SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 16: 3706-3714, 2018

si-TP73-AS1 suppressed proliferation and increased the chemotherapeutic response of GC cells to cisplatin

JIANJUN PENG

Department of Gastrointestinal Surgery, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong 510080, P.R. China

Received July 29, 2017; Accepted April 16, 2018

DOI: 10.3892/ol.2018.9107

Abstract. Previous studies have revealed that long noncoding RNAs (lncRNAs) function as crucial regulators in various biological processes, including tumorigenesis. Although the expression of lncRNA TP73-antisense RNA1 (AS1) has been identified in hepatocellular carcinoma and glioma, the biological function of TP73-AS1 in gastric cancer (GC) remains unclear. Thus, the present study employed a comprehensive analysis on the function of lncRNA TP73-AS1 in GC. The aim of the present study was to determine the clinical significance and biological function of TP73-AS1 in human GC tissues and cells. Additionally, the expression of TP73-AS1 was increased in GC tissues and cell lines and increased expression level of TP73-AS1 was associated with poor prognosis in patients with GC. Functional assays revealed that silencing of TP73-AS1 may suppress cell proliferation and enhance the chemotherapeutic response of GC cells to cisplatin through targeting the high mobility group 1/receptor for advanced glycation endproducts signaling pathway. Collectively, the results of the present study demonstrated that TP73-AS1 may be a novel lncRNA for the clinical prognosis of GC and a potential therapeutic target for the treatment of GC.

Introduction

Gastric cancer (GC) is the third leading cause of cancer-associated mortality worldwide (1-3). Due to the lack of effective techniques for early diagnosis, the majority the patients with GC are diagnosed at late stages of GC. Despite advances in the diagnosis and treatment of GC, the 5-year overall survival rate of patients with GC remains low (4). Chemotherapy is the primary treatment for GC. However, chemoresistance remains to be a major obstacle for the clinical treatment of the disease. Therefore, investigating the molecular mechanism underlying chemoresistance is essential for effective treatments in patients with GC.

Long noncoding RNAs (lncRNAs, >200 nucleotides in length) are dysregulated in various human diseases and disorders, including cancer (5-13). LncRNA metastasis-associated lung adenocarcinoma transcript 1 promotes the development of hepatocellular carcinoma by upregulating serine/arginine-rich splicing factor 1 and activating mammalian target of rapamycin (14). LncRNA FEZF1 antisense RNA1 (AS1) may repress the expression of p21 and promote the proliferation of GC cells through lysine-specific demethylase 1-mediated H3K4 dimethylation (15). LncRNA SPRY4-intronic transcript 1 may lead to microRNA-101-3p-mediated proliferation and metastasis of bladder cancer cells through upregulating enhancer of zeste homolog 2 (16). These studies suggest that lncRNAs may be involved in tumor development and progression.

P73 antisense RNA 1T also known as TP73-AS1 or PDAM, is a long noncoding RNA which may regulate apoptosis via p53-dependent anti-apoptotic genes, and may be deregulated in cancer (17,18). To the best of our knowledge, the biological function of TP73-AS1 in patients with GC has not been examined. Additionally, the function of TP73-AS1 in cisplatin resistance of GC remains unclear.

Reverse-transcription quantitative polymerase chain reaction (RT-qPCR) analysis was conducted to detect the expression levels of TP73-AS1 in GC tissues and cell lines. Following transfection, loss-of function assays were conducted in GC cells, to measure the effects of silenced TP73-AS1 on cell growth and the chemosensitivity of GC cells. Mechanism experiments were performed to examine the functional mechanism underlying TP73-AS1 and mobility group 1 (HMGB1)/receptor for advanced glycation endproducts (RAGE) signaling pathway in GC; therefore, the study investigated the function and mechanism underlying TP73-AS1 in GC.

Materials and methods

Clinical tissues. A total of 58 patients with GC underwent surgery at the Department of Gastrointestinal Surgery, the First Affiliated Hospital of Sun Yat-Sen University (Guangdong, China) and were enrolled in the present study. In total, 58 pairs of GC tissues and adjacent non-tumor tissues were collected

Correspondence to: Dr Jianjun Peng, Department of Gastrointestinal Surgery, The First Affiliated Hospital of Sun Yat-Sen University, 2 Zhongshan Street, Guangzhou, Guangdong 510080, P.R. China
E-mail: jianjunpeng_q57@163.com

Key words: gastric cancer, TP73-antisense RNA 1T, chemosensitivity, cell cycle, apoptosis

between September 2008 and September 2011 and stored at -80˚C. Patients who were not diagnosed with gastric cancer were excluded from the present study. Patients who received previous treatment were excluded from this study. The clinicopathological characteristics of patients with GC are presented in Table I. Tumor differentiation was defined based on the cellular differentiation degree, which may be divided into three grades including well differentiation, moderate differentiation and poor differentiation (19). The present study was approved by the Department of Gastrointestinal Surgery, the First Affiliated Hospital of Sun Yat-Sen University (Guangdong, China). Written informed consent was obtained from all participants.

Cell culture. GC cell lines, including AGS, SGC-7901, BGC-823 and MGC-803 and a normal gastric epithelial cell line (GES-1) were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) medium supplemented with 10% of fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 mg/ml streptomycin at 37˚C in a humidified atmosphere containing 5% CO_2.

Plasmid construction and transfection. The full-length TP73-AS1 sequence was synthesized and then sub-cloned into pcDNA3.1 vector (Thermo Fisher Scientific, Inc.) to construct the pcDNA3.1-TP73-AS1 vector. The blank vector was obtained from the Thermo Fisher Scientific, Inc.. Subsequently, the pcDNA3.1-TP73-AS1 vector (2 μg) or the empty vector (2 μg) was transfected into the cells using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) in Hybridoma serum-free medium (Gibco, Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions.

The primers (Thermo Fisher Scientific, Inc.) were as follows: TP73-AS1, 5'-TCAGGTTCGTAACGGTGCGTT-3' (forward) and 5'-TCGTATCTCGCGACTCTTCC-3' (reverse). The empty pcDNA3.1 vector was used as a negative control. The small interfering RNA (siRNA) sequence for TP73-AS1 was as follows: 5'-CCTGCTGCCTCTCCAAGAGACTGC TATTA-3'. The plasmid of pcDNA/TP73-AS1 was transfected into GES-1 cells (90%) at a density of $0.8x10^6$ cells at a final concentration of 2 μg/ml using Lipofectamine 2000 (Thermo Fisher Scientific, Inc.), and were incubated for 48 h.

