Original Article

Long non-coding RNA *SNHG17* promotes gastric cancer progression by inhibiting *P15* and *P16*

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Background: The dysregulated long non-coding RNA (lncRNA) small nucleolar RNA host genes (*SNHGs*) have been demonstrated to be involved in gastric carcinogenesis and progression; however, the role of *SNHG17* in gastric carcinoma remains to be investigated. We aimed to ascertain the expression of *SNHG17* in gastric carcinoma tissues and cell lines, and to investigate its mechanistic role in this malignancy.

Methods: The expression levels of *SNHG17*, *P15*, *P16*, *P18*, *P19* and cyclin dependent kinases-4 (CDK4) were determined by real-time quantitative polymerase chain reaction (RT-qPCR) and/or western blotting in human gastric cancer tissues and cell lines. Correlations between *SNHG17* levels and clinicopathological features were evaluated. siRNAs were used to silence *SNHG17* in cell lines, and then Cell Counting Kit-8, colony formation, and transwell migration assays were used to assess proliferation, clonogenic potential, and migration, respectively. Flow cytometry was used to analyze cell cycle distributions and apoptosis. *In vivo* tumorigenicity was evaluated using xenografts in nude mice.

Results: Analysis of The Cancer Genome Atlas (TCGA) database revealed that *SNHG17* expression was remarkably higher in gastric carcinoma tissues than normal stomach mucosae ($P=4.85\times10^{-10}$). We confirmed that *SNHG17* was overexpressed in gastric cancer tissues ($P<0.0001$) and cell lines ($P<0.01$) compared with corresponding noncancerous tissues and gastric epithelial cell line, respectively. Furthermore, *SNHG17* levels in tumor tissues were associated with lymph node metastasis ($P=0.0006$), pTNM stage ($P=0.0061$), and lymphovascular invasion ($P=0.0005$), but were not associated with overall survival (OS) ($P=0.888$). Loss-of-function studies indicated that *SNHG17* promoted gastric carcinoma cell proliferation *in vitro* and *in vivo* ($P<0.01$), and that *SNHG17* enhanced gastric cancer cell migration ($P<0.01$). Mechanistically, we found that *SNHG17* inhibited *P15* and *P16*, and enhanced CDK4 expression, resulting in a G0/G1 cell cycle arrest, and that *SNHG17* inhibited cell apoptosis.

Conclusions: These preliminary findings highlight the role of *SNHG17* in gastric cancer, and suggest that it may be a novel indicator and/or a potential therapeutic target for diagnosing and/or treating gastric cancer.

Keywords: Gastric cancer; small nucleolar RNA host genes (*SNHGs*); cell cycle; proliferation; migration; apoptosis

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Introduction

Gastric carcinoma remains one of the most prevalent tumors of digestive system, and the incidence of stomach cancer in China, Japan, Latin America, and Eastern Europe is relatively higher than in other countries. Approximately 72 million people die from this disease every year worldwide (1,2). Gastric cancer endangers human life, and radical surgery is the only potential curative approach. With the increase of early gastric cancer detection and the progress of comprehensive treatment, the prognosis of gastric cancer patients has greatly improved; however, the long-term survival of advanced gastric cancer patients remains poor (3). Gastric cancer development is a complex and multistage biological process that involves multiple oncogenes and tumor suppressors (4,5). The goal of genomic studies in gastric cancer is to find early diagnostic biomarkers and/or novel therapeutic targets.

Human genome analyses have indicated that 85% of DNA can be transcribed into RNA, but less than 2% of RNA can be translated into protein. The RNAs that cannot be translated into proteins are called non-coding RNAs (ncRNAs) (6). NcRNAs can be classified into short ncRNAs, mid-sized ncRNAs, and long ncRNAs (lncRNAs) on the basis of molecular length (7). LncRNAs are defined as being >200 bp in length, and >15,000 different types of lncRNAs have been identified by gene chip or high-throughput sequencing technologies (8). LncRNAs regulate cellular functions at the gene level by participating in genomic imprinting, chromatin packaging, differentiation, maintaining genomic integrity, and affecting embryo development (9,10). Abnormal lncRNA expression in tumor cells is usually associated with increased proliferation, invasion, and angiogenesis; however, other studies have shown that lncRNAs can also inhibit apoptosis and affects cell cycle progression in tumor cells (11-13).

