to verify whether TUG1 can directly target miR-145-5p, and the results indicated that miR-145-5p overexpression dramatically inhibited luciferase activity of pGL3-TUG1-WT in BGC-823 and SGC-7901 cells. However, an obvious inhibitory effect on the luciferase activity of pGL3-TUG1-MUT was observed (Fig. 3C and D). Cotransfection with miR-145-5p and NC or TUG1 in BGC-823 and SGC-7901 cells was used to investigate whether TUG1 can regulate the expression of miR-145-5p. The qRT-PCR results demonstrated that TUG1 overexpression significantly impeded the expression of miR-145-5p (Fig. 3E and F). The results demonstrated that TUG1 may serve as a molecular sponge for miR-145-5p.

TUG1 Overturns the miR-145-5p-Induced Inhibitory Effect on Proliferation and Invasion of GC Cells Both In Vitro and In Vivo

In consideration of the inhibitory effect of TUG1 on miR-145-5p expression in GC, whether TUG1 can inhibit the biological function of miR-145-5p was further investigated in BGC-823 and SGC-7901 cells transfected with miR-145-5p, miR-NC, miR-145-5p+NC, or miR-145-5p+TUG1. The MTT assay suggested that miR-145-5p overexpression obviously inhibited cell viability at 48, 72, and 96 h in BGC-823 and SGC-7901 cells compared with the miR-NC-transfected group, whereas TUG1 markedly relieved the inhibitory effect on cell proliferation mediated by miR-145-5p overexpression in vitro (Fig. 4A and B). Likewise, cell invasiveness was dramatically reduced by miR-145-5p overexpression, and this reduction was strikingly reversed by TUG1 overexpression in BGC-823 (Fig. 4C) and SGC-7901 (Fig. 4D) cells compared with the miR-NC-transfected groups in vitro. Furthermore, miR-145-5p remarkably reduced tumor volume at indicated times (Fig. 5A) and tumor weights (Fig. 5B), which were significantly rescued by TUG1 overexpression.