RNA extraction and RT-qPCR. Total RNA was isolated from cells and tissues using TRIzol reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. According to the manufacturer's protocols, PrimeScript™ RTMaster Mix (Takara Biotechnology Co., Ltd., Dalian, China) was used to reverse transcribe RNA to cDNA. RT-qPCR was performed using random primers from Augct DNA-Syn Biotechnology Co., Ltd. (Beijing, China). Real time PCR conditions were: 1 cycle of 2 min at 50˚C; 1 cycle of 10 min at 95˚C; and 40 cycles of 15 sec at 95˚C and 1 min at 60˚C. RT-qPCR was performed using ABI 7300 Real-time PCR system and Power SYBR-Green PCR master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The primers were as follows: TP73-AS1, 5'-CCGGTTTTCCAG TTCTTGCAC-3' (forward) and 5'-GCCTCACAGGGAAAC TTCATGC-3' (reverse); GAPDH, 5'-GTCAACGGATTTGGT CTGTATT-3' (forward) and 5'-AGTCTTCTGGGTGGCAGT GAT-3' (reverse). Relative expression levels were determined using the $2^{-\Delta\Delta Cq}$ method (20). StepOne™ Software Version 2.1 (Applied Biosystems; Thermo Fisher Scientific, Inc.) was utilized to analyze Cq value. GAPDH was used as the internal reference. All experiments were performed in triplicate.

Cell viability. GC cells ($3\text{-}6x10^3$) were incubated at 37˚C in a 96-well plate in DMEM (200 μl/well) in a humidified atmosphere containing 5% CO_2 for 24-72 h and 20 μl MTT solution (5 mg/ml; Merck KGaA, Darmstadt, Germany) was added into each well. At 4 h, the culture medium was discarded and dimethyl sulfoxide (DMSO; 150 μl) (Merck KGaA) was added into each well and mixed for 10 min to dissolve crystallization. Absorbance values were determined using a microplate reader at a wavelength of 570 nm at indicated time points (12, 24, 48, 72 and 96 h). All experiments were performed in triplicate. The chemosensitivity was determined using an MTT assay (5 mg/ml; Merck KGaA, Darmstadt, Germany). Cells were cultured in 96-well plates and were treated with cisplatin (0, 5, 10, 15 and 20 μm/ml; BioVision, Inc., Milpitas, CA, USA). At 48 h post-treatment, MTT solution was added into each well. At 4 h, the medium was removed and 100 μl DMSO was added into each well. Absorbance values were determined using a microplate reader at a wavelength of 560 nm. All experiments were performed in triplicate.

Colony formation assay. Cells (500 cells/well) were plated in 6-well plates and incubated in RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% of FBS at 37˚C for 2 weeks. Following incubation, cells were fixed with 4% methanol for 15 min at room temperature and stained with 0.1% of crystal violet at room temperature for 30 min. The number of visible colonies was counted manually using an Olympus optical microscope (DSX100; Olympus Corporation, Tokyo, Japan).

Flow cytometric analysis of apoptosis. Cells were transfected with indicated plasmids (pcDNA/TP73-AS1) or negative control for 48 h as aforementioned. Cells were stained using an Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) kit (BD Biosciences, San Jose, CA, USA), according to the manufacturer's protocol. Cells were analyzed using a flow cytometer and CellQuest software version 0.9.3.1 (BD Biosciences). All experiments were performed in triplicate.

Flow cytometric analysis of cell cycle distribution. Cells were collected at 48 h post-transfection, washed with ice-cold phosphate-buffered saline (PBS). Following this, the cells were fixed with 70% ethanol at 4˚C for 2 h. Fixed cells were rehydrated in PBS for 10 min and then were incubated in RNase A (1 mg/ml) for 30 min at 37˚C, and stained with PI/RNase (1 ml) at 4˚C overnight in a dark place. Cells were analyzed using a flow cytometer (BD Biosciences). All experiments were performed in triplicate.

Western blot analysis. Total protein was isolated from cells using radioimmunoprecipitation assay buffer (Merck

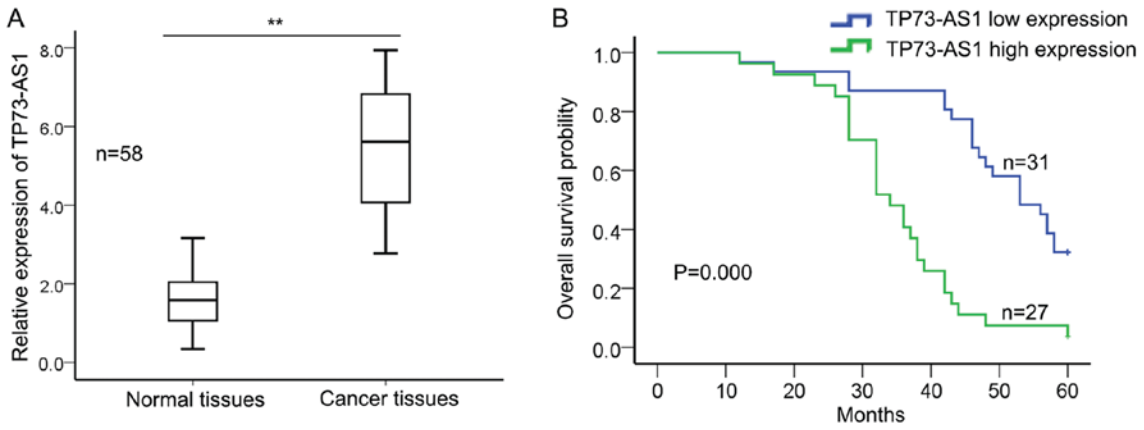

Figure 1. TP73-AS1 is upregulated in human GC tissues. (A) Reverse-transcription quantitative polymerase chain reaction was performed to evaluate the expression level of TP73-AS1 in human GC tissues and adjacent normal tissues. (B) Survival analysis was performed using the Kaplan-Meier method and log-rank test to determine the association between the expression of TP73-AS1 and the overall survival of patients. **P<0.01. GC, gastric cancer; AS1, antisense RNA1.