Accumulating evidence has demonstrated that cancer involves a disorder of cell cycle regulatory mechanisms (14-16). There are three major protein families that regulate cell cycle progression: the cyclins, the cyclin dependent kinases (CDKs), and the CDK inhibitors (CKIs). Cyclins combine with CDKs to form a complex that drives cell cycle progression, and CKIs represses cyclin-CDK complexes (17). Currently, seven CKIs have been identified, and they are divided into two families: the INK4 and CIP/KIP families. The former includes *P15*, *P16*, *P18* and *P19*, which competes with cyclin D1-, D2-, and D3-CDK4/6 complexes in the G1 phase of the cell cycle to block cell cycle progression. CIP/KIP family includes *P21*, *P27*, and *P57*, which bind to cyclin-CDK2/7/9/10 complexes, acting on the G0/G1, S, and G2/M phases (18-20). Recently, CKIs have been shown to acquire tumor-suppressive properties in certain environments (21), while lncRNAs have been shown to promote tumor cell proliferation and migration by decreasing the expression of CKIs (22-24).

The roles of some small nucleolar RNA host genes (*SNHGs*) in cancer have attracted much attention recently. *SNHG5* suppresses gastric cancer progression (25), while *SNHG6* is related to poor prognosis in stomach carcinoma and promotes cell proliferation (22). However, the roles of *SNHG17* in stomach cancer remain to be investigated. In the present study, we first consulted The Cancer Genome Atlas (TCGA), and detected that *SNHG17* expression was remarkably higher in the gastric cancer tissues than in normal stomach mucosae. We found that *SNHG17* was overexpressed in stomach cancer tissues comparing to corresponding normal tissues, and that *SNHG17* was associated with lymph node metastasis, pTNM stage, and lymphovascular invasion. Loss-of-function assays showed that *SNHG17* inhibited *P15* and *P16* and upregulated CDK4, resulting in cell cycle arrest at the G0/G1 phase. Subsequently, *SNHG17* promoted gastric cancer cell proliferation and migration. This preliminary study elucidated the roles and mechanisms of the lncRNA *SNHG17* in gastric cancer. These results could provide a new biomarker or target for diagnosing or treating gastric cancer.

Methods

Human gastric cancer specimens

Patients with stomach cancer (n=80; median age: 59 years; range, 36–76 years) from January 2012 to December 2013 at the First Affiliated Hospital of Nanjing Medical University were randomly enrolled in this study. All these patients were pathologically diagnosed as gastric adenocarcinoma according to the American Joint Committee on Cancer criteria. All these patients had not received preoperative chemotherapy or radiotherapy. Samples of tumor tissues and corresponding noncancerous mucosae were collected immediately following resection and snap frozen in liquid nitrogen. All patients were followed up until March 2018, with a median follow-up of 48 months. Overall survival (OS) was defined as the interval between the dates of surgery and death. Distant metastases were not observed

preoperatively in these patients. This study was approved by the Nanjing Medical University Institutional Review Board. All the patients provided the written informed consent for their information and samples to be stored in the hospital database and used for researches. This study was compliant with the Helsinki Declaration.

Cell culture

We applied the normal human stomach epithelial cell line GES-1 (CBTCCCAS, Shanghai, China) and the human gastric adenocarcinoma cell lines AGS, SGC7901, BGC803, and BGC823 (ATCC, Manassas, VA, USA) in this study, and these cell lines were cultured in DMEM and RPMI-1640 (Invitrogen, Carlsbad, CA, USA), respectively at 37 ℃ in a humidified atmosphere with 5% CO_2, which was supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), penicillin/streptomycin (1:100 dilution; Invitrogen), and 4 mM glutamine (Life Technologies, Gibco BRL).