DISCUSSION

Recent advances in the noncoding part of human genome analysis have indicated that lncRNAs, which lack protein-coding potential, can regulate protein-coding genes at epigenetic, transcriptional, and posttranscriptional levels[28]. It is gradually apparent that lncRNAs play considerable functional roles in diverse biological processes, including cancer development and metastasis[29]. Recent studies have indicated that numerous lncRNA expressions are significantly altered in GC cells and are closely associated with the occurrence and development
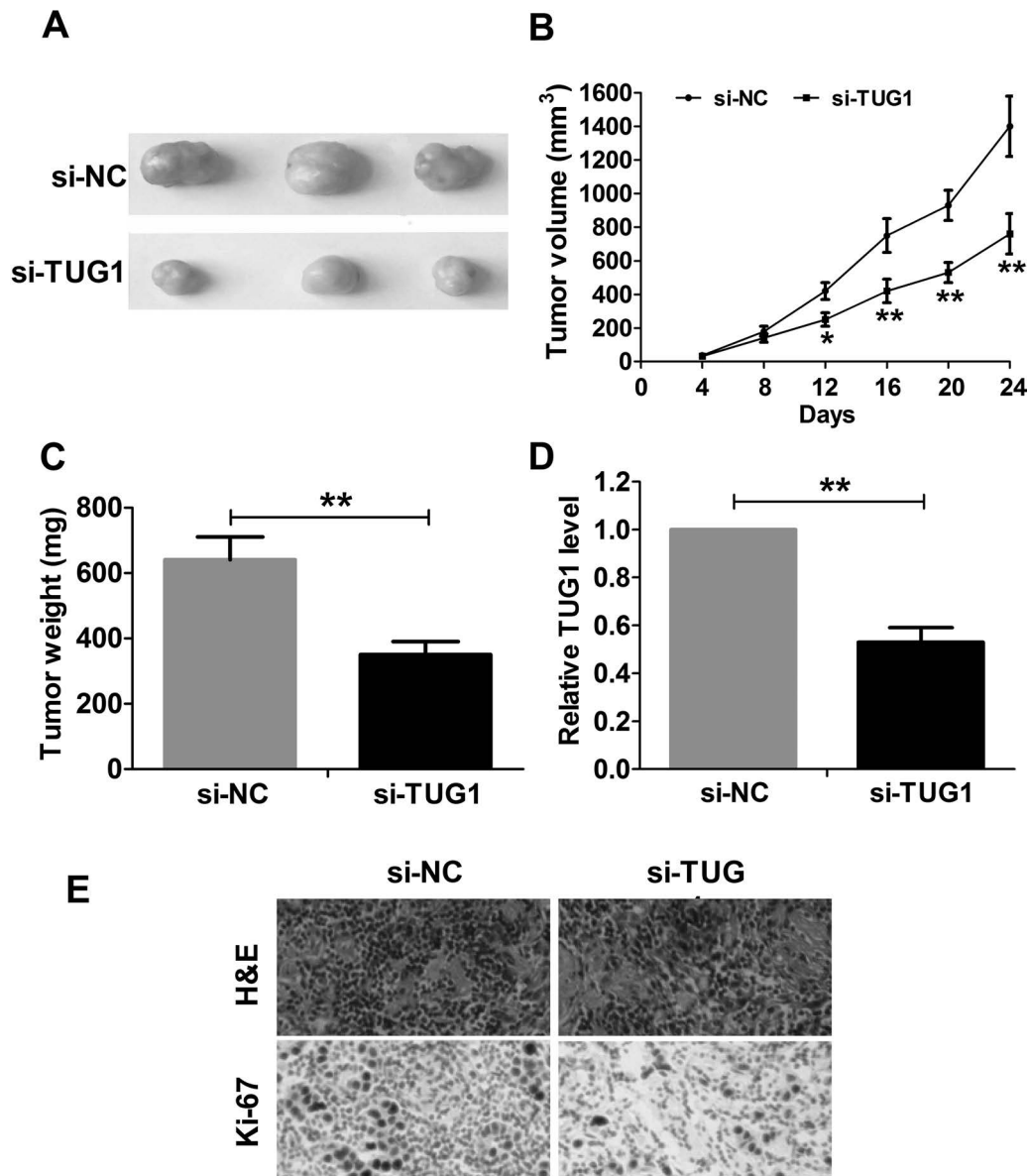
Figure 2. Effect of TUG1 knockdown on GC cell growth in vivo. Xenograft model was applied by injecting si-NC- or si-TUG1-transfected SGC-7901 cells into nude mice. (A) The image of tumor xenografts after 24 days of incubation. (B) Tumor volume was monitored and calculated every 4 days for 24 days after injection. (C) Tumor xenograft weight was measured at 24 days after injection. (E) Immunohistochemical analysis of proliferating cell nuclear antigen (Ki-67) protein expression in GC tissue sections from five randomly selected fields. Upper: H&E staining; lower: immunostaining (200×). *$p<0.05$, **$p<0.01$.

of GC[30]. For example, Xia et al. reported that lncRNA MALAT1 was highly expressed and functioned as an oncogene in GC[31]. More recently, Zhang et al. found that lncRNA LINC00628 acted as a tumor suppressor by inhibiting cell proliferation, migration, and colony formation in GC both in vitro and in vivo[32].