KGaA) with Complete Protease Inhibitor Cocktail (Roche Diagnostics GmbH, Mannheim, Germany) and stored at -20˚C. Protein concentration was evaluated with the BCA protein assay kit (Thermo Fisher Scientific, Inc.). Each sample (40 mg/lane) was isolated by 10% SDS-PAGE electrophoresis and transferred to polyvinylidene fluoride membranes (Sangon Biotech Co., Ltd.). The membranes were blocked with 5% skimmed milk at 37˚C for 1 h. The membranes were incubated with the following primary antibodies: Anti-HMGB1 (1:1,000; ab79823), anti-RAGE (1:1,000: ab3611), nuclear factor (NF)-κB (1:1,000; ab222497), anti-p21 (1:1,000; ab109520), anti-cyclin-dependent kinases (CDK)2 (1:1,000; ab208697), anti-CDK4 (1:1,000; ab199728), anti-CDK6 (1:1,000; ab151247) and anti-GAPDH (1:1,000; ab9485) at 4˚C overnight and with horseradish peroxidase-conjugated goat anti-mouse IgG H&L (1:2,000; ab6789) at 37˚C for 1 h. All antibodies used in this experiment were obtained from Abcam (Cambridge, UK). The molecular weight of candidate proteins was referred to the Pre-stained SeeBlue Rainbow marker (Thermo Fisher Scientific, Inc.) loaded in parallel. The blots were visualized using the enhanced chemiluminescence detection reagent (Thermo Fisher Scientific, Inc.). The results were analyzed with Quantity One software (V4.4.0; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis. Data were analyzed using SPSS software (version 19.0; IBM Corp., Armonk, NY, USA). The relevant data are expressed as the mean ± standard deviation (SD). The Chi-square test was used to assess the association between TP73-AS1 expression and clinicopathological factors. Differences between two groups were analyzed using Student's t-test. One-way analysis of variance (Least-Significance-Difference post-hoc test) was performed when multiple comparisons were performed. Survival analysis was performed using the Kaplan-Meier method and the log-rank test. Cox proportional hazards regression model was generated to identify factors associated with overall survival through a multivariate survival analysis. Correlation among the expression levels of TP73-AS1, HMGB1 and RAGE in 58 cases of GC s were analyzed using Spearman's correlation analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

TP73-AS1 is upregulated in human GC tissues and is associated with poor prognosis. To explore the biological function of TP73-AS1 in GC, the expression level of TP73-AS1 was examined in 58 GC tissues and adjacent normal tissues using RT-qPCR. Fig. 1A demonstrated that the relative expression of TP73-AS1 was significantly increased in GC tissues compared with that in adjacent normal tissues. Then, the association between the expression level of TP73-AS1 and the clinicopathological parameters of 58 patients with GC was evaluated. The mean value of TP73-AS1 in GC tissues was used as a cutoff value and patients with GC were divided into two groups (high expression group, n=27; low expression group, n=31). Table I demonstrated that increased expression level of TP73-AS1 was significantly associated with tumor stage (P=0.001), lymph node metastasis (P=0.008), distant metastasis (P=0.034) and differentiation (P=0.017), but was not significantly associated with age, sex and tumor size (P>0.05). Furthermore, Kaplan-Meier method analysis and log-rank test was performed to determine the association between TP73-AS1 expression and overall survival of patients with GC. Fig. 1B demonstrates that patients with increased expression of TP73-AS1 exhibited a significantly shorter overall survival compared with those with low expression level of TP73-AS1 (P=0.000). Cox's proportional hazards analysis revealed that the increased expression level of TP73-AS1 (P=0.012; Table II) may be a prognostic factor in GC. These results suggest that TP73-AS1 may act as an oncogene in GC and may be considered as a specific biomarker for poor prognosis in GC.

Knockdown of TP73-AS1 suppresses cell proliferation and increases the sensitivity of GC cells to cisplatin. To determine the biological function of TP73-AS1 in GC, the expression level of TP73-AS1 was evaluated in GC cell lines (AGS, SGC-7901, BGC-823 MGC-803) and a normal gastric epithelial cell line

Table I. Association between the expression of lncRNA-TP73-AS1 and clinical features in gastric cancer.

	LncRNA-TP73-AS1 expression, n		
Variable	Low	High	P-value
Sex			0.113
Male	20	11	
Female	11	16	
Age, years			0.428
<60	12	14	
≥60	19	13	
T stage			0.001
T1-T2	22	7	
T3-T4	9	20	
Lymph node metastasis			0.008
No	21	8	
Yes	10	19	
Distant metastasis			0.034
No	17	7	
Yes	14	20	
Tumor size, cm			0.124
<5	18	10	
≥5	13	17	

Low/high expression was determined by the mean of TP73-AS1. AS1, antisense RNA1; lncRNAs, long noncoding RNAs.

Table II. Multivariate analysis of prognostic parameters in patients with gastric cancer by Cox's proportional hazard model analysis.

Variable	P-value
Sex	0.459
Male	
Female	
Age, years	0.494
<60	
≥60	
T stage	0.897
T1-T2	
T3-T4	
Lymph node metastasis	0.652
No	
Yes	
Distant metastasis	0.257
No	
Yes	
Differentiation	0.002
Well/moderate	
Poor	
Tumor size, cm	0.602
<5	
≥5	
TP73-AS1 expression	0.012
Low	
High	

AS1, antisense RNA1.

(GES-1). Fig. 2A demonstrates that the expression level of TP73-AS1 in GC cells was significantly increased compared with that in the normal gastric epithelial cell line. Among the four GC cells, the expression of TP73-AS1 was increased in AGS and BGC-823 cells compared with that in the remaining cell lines. Therefore, AGS and BGC-823 cells were selected for subsequent experiments.

AGS and BGC-823 cells were transfected with TP73-AS1 specific siRNA in order to downregulate the endogenous level of TP73-AS1. GES-1 cells were transfected with TP73-AS1 expression vector (pcDNA3.1/TP73-AS1) to enhance the expression level of TP73-AS1. The results demonstrated that the expression of TP73-AS1 was downregulated in AGS and BGC-823 cells following transfection with si-TP73-AS1 compared with that in the negative control (Fig. 2B). Additionally, the expression level of TP73-AS1 in pcDNA3.1/TP73-AS1-transfected GES-1 cells was increased compared with that in the negative control (pcDNA3.1) (Fig. 2B).