Real-time quantitative polymerase chain reaction (RT-qPCR)

RT-qPCR analyses were performed with reference to our previous report (26). The following primer pairs were applied in this study: *SNHG17*: 5'-TGCTTGTAAGGCAGGGTCTC-3' (sense) and 5'-ACAGCCACTGAAAGCATGTG-3' (antisense); *P15*: 5'-GGACTAGTGGAGAAGGTGCG-3' (sense) and 5'-GGGCGCTGCCCATCATCATG-3' (antisense); *P16*: 5'-ATGGAGCCTTCGGCTGAC-3' (sense) and 5'-GGCCTCCGACCGTAACTA-3' (antisense); *P18*: 5'-CGCGGATCCGCGCATG-3' (sense) and 5'-CCGCTCGAGCGGTCAC-3' (antisense); *P19*: 5'-GCGCCTGGTCACCAGGGC-3' (sense) and 5'-CACCTATAAGCCACAAAC-3' (antisense); and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*): 5'-GAAGAGAGAGACCCTCACGCT-3' (sense) and 5'-ACTGTGAGGAGGGGAGATTCA-3' (antisense). *GAPDH* served as the reference, and the observed gene expression levels were normalized to *GAPDH*. All assays were performed in triplicate.

Western blotting

Western blotting was used to analyze protein expression with reference to our previous report (26). The following antibodies were used: mouse monoclonal anti-P15, anti-P16, anti-CDK4, and rabbit monoclonal anti-GAPDH antibodies (Cell Signaling Technology, Danvers, MA, USA). Protein expression was quantified and normalized to *GAPDH* by densitometric analysis.

Transduction of SNHG17 siRNAs

Two *SNHG17* siRNAs, siRNA-*SNHG17*#1 (AAACGAGCGTAGCTTCCTT) and siRNA-*SNHG17*#2 (GCAGTGTCTCGTCCTCTTT), were designed and synthesized. *SNHG17* siRNA (siRNA-*SNHG17*) transduction was performed using riboFECTTM CP (RiboBio, Guangzhou, China) with reference to the manufacturer's recommended protocol.

Cell proliferation assay

The Cell Counting Kit-8 assay (Sigma-Aldrich, St. Louis, MO, USA) was performed to assay cell proliferation. Results are plotted as mean ± standard error of three separate experiments for each condition.

Colony formation assay

The clonogenic potential of the cells was assessed by colony formation assays. Briefly, 500 single viable cells in RPMI-1640 or DMEM containing 10% FBS were plated in 6-well plates, and were incubated at 37 ℃ in an atmosphere of 5% CO_2 for 14 d. Subsequently, the colonies formed were stained with 0.1% Crystal Violet Solution (Sigma-Aldrich), washed, and counted.

Transwell migration assay

According to the manufacturer's protocol, cells (3×10^5 per well) were seeded in the upper chambers of 24-well Transwell inserts (8.0-μm pore size; Corning, NY, USA) with 200 μL RMPI-1640 or DMEM containing 1% fetal bovine serum and 0.2% bovine serum albumin. After culture, migrated cells were stained and counted.

Flow cytometry

Cells were stained with both FITC-Annexin V and propidium iodide (PI) using the FITC-Annexin V Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ, USA), and were analyzed by flow cytometry using a FACScan with CellQuest software (BD Biosciences). Cells were classified into viable cells, dead cells, early apoptotic cells, and

apoptotic cells. The relative ratio of early apoptotic cells was compared with that of the controls for each experiment, and all samples were assayed at least in triplicate.