Previous studies have demonstrated that TUG1 is abnormally expressed and associated with tumor progression and development[28]. For example, TUG1 was reported to be highly expressed and promoted cell proliferation, migration, and invasion in colorectal cancer in vitro[33]. Zhai et al. indicated that TUG1 expression was obviously higher in colon cancer and contributed to promoting cell proliferation and migration in colon cancer[34]. Zhang et al. noted that TUG1 expression was significantly enhanced and predicted a poor prognosis in GC. Moreover, forced expression of TUG1 promoted cell proliferation by silencing p57 in GC[24]. In line with previous studies, this study confirmed that TUG1 expression was significantly upregulated in GC cells compared with
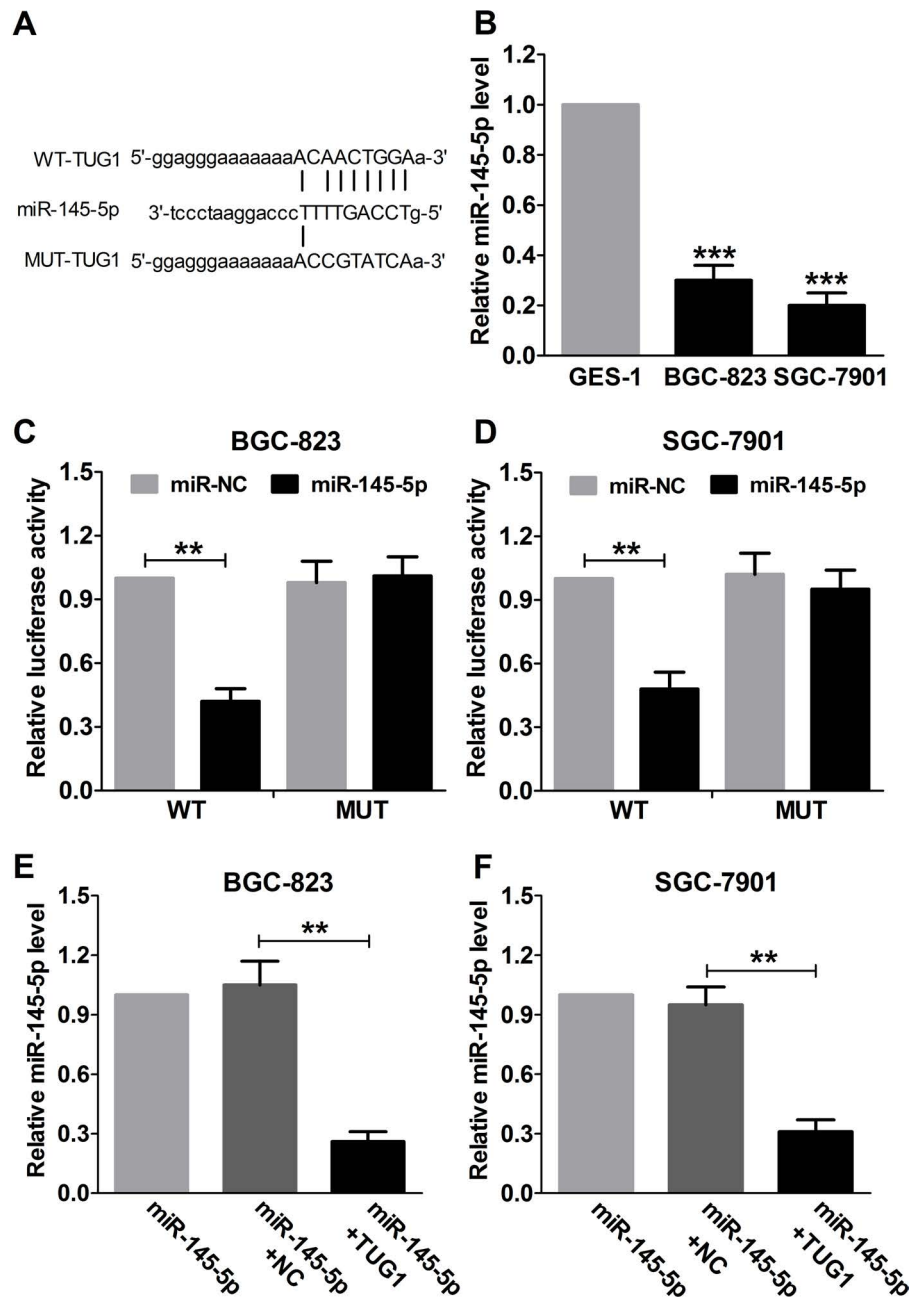
Figure 3. Regulatory relationship between TUG1 and miR-145-5p. (A) Bioinformatics analysis of the predicted target sites for miR-145-5p in TUG1. (B) qRT-PCR was used to analyze the expression of miR-145-5p in GC cell lines (BGC-823 and SGC-7901) and nonmalignant gastric epithelial cell line GES-1. GAPDH was used as the internal control. Luciferase activity in BGC-823 (C) and SGC-7901 (D) cells cotransfected with miR-145-5p or miR-NC and WT or MUT was analyzed by luciferase reporter assay. *Renilla* luciferase activity was used for normalization. qRT-PCR was used to detect the miR-145-5p levels in GC BGC-823 (E) and SGC-7901 (F) cells transfected with miR-145-5p, miR-145-5p+NC, or miR-145-5p+TUG1. **$p<0.01$, ***$p<0.001$.