MTT and colony formation assays were also performed. The results demonstrated that the cell proliferation was impaired in AGS and BGC-823 cells transfected with siRNA compared with that in the negative control (Fig. 2C). Additionally, overexpression of TP73-AS1 in GES-1 cells promoted cellular proliferative ability compared with that in the negative control (Fig. 2C). The colony formation ability of AGS and BGC-823 cells transfected with siRNA was decreased compared with that in the negative control (Fig. 2D), whereas an increased colony formation ability was observed in GES-1 cells transfected with pcDNA3.1/TP73-AS1 (Fig. 2D). Additionally, downregulation of TP73-AS1 increased the sensitivity of AGS and BGC-823 cells to cisplatin compared with control-transfected cells, whereas overexpressed TP73-AS1 significantly decreased the sensitivity of GES-1 cells to cisplatin (Fig. 2E). These results indicated that TP73-AS1 may be involved in the progression of GC.

Silencing of TP73-AS1 inhibits cell proliferation and increases chemosensitivity through regulating cell cycle and apoptosis. Flow cytometric analyses were conducted to investigate the effects of dysregulated TP73-AS1 on cell apoptosis and cell cycle in GC. As demonstrated in Fig. 3A, knockdown of TP73-AS1 in AGS and BGC-823 cells induced cell cycle arrest at G1 phase, whereas overexpressed TP73-AS1 promoted cell cycle progression. CDKs are key factors in G1/S phase transition. The dysregulation of the cell cycle may be mediated by deregulation of CDKs (21). To determine the mechanism by which TP73-AS1 may regulate cell cycle, the expression

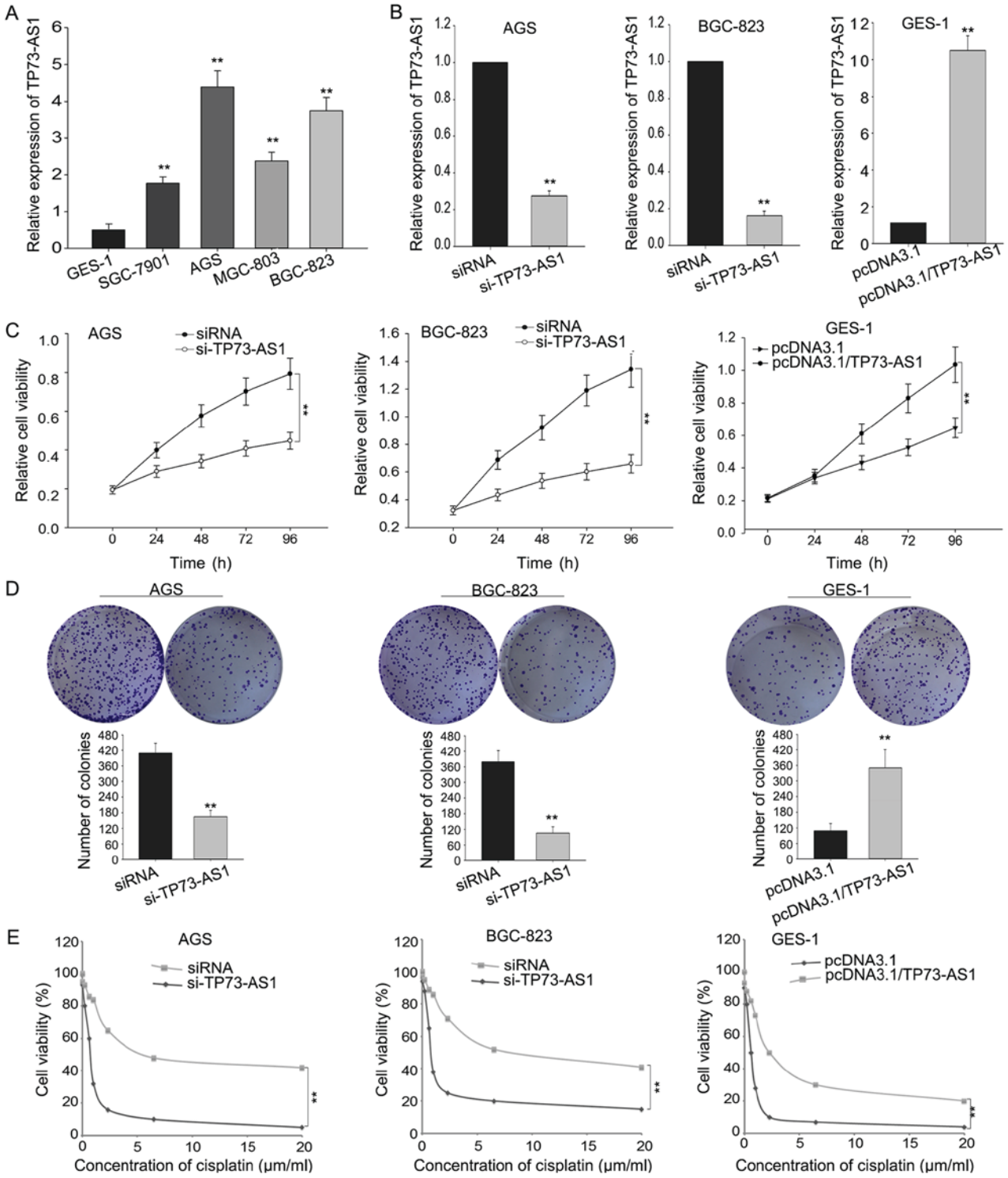

Figure 2. Knockdown of TP73-AS1 suppresses cell proliferation and enhances the sensitivity of GC cells to cisplatin. (A) RT-qPCR was performed to determine the expression level of TP73-AS1 in GC cells. (B) AGS and BGC-823 cells were transfected with TP73-AS1 specific siRNA and the expression level of TP73-AS1 was evaluated using RT-qPCR. (C) MTT and (D) colony formation assays were employed to assess the effect of downregulation or upregulated TP73-AS1 on the proliferative ability of GC cells. (E) MTT assay was performed to evaluate the chemosensitivity of GC cells to cisplatin when TP73-AS1 was dysregulated. **P<0.01. RT-qPCR, reverse-transcription quantitative polymerase chain reaction; GC, gastric cancer; AS1, antisense RNA1; siRNA, small interfering RNA.

level of CDKs (CDK2, 4 and 6) was determined. The results demonstrated that CDK2 may be positively regulated by TP73-AS1 (Fig. 3B).