Tumorigenicity in vivo

All animal studies were conducted under the guidelines of the Nanjing Medical University Institutional Animal Care and Use Committee. A total of 16 4-week-old male BALB/c nude mice (Vitalriver, Beijing, China) were grouped randomly. For the analysis of tumorigenicity, 5×10^5 cells from AGS-si-NC, or AGS-si-*SNHG17* stable cell lines were inoculated subcutaneously into the flanks of the mice. Tumor volumes were measured every 4 d and were calculated using the following formula: volume = (length × width^2) ×0.5. On 40 d of inoculation, mice were euthanized, and tumor tissues were removed, weighed, and snap frozen in liquid nitrogen.

Statistical analysis

All statistical analyses were performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard error unless indicated. All values in protein expression are representative of three independent experiments with similar results. Associations of lncRNA levels with various clinicopathological parameters were evaluated with Pearson's χ^2 test. Quantitative data between the control and treatment groups were analyzed by variance analysis. P less than 0.05 was defined as statistically significant.

Results

SNHG17 was overexpressed in human gastric cancer cell lines and tissues and associated with lymph node metastasis, pTNM stage, and lymphovascular invasion

SNHG17 expression was evaluated by RT-qPCR in GES-1, AGS, SGC7901, BGC803, and BGC823 cells. As indicated in *Figure 1A*, *SNHG17* levels were higher in human gastric adenocarcinoma cell lines than in the human normal stomach epithelial cell line (P<0.01). We next analyzed TCGA database (www.cancergenome.nih.gov. version 2017-09-08). There were 375 cases of gastric cancer with *SNHG17* value in tumor tissues and 32 of them had *SNHG17* value in normal mucosae simultaneously, and analysis revealed that *SNHG17* value in tumor tissues was higher than in normal mucosae (*Figure 1B*, P=4.85×10^{-10}), indicating that *SNHG17* was overexpressed in human stomach tumor tissues compared with noncancerous mucosae.

Furthermore, we examined *SNHG17* expression in 80 human gastric cancer specimens, which indicated that *SNHG17* levels were significantly higher in stomach tumor tissues than in corresponding normal mucosae (*Figure 1C*, P<0.0001). According to the median *SNHG17* expression level, the 80 gastric cancer patients were divided into high group (above the median value, n=46) and low group (below the median value, n=34). Correlations between *SNHG17* expression and clinicopathological characteristics were investigated. As indicated in *Table 1*, high *SNHG17* expression in tumor tissue was associated with lymph node metastasis (P=0.0006), pTNM stage (P=0.0061), and lymphovascular invasion (P=0.0005), and was not significantly associated with gender, age, tumor location, tumor size, or OS (*Figure 1D*, P=0.888).

Silencing SNHG17 inhibited gastric cancer cell proliferation and migration

To probe the effects of *SNHG17* on cell behaviors, two *SNHG17*-targeted siRNAs, siRNA-*SNHG17*#1 and siRNA-*SNHG17*#2, were designed and synthesized. As shown in *Figure 2A*, both effectively silenced *SNHG17* in AGS and SGC7901 cells (P<0.01).

Colony formation assays indicated that colony numbers were significantly lower in *SNHG17*-silenced AGS and SGC7901 cells compared with controls (*Figure 2B*, P<0.01). Cell Counting Kit-8 assays also showed that silencing *SNHG17* significantly reduced proliferation (*Figure 2C*, P<0.01). Transwell assays demonstrated that siRNA treatment significantly reduced the number of gastric tumor cells that migrated through Matrigel compared with the scrambled siRNA groups (*Figure 2D*, P<0.01). Together, these experimental and clinical results demonstrated that *SNHG17* promoted gastric cancer progression.