that of nonmalignant gastric epithelial cells. In addition, the biological function of TUG1 in GC development was investigated by si-TUG1. It was shown that a remarkable inhibition of GC cell proliferation and invasion induced by TUG1 knockdown in vitro was observed. More importantly, TUG1 knockdown dramatically inhibited

tumor growth in vivo. These results indicated that TUG1 may have a strong correlation with GC progression.

It is well known that miR-145-5p is frequently downregulated and has an antitumor function in various tumors. For example, Matsushita et al. reported that miR-145-5p was significantly downregulated in bladder
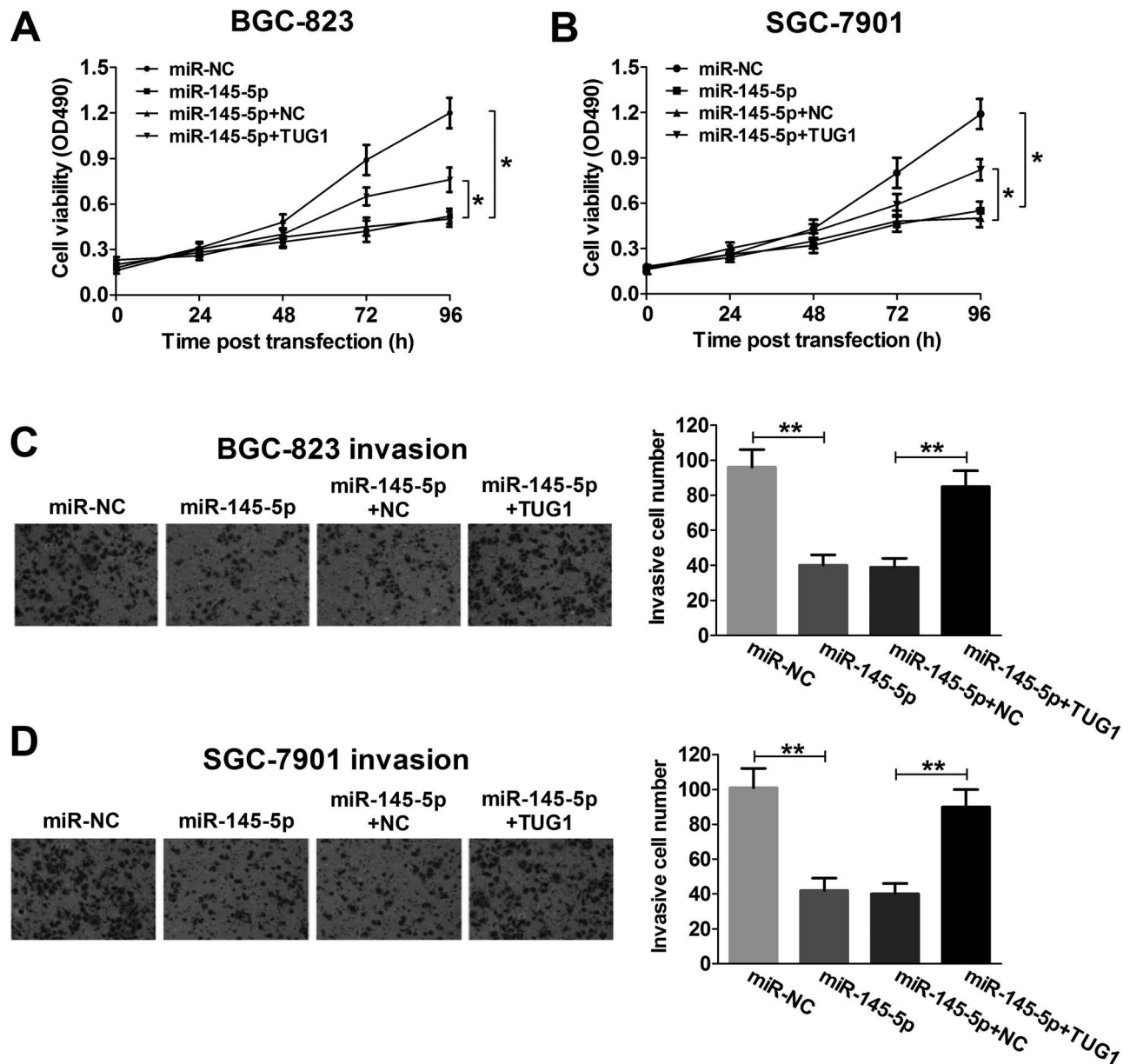
Figure 4. TUG1 reverses the miR-145-5p-induced inhibitory effect on proliferation and invasion of GC cells. BGC-823 and SGC-7901 cells were transfected with miR-145-5p, miR-NC, miR-145-5p+NC, or miR-145-5p+TUG1. Cell viability was determined by MTT assay in transfected BGC-823 (A) and SGC-7901 (B) cells at 24, 48, 72, and 96 h. Cell invasiveness was evaluated by Transwell invasion assay in transfected BGC-823 (C) and SGC-7901 (D) cells. *$p < 0.05$, **$p < 0.01$.