CDK2 may serve a crucial function in cell cycle progression and apoptosis and its activity may be regulated by the CDK inhibitor p21 (22). Therefore, the expression level of p21 was evaluated in TP73-AS1-downregulated/overexpressed GC cells. The results demonstrated that p21 may be negatively regulated by TP73-AS1 (Fig. 3B). These results indicated that TP73-AS1 may affect the cell cycle through targeting p21

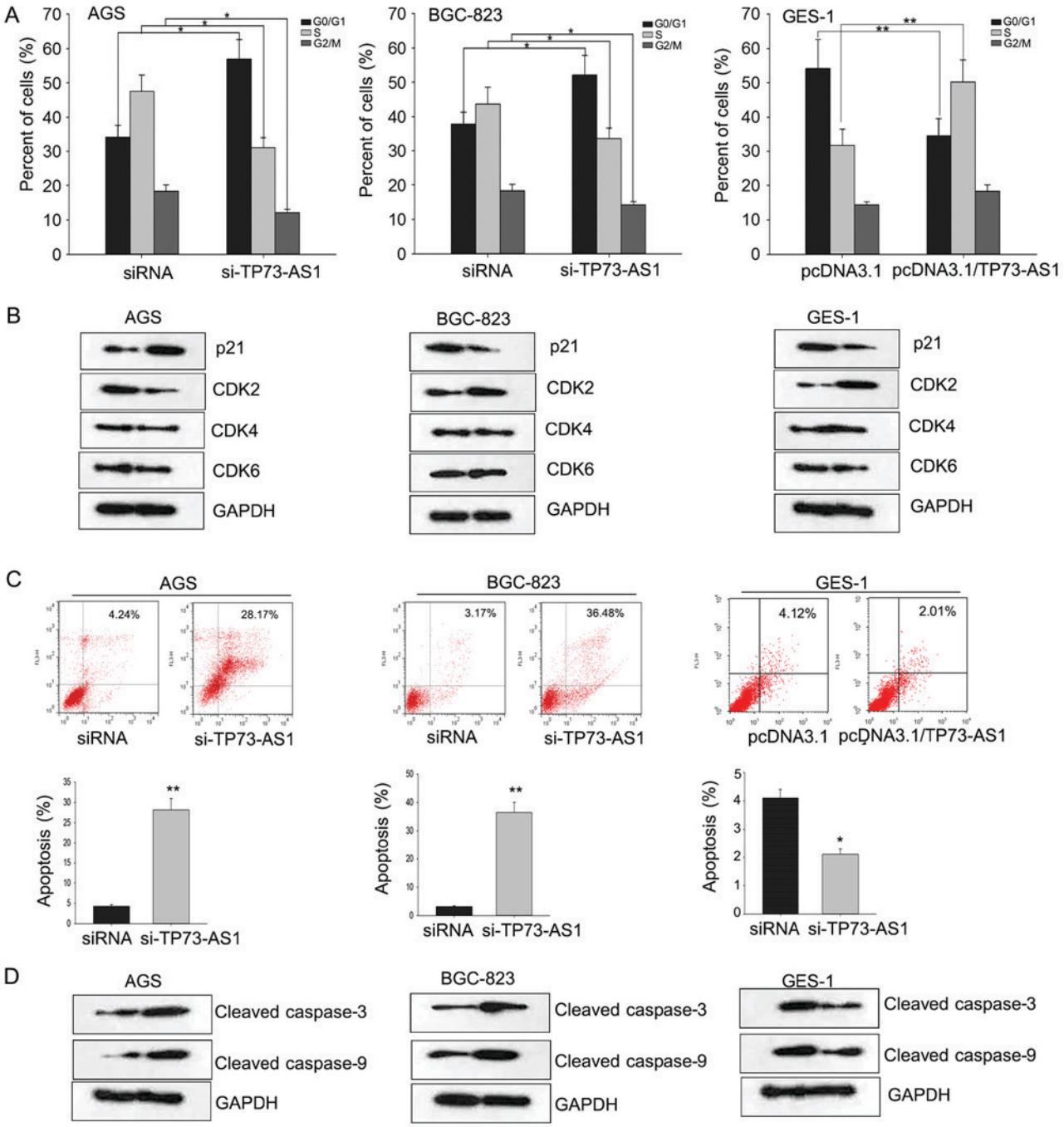

Figure 3. Silencing of TP73-AS1 inhibits cell proliferation and increases chemosensitivity through regulating cell cycle and apoptosis. (A) Flow cytometry and (B) western blot analysis were used to analyze the effect of TP73-AS1 on cell cycle and the expression of CDKs. (C) Flow cytometric and (D) western blot analysis were used to analyze the effect of TP73-AS on cell apoptosis and the expression apoptosis-associated proteins. *P<0.05, **P<0.01. GC, gastric cancer; AS1, antisense RNA1; siRNA, small interfering RNA; CDK, cyclin-dependent kinase.

in GC. Additionally, downregulation of TP73-AS1 significantly increased the apoptosis rate of AGS and BGC-823 cells (Fig. 3C). The levels of apoptosis-associated proteins (cleaved caspase-3 and -9) were examined in indicated GC cells (Fig. 3D). These results indicated that downregulation of TP73-AS1 inhibited cell proliferation and increased chemosensitivity, which may be mediated through the regulation of cell cycle and apoptosis.

Downregulation of TP73-AS1 increased the sensitivity of GC cells to cisplatin through targeting the HMGB1 signaling pathway. HMGB1 is an evolutionarily ancient and critical regulator for cell death and survival. It has been revealed that HMGB1 may activate the RAGE signaling pathway and induce the activation of NF-κB to promote cellular processes (23). Previous studies demonstrated that the HMGB1/RAGE signaling pathway may be involved in the biological function of TP73-AS1 in hepatocellular carcinoma and glioma (17,24). To determine whether the HMGB1/RAGE signaling pathway was involved in TP73-AS1-mediated effects in GC, the levels of HMGB1, RAGE and NF-κB were evaluated in response to downregulation or upregulation of TP73-AS1. Fig. 4A