Silencing SNHG17 resulted in G0/G1 cell cycle arrest and increased apoptosis in gastric cancer cell lines

Then, we investigated the effects of *SNHG17* on gastric carcinoma cell cycle progression and apoptosis. As shown in *Figure 3A*, silencing *SNHG17* with siRNA-*SNHG17*#1 or siRNA-*SNHG17*#2 resulted in a G0/G1 cell cycle arrest in AGS and SGC7901 cells (P<0.05). Additionally,

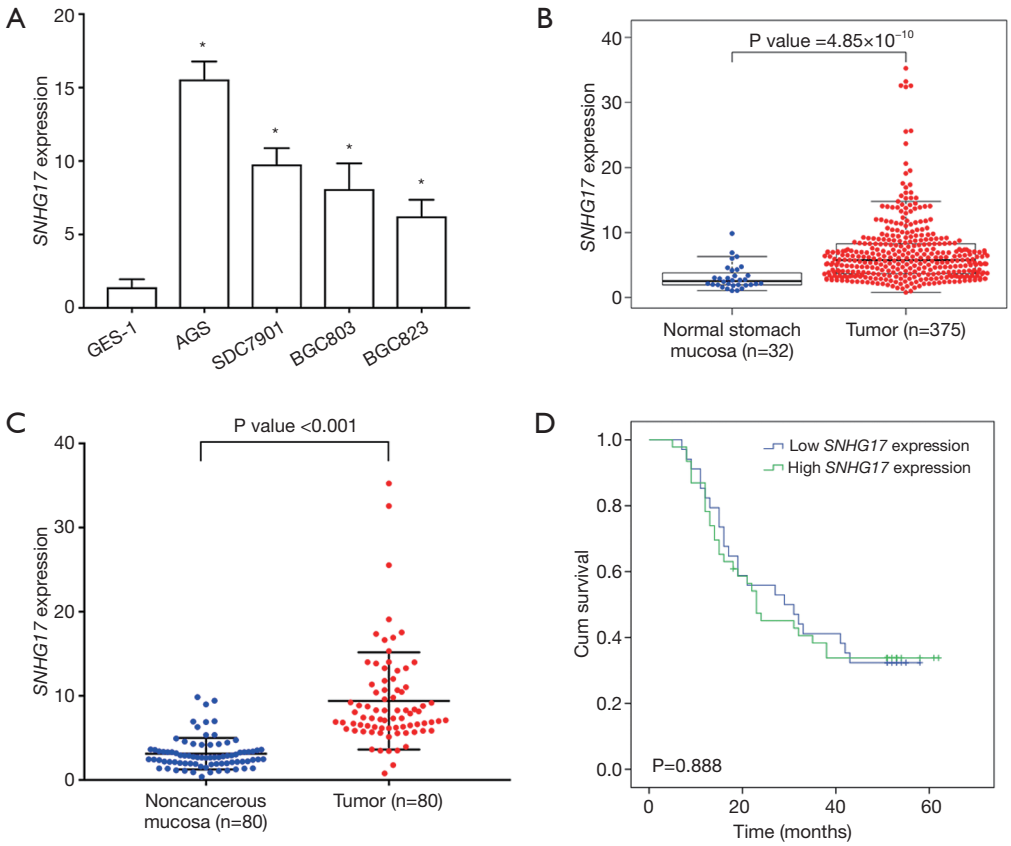

Figure 1 *SNHG17* was overexpressed in human stomach adenocarcinoma cell lines and tissues. (A) Compared with the normal human stomach epithelial cell line GES-1, *SNHG17* was overexpressed in the human gastric cancer cell lines, AGS, SGC7901, BGC802, and BGC823 (*, P<0.01 *vs.* GES-1, respectively); (B) TCGA analysis revealed that *SNHG17* was overexpressed in human stomach tumor tissues compared with noncancerous mucosae ($P=4.85\times10^{-10}$); (C) *SNHG17* levels were remarkably higher in stomach tumor tissues than in corresponding noncancerous mucosae (P<0.0001); (D) *SNHG17* levels in tumor tissues were not significantly associated with OS in gastric cancer patients (P=0.888). TCGA, The Cancer Genome Atlas; OS, overall survival.

flow cytometry indicated that silencing *SNHG17* in AGS and SGC7901 cells significantly increased the percentage of apoptotic cells (*Figure 3B*, P<0.01). These findings suggested that *SNHG