cancer, and ectopic expression of miR-145-5p dramatically inhibited cell growth, migration, and invasion by regulating UHRF1 in bladder cancer[13]. Ozen et al. discovered that miR-145-5p was obviously overexpressed in prostate cancer cells, and its overexpression led to a significant inhibition of proliferation, apoptosis, and migration by reducing SOX2 expression in prostate cancer[35]. Additionally, it has been reported that the expression level of miR-145 was reduced in GC, and overexpression of miR-145-5p caused a significant inhibitory effect

on growth in GC[12]. Consistent with the above report, our research revealed that miR-145-5p was significantly downregulated in GC cells compared with thatof nonmalignant gastric epithelial cells. Furthermore, miR-145-5p overexpression resulted in a remarkable suppression of cell proliferation and invasion in GC in vitro, as well as tumor growth inhibition in vivo.

Intriguingly, accumulating evidence demonstrates that lncRNAs serve as molecular sponges to competitively inhibit miRNAs[36]. Recently, several reports have revealed
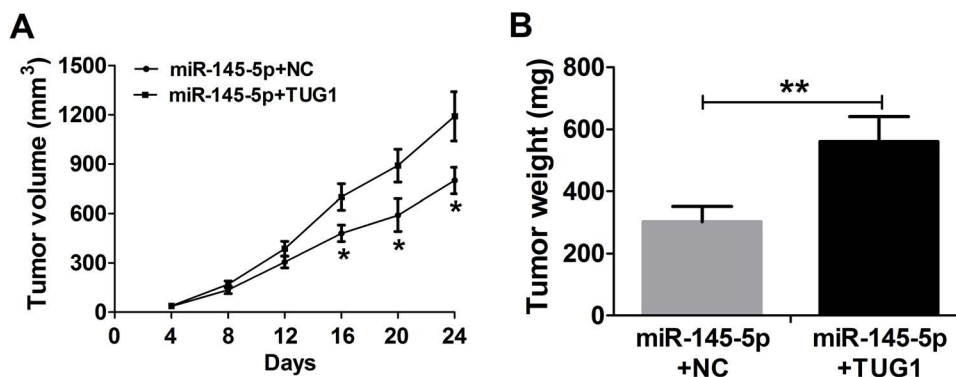
Figure 5. TUG1 reverses the miR-145-5p-induced inhibitory effect on tumor xenografts. (A) Tumor volume developed from miR-145-5p+NC- or miR-145-5p+TUG1-transfected SGC-7901 cells was calculated every 4 days for 24 days after injection. (B) Tumor weight developed from miR-145-5p+NC- or miR-145-5p+TUG1-transfected SGC-7901 cells was measured at 24 days after injection. *$p < 0.05$, **$p < 0.01$.

the interaction between TUG1 and miRNAs. For example, TUG1 was discovered to be highly expressed and promoted the transferring and invading capacities of GC by inhibiting the expression of miR-144/c-Met axis in GC[37] and inhibited blood–tumor barrier permeability by binding to miR-144[38]. In this study, bioinformatics analysis revealed that TUG1 could bind to miR-145-5p. Thus, we speculated that TUG1 may interact with miR-145-5p, functioning as a molecular sponge. As expected, luciferase reporter assay verified the direct binding relationship between TUG1 and miR-145-5p. Further studies suggested that TUG1 could dramatically suppress miR-145-5p expression and markedly overturn the inhibitory effect on cell proliferation and invasion, suggesting that TUG1 exerted its biological function by negatively regulating miR-145-5p in GC.

Taken together, our study confirmed that TUG1 was significantly overexpressed and miR-145-5p was dramatically decreased in GC cells. In addition, TUG1 exerted its tumor-promoting effect by negatively regulating miR-145-5p in GC, which undoubtedly contributed to understanding the mechanism of the GC occurrence and development.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65:5–29.