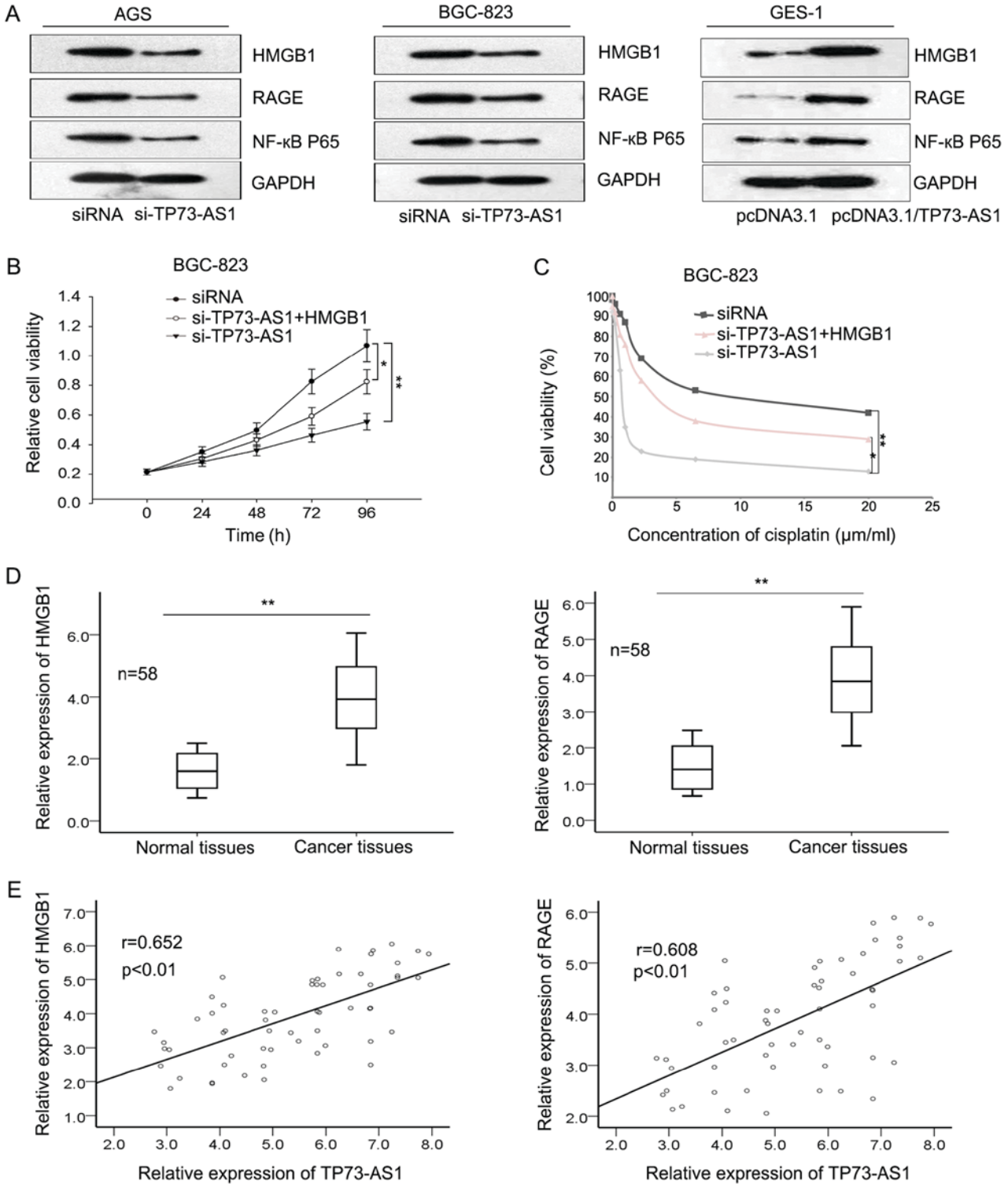

Figure 4. TP73-AS1 is upregulated in human GC tissues and is associated with poor prognosis. (A) Western blot analysis was used to evaluate the expression level of HMGB1, RAGE and NF-κB in response to downregulation or upregulation of TP73-AS1. MTT assay was performed to determine (B) the proliferative ability and (C) sensitivity to cisplatin of cells co-transfected with si-TP73-AS1 and HMGB1 overexpression vector. (D) Reverse-transcription quantitative polymerase chain reaction was used to determine the expression level of HMGB1 and RAGE in GC tissues. (E) The correlation among the expression levels of TP73-AS1 and HMGB1 or RAGE in GC tissues was analyzed by Spearman's correlation analysis. *P<0.05, **P<0.01. GC, gastric cancer; AS1, antisense RNA1; siRNA, small interfering RNA; HMGB1, high mobility group 1; RAGE, receptor for advanced glycation endproducts; NF, nuclear factor.

demonstrates that the protein levels of HMGB1, RAGE and NF-κB were significantly decreased in AGS and BGC-823 cells following knockdown of TP73-AS1 (achieved by si-TP73-AS1) whereas their protein levels were increased in GES-1 cells with an overexpression of TP73-AS1. Rescue assays were performed to confirm the association between TP73-AS1 and HMGB1. Fig. 4B and C demonstrate that co-transfection with HMGB1 overexpression vector may restore the proliferative ability and the sensitivity to cisplatin mediated by si-TP73-AS1 in BGC-823 cells. Additionally, the expression levels of HMGB1 and RAGE

were evaluated in GC tissues. The results demonstrated that the expression of HMGB1 and RAGE was upregulated in GC tissues (Fig. 4D), and were positively correlated with TP73-AS1 (Fig. 4E). Collectively, the results revealed that TP73-AS1 regulated the sensitivity of GC cells to cisplatin through the HMGB1/RAGE signaling pathway.

Discussion

GC is a common malignancy in humans and is associated with an increased incidence in China (25). Several studies have investigated strategies for improving the diagnostic methods in GC. Dakal *et al* (26) revealed that the deregulation of IL-8 may be an important prognostic marker for patients with GC. Due to the lack of effective techniques for early diagnosis, the majority the patients with GC are diagnosed at late stages of GC. Chemotherapy is the primary treatment for GC and is used in patients at advanced stage of GC. However, chemoresistance remains to be a major obstacle for clinical treatment of GC. The molecular mechanism underlying chemoresistance is complex and involves a deregulation of various biological processes involved in drug metabolism and transport, apoptosis and DNA repair (27-32). Despite several advances, the molecular mechanisms underlying chemoresistance remain unclear. Therefore, further investigation on the molecular mechanism underlying the chemoresistance in GC is required.