2. Kang C, Song JJ, Lee J, Kim MY. Epigenetics: An emerging player in gastric cancer. World J Gastroenterol. 2014;20: 6433–47.
3. Kagawa S, Shigeyasu K, Ishida M, Watanabe M, Tazawa H, Nagasaka T, Shirakawa Y, Fujiwara T. Molecular diagnosis and therapy for occult peritoneal metastasis in gastric cancer patients. World J Gastroenterol. 2014;20:17796–803.
4. Liu HS, Xiao HS. MicroRNAs as potential biomarkers for gastric cancer. World J Gastroenterol. 2014;20:12007–17.
5. Schirren R, Reim D, Novotny AR. Adjuvant and/or neoadjuvant therapy for gastric cancer? A perspective review. Ther Adv Med Oncol. 2015;7:39–48.
6. Tan J, Qiu K, Li M, Liang Y. Double-negative feedback loop between long non-coding RNA TUG1 and miR-145 promotes epithelial to mesenchymal transition and radioresistance in human bladder cancer cells. FEBS Lett. 2015;589:3175–81.
7. Zhou X, Ye F, Yin C, Zhuang Y, Yue G, Zhang G. The interaction between MiR-141 and lncRNA-H19 in regulating cell proliferation and migration in gastric cancer. Cell Physiol Biochem. 2015;36:1440–52.
8. Pereira DM, Rodrigues PM, Borralho PM, Rodrigues CM. Delivering the promise of miRNA cancer therapeutics. Drug Discov Today 2013;18:282–9.
9. Liu G, Jiang C, Li D, Wang R, Wang W. MiRNA-34a inhibits EGFR-signaling-dependent MMP7 activation in gastric cancer. Tumour Biol. 2014;35:9801–6.
10. Mei Q, Li F, Quan H, Liu Y, Xu H. Busulfan inhibits growth of human osteosarcoma through miR-200 family microRNAs in vitro and in vivo. Cancer Sci. 2014;105: 755–62.
11. Wang YW, Shi DB, Chen X, Gao C, Gao P. Clinicopathological significance of microRNA-214 in gastric cancer and its effect on cell biological behaviour. PLoS One 2014;9:e91307.
12. Takagi T, Iio A, Nakagawa Y, Naoe T, Tanigawa N, Akao Y. Decreased expression of microRNA-143 and -145 in human gastric cancers. Oncology 2009;77:12–21.
13. Matsushita R, Yoshino H, Enokida H, Goto Y, Miyamoto K, Yonemori M, Inoguchi S, Nakagawa M, Seki N. Regulation of UHRF1 by dual-strand tumor-suppressor microRNA-145 (miR-145-5p and miR-145-3p): Inhibition of bladder cancer cell aggressiveness. Oncotarget 2016;7: 28460–87.
14. Zhang Y, Wen X, Hu XL, Cheng LZ, Yu JY, Wei ZB. Downregulation of miR-145-5p correlates with poor prognosis in gastric cancer. Eur Rev Med Pharmacol Sci. 2016;20:3026–30.
15. Chen X, Gong J, Zeng H, Chen N, Huang R, Huang Y, Nie L, Xu M, Xia J, Zhao F, Meng W, Zhou Q. MicroRNA145 targets BNIP3 and suppresses prostate cancer progression. Cancer Res. 2010;70:2728–38.

16. Liang L, Stone RC, Stojadinovic O, Ramirez H, Pastar I, Maione AG, Smith A, Yanez V, Veves A, Kirsner RS, Garlick JA, Tomic-Canic M. Integrative analysis of miRNA and mRNA paired expression profiling of primary fibroblast derived from diabetic foot ulcers reveals multiple impaired cellular functions. Wound Repair Regen. 2016;24(6): 943–53.

17. Yang F, Huo XS, Yuan SX, Zhang L, Zhou WP, Wang F, Sun SH. Repression of the long noncoding RNA-LET by histone deacetylase 3 contributes to hypoxia-mediated metastasis. Mol Cell 2013;49:1083–96.

18. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell 2011;43:904–14.

19. Fatica A, Bozzoni I. Long non-coding RNAs: New players in cell differentiation and development. Nat Rev Genet. 2014;15:7–21.

20. Zhang EB, Kong R, Yin DD, You LH, Sun M, Han L, Xu TP, Xia R, Yang JS, De W, Chen JF. Long noncoding RNA ANRIL indicates a poor prognosis of gastric cancer and promotes tumor growth by epigenetically silencing of miR-99a/miR-449a. Oncotarget 2014;5:2276–92.

21. Young TL, Matsuda T, Cepko CL. The noncoding RNA taurine upregulated gene 1 is required for differentiation of the murine retina. Curr Biol. 2005;15:501–12.

22. Zhang EB, Yin DD, Sun M, Kong R, Liu XH, You LH, Han L, Xia R, Wang KM, Yang JS, De W, Shu YQ, Wang ZX. P53-regulated long non-coding RNA TUG1 affects cell proliferation in human non-small cell lung cancer, partly through epigenetically regulating HOXB7 expression. Cell Death Dis. 2014;5:e1243.

23. Sun J, Ding C, Yang Z, Liu T, Zhang X, Zhao C, Wang J. The long non-coding RNA TUG1 indicates a poor prognosis for colorectal cancer and promotes metastasis by affecting epithelial-mesenchymal transition. J Transl Med. 2016;14:42.