Accumulating evidence suggest that lncRNAs are associated with various biological processes (32-37). The prognostic potential of lncRNAs has been demonstrated in several types of cancer, including GC. Wu *et al* (38) demonstrated that increased expression of long noncoding RNA colon cancer-associated transcript 2 indicated poor prognosis of GC. Tan *et al* (39) revealed that plasma lncRNA-gastric cancer associated transcript 2 may be a valuable marker for the screening of GC. Moreover, Liu and Shangguan (40) demonstrated that the upregulation of lncRNA CARLo-5 was associated with poor prognosis in patients with GC. However, whether additional lncRNAs may be associated with chemoresistance remains to be investigated. TP73-AS1, a lncRNA transcribed from chromosome 1p36, has been reported to be associated with cell proliferation and tumor progress (17,18). Previous studies predicted that TP73-AS1 may be upregulated in glioma and esophageal squamous cell carcinoma and was associated with the progression and prognosis of cancer (17,18). However, its biological function in GC still remains unclear.

The results of the present study demonstrated that TP73-AS1 was differentially expressed in the GC tissues and cell lines compared with those of controls, and increased expression level of TP73-AS1 was associated with poor prognosis of GC. Cox's proportional hazards analysis revealed that increased expression of TP73-AS1 may be considered as a specific biomarker for the poor prognosis of GC. Furthermore, cellular transfection experiments revealed that knockdown of TP73-AS1 significantly suppressed the proliferative ability and increased the sensitivity to cisplatin of GC cells. Flow cytometric analysis revealed that downregulation of TP73-AS1 may induce cell cycle arrest and promote cell apoptosis. The results demonstrated that the HMGB1/RAGE signaling pathway was involved in TP73-AS1-mediated function in GC. Taken together, the results of the present study investigated the lncRNA-mediated regulation of chemoresistance in GC and provide a potential candidate for novel therapeutic strategies in GC.

Acknowledgements

The author would like to thank the laboratory members, who assisted the author to finish the experiments.

Funding

No funding was received.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

JP wrote the present manuscript. All experiments were designed and conducted by JP. Data were collected by JP.

Ethics approval and consent to participate

The present study was approved by the Department of Gastrointestinal Surgery, the First Affiliated Hospital of Sun Yat-Sen University (Guangdong, China). Written informed consent was obtained from all participants.

Patient consent for publication

All patients have provided written informed consent for publication.

Competing interests

The authors declare that they have no competing interests.

References

1. Niccolai E, Taddei A, Prisco D and Amedei A: Gastric cancer and the epoch of immunotherapy approaches. World J Gastroenterol 21: 5778-5793, 2015.
2. Yang L: Incidence and mortality of gastric cancer in China. World J Gastroenterol 12: 17-20, 2006.
3. Forman D and Burley VJ: Gastric cancer: Global pattern of the disease and an overview of environmental risk factors. Best Pract Res Clin Gastroenterol 20: 633-649, 2006.
4. Terry MB, Gaudet MM and Gammon MD: The epidemiology of gastric cancer. Semin Radiat Oncol 12: 111-127, 2002.
5. Jin Y, Cui Z, Li X, Jin X and Peng J: Upregulation of long non-coding RNA PlncRNA-1 promotes proliferation and induces epithelial-mesenchymal transition in prostate cancer. Oncotarget 8: 26090-26099, 2017.
6. Conte F, Fiscon G, Chiara M, Colombo T, Farina L and Paci P: Role of the long non-coding RNA PVT1 in the dysregulation of the ceRNA-ceRNA network in human breast cancer. PLoS One 12: e0171661, 2017.
7. Zhang Y, Sun L, Xuan L, Pan Z, Li K, Liu S, Huang Y, Zhao X, Huang L, Wang Z, *et al*: Reciprocal changes of circulating long non-coding RNAs ZFAS1 and CDR1AS predict acute myocardial infarction. Sci Rep 6: 22384, 2016.
8. Wei X, Wang C, Ma C, Sun W, Li H and Cai Z: Long noncoding RNA ANRIL is activated by hypoxia-inducible factor-1α and promotes osteosarcoma cell invasion and suppresses cell apoptosis upon hypoxia. Cancer Cell Int 16: 73, 2016.