24. Zhang E, He X, Yin D, Han L, Qiu M, Xu T, Xia R, Xu L, Yin R, De W. Increased expression of long noncoding RNA TUG1 predicts a poor prognosis of gastric cancer and regulates cell proliferation by epigenetically silencing of p57. Cell Death Dis. 2016;7:e2109.

25. Han Y, Liu Y, Gui Y, Cai Z. Long intergenic non-coding RNA TUG1 is overexpressed in urothelial carcinoma of the bladder. J Surg Oncol. 2013;107:555–9.

26. Xu Y, Wang J, Qiu M, Xu L, Li M, Jiang F, Yin R, Xu L. Upregulation of the long noncoding RNA TUG1 promotes proliferation and migration of esophageal squamous cell carcinoma. Tumour Biol. 2015;36:1643–51.

27. Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, Tramontano A, Bozzoni I. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell 2011;147: 358–69.

28. Wang F, Ying HQ, He BS, Pan YQ, Deng QW, Sun HL, Chen J, Liu X, Wang SK. Upregulated lncRNA-UCA1 contributes to progression of hepatocellular carcinoma through inhibition of miR-216b and activation of FGFR1/ERK signaling pathway. Oncotarget 2015;6:7899–917.

29. Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010; 464:1071–6.

30. Fang XY, Pan HF, Leng RX, Ye DQ. Long noncoding RNAs: Novel insights into gastric cancer. Cancer Lett. 2015;356:357–66.

31. Xia H, Chen Q, Chen Y, Ge X, Leng W, Tang Q, Ren M, Chen L, Yuan D, Zhang Y, Liu M, Gong Q, Bi F. The lncRNA MALAT1 is a novel biomarker for gastric cancer metastasis. Oncotarget 2016;7(35):56209–18.

32. Zhang ZZ, Zhao G, Zhuang C, Shen YY, Zhao WY, Xu J, Wang M, Wang CJ, Tu L, Cao H, Zhang ZG. Long noncoding RNA LINC00628 functions as a gastric cancer suppressor via long-range modulating the expression of cell cycle related genes. Sci Rep. 2016;6:27435.

33. Wang L, Zhao Z, Feng W, Ye Z, Dai W, Zhang C, Peng J, Wu K. Long non-coding RNA TUG1 promotes colorectal cancer metastasis via EMT pathway. Oncotarget 2016;7(32):51713–9.

34. Zhai HY, Sui MH, Yu X, Qu Z, Hu JC, Sun HQ, Zheng HT, Zhou K, Jiang LX. Overexpression of long non-coding RNA TUG1 promotes colon cancer progression. Med Sci Monit. 2016;22:3281–7.

35. Ozen M, Karatas OF, Gulluoglu S, Bayrak OF, Sevli S, Guzel E, Ekici ID, Caskurlu T, Solak M, Creighton CJ, Ittmann M. Overexpression of miR-145-5p inhibits proliferation of prostate cancer cells and reduces SOX2 expression. Cancer Invest. 2015;33:251–8.

36. Chen DL, Ju HQ, Lu YX, Chen LZ, Zeng ZL, Zhang DS, Luo HY, Wang F, Qiu MZ, Wang DS, Xu DZ, Zhou ZW, Pelicano H, Huang P, Xie D, Wang FH, Li YH, Xu RH. Long non-coding RNA XIST regulates gastric cancer progression by acting as a molecular sponge of miR-101 to modulate EZH2 expression. J Exp Clin Cancer Res. 2016; 35:142.

37. Ji TT, Huang X, Jin J, Pan SH, Zhuge XJ. Inhibition of long non-coding RNA TUG1 on gastric cancer cell transference and invasion through regulating and controlling the expression of miR-144/c-Met axis. Asian Pac J Trop Med. 2016;9:508–12.

38. Cai H, Xue Y, Wang P, Wang Z, Li Z, Hu Y, Li Z, Shang X, Liu Y. The long noncoding RNA TUG1 regulates blood-tumor barrier permeability by targeting miR-144. Oncotarget 2015;6:19759–79.