9. Peng W, Wang Z and Fan H: LncRNA NEAT1 impacts cell proliferation and apoptosis of colorectal cancer via regulation of Akt signaling. Pathol Oncol Res 23: 651-656, 2017.
10. Tao H, Cao W, Yang JJ, Shi KH, Zhou X, Liu LP and Li J: Long noncoding RNA H19 controls DUSP5/ERK1/2 axis in cardiac fibroblast proliferation and fibrosis. Cardiovasc Pathol 25: 381-389, 2016.
11. Mizrahi I, Mazeh H, Grinbaum R, Beglaibter N, Wilschanski M, Pavlov V, Adileh M, Stojadinovic A, Avital I, Gure AO, *et al*: Colon cancer associated transcript-1 (CCAT1) expression in adenocarcinoma of the stomach. J Cancer 6: 105-110, 2015.
12. Zhao Y, Guo Q, Chen J, Hu J, Wang S and Sun Y: Role of long non-coding RNA HULC in cell proliferation, apoptosis and tumor metastasis of gastric cancer: A clinical and in vitro investigation. Oncol Rep 31: 358-364, 2014.
13. Kallen AN, Zhou XB, Xu J, Qiao C, Ma J, Yan L, Lu L, Liu C, Yi JS, Zhang H, *et al*: The imprinted H19 lncRNA antagonizes let-7 microRNAs. Mol Cell 52: 101-112, 2013.
14. Malakar P, Shilo A, Mogilevsky A, Stein I, Pikarsky E, Nevo Y, Benyamini H, Elgavish S, Zong X, Prasanth KV and Karni R: Long noncoding RNA MALAT1 promotes hepatocellular carcinoma development by SRSF1 upregulation and mTOR activation. Cancer Res 77: 1155-1167, 2017.
15. Liu YW, Xia R, Lu K, Xie M, Yang F, Sun M, De W, Wang C and Ji G: LincRNAFEZF1-AS1 represses p21 expression to promote gastric cancer proliferation through LSD1-Mediated H3K4me2 demethylation. Mol Cancer 16: 39, 2017.
16. Liu D, Li Y, Luo G, Xiao X, Tao D, Wu X, Wang M, Huang C, Wang L, Zeng F and Jiang G: LncRNA SPRY4-IT1 sponges miR-101-3p to promote proliferation and metastasis of bladder cancer cells through up-regulating EZH2. Cancer Lett 388: 281-291, 2017.
17. Zhang R, Jin H and Lou F: The long non-coding RNA TP73-AS1 interacted with miR-142 to modulate brain glioma growth through HMGB1/RAGE pathway. J Cell Biochem 119: 3007-3016, 2018.
18. Zang W, Wang T, Wang Y, Chen X, Du Y, Sun Q, Li M, Dong Z and Zhao G: Knockdown of long non-coding RNA TP73-AS1 inhibits cell proliferation and induces apoptosis in esophageal squamous cell carcinoma. Oncotarget 7: 19960-19974, 2016.
19. Peng XC, Zeng Z, Huang YN, Deng YC and Fu GH: Clinical significance of TM4SF1 as a tumor suppressor gene in gastric cancer. Cancer Med 7: 2592-2600, 2018.
20. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
21. Ansari SS, Sharma AK, Zepp M, Ivanova E, Bergmann F, Konig R and Berger MR: Upregulation of cell cycle genes in head and neck cancer patients may be antagonized by erufosine's down regulation of cell cycle processes in OSCC cells. Oncotarget 9: 5797-5810, 2018.
22. Huang H, Regan KM, Lou Z, Chen J and Tindall DJ: CDK2-dependent phosphorylation of FOXO1 as an apoptotic response to DNA damage. Science 314: 294-297, 2006.
23. Chen RC, Yi PP, Zhou RR, Xiao MF, Huang ZB, Tang DL, Huang Y and Fan XG: The role of HMGB1-RAGE axis in migration and invasion of hepatocellular carcinoma cell lines. Mol Cell Biochem 390: 271-280, 2014.
24. Li S, Huang Y, Huang Y, Fu Y, Tang D, Kang R, Zhou R and Fan XG: The long non-coding RNA TP73-AS1 modulates HCC cell proliferation through miR-200a-dependent HMGB1/RAGE regulation. J Exp Clin Cancer Res 36: 51, 2017.
25. Coupland VH, Lagergren J, Lüchtenborg M, Jack RH, Allum W, Holmberg L, Hanna GB, Pearce N and Møller H: Hospital volume, proportion resected and mortality from oesophageal and gastric cancer: A population-based study in England, 2004-2008. Gut 62: 961-966, 2013.
26. Dakal TC, Kala D, Dhiman G, Yadav V, Krokhotin A and Dokholyan NV: Predicting the functional consequences of non-synonymous single nucleotide polymorphisms in IL8 gene. Sci Rep 7: 6525, 2017.
27. Rassy EE, Assi T, Rizkallah J and Kattan J: Diffuse edema suggestive of cytokine release syndrome in a metastatic lung carcinoma patient treated with pembrolizumab. Immunotherapy 9: 309-311, 2017.
28. Jung KH, Choi IK, Lee HS, Yan HH, Son MK, Ahn HM, Hong J, Yun CO and Hong SS: Oncolytic adenovirus expressing relaxin (YDC002) enhances therapeutic efficacy of gemcitabine against pancreatic cancer. Cancer Lett 396: 155-166, 2017.
29. Hsieh MJ, Lin CW, Yang SF, Sheu GT, Yu YY, Chen MK and Chiou HL: A combination of pterostilbene with autophagy inhibitors exerts efficient apoptotic characteristics in both chemosensitive and chemoresistant lung cancer cells. Toxicol Sci 156: 549, 2017.
30. Fong Y, Wu CY, Chang KF, Chen BH, Chou WJ, Tseng CH, Chen YC, Wang HD, Chen YL and Chiu CC: Dual roles of extracellular signal-regulated kinase (ERK) in quinoline compound BPIQ-induced apoptosis and anti-migration of human non-small cell lung cancer cells. Cancer Cell Int 17: 37, 2017.
31. Elias KM, Harvey RA, Hasselblatt KT, Seckl MJ and Berkowitz RS: Type I interferons modulate methotrexate resistance in gestational trophoblastic neoplasia. Am J Reprod Immunol 77: e12666, 2017.
32. Cai W, Chen G, Luo Q, Liu J, Guo X, Zhang T, Ma F, Yuan L, Li B and Cai J: PMP22 regulates self-renewal and chemoresistance of gastric cancer cells. Mol Cancer Ther 16: 1187-1198, 2017.
33. Wu J, Cheng G, Zhang C, Zheng Y, Xu H, Yang H and Hua L: Long noncoding RNA LINC01296 is associated with poor prognosis in prostate cancer and promotes cancer-cell proliferation and metastasis. Onco Targets Ther 10: 1843-1852, 2017.
34. Rao AKDM, Rajkumar T and Mani S: Perspectives of long non-coding RNAs in cancer. Mol Biol Rep 44: 203-218, 2017.
35. Qian L, Xu F, Wang X, Jiang M, Wang J, Song W, Wu D, Shen Z, Feng D, Ling B, *et al*: LncRNA expression profile of ΔNp63α in cervical squamous cancers and its suppressive effects on LIF expression. Cytokine 96: 114-122, 2017.
36. Li S, Li B, Zheng Y, Li M, Shi L and Pu X: Exploring functions of long noncoding RNAs across multiple cancers through co-expression network. Sci Rep 7: 754, 2017.
37. Chang S, Chen B, Wang X, Wu K and Sun Y: Long non-coding RNA XIST regulates PTEN expression by sponging miR-181a and promotes hepatocellular carcinoma progression. BMC Cancer 17: 248, 2017.
38. Wu SW, Hao YP, Qiu JH, Zhang DB, Yu CG and Li WH: High expression of long non-coding RNA CCAT2 indicates poor prognosis of gastric cancer and promotes cell proliferation and invasion. Minerva Med 108: 317-323, 2017.
39. Tan L, Yang Y, Shao Y, Zhang H and Guo J: Plasma lncRNA-GACAT2 is a valuable marker for the screening of gastric cancer. Oncol Lett 12: 4845-4849, 2016.
40. Liu JN and Shangguan YM: Long non-coding RNA CARLo-5 upregulation associates with poor prognosis in patients suffering gastric cancer. Eur Rev Med Pharmacol Sci 21: 530-534, 2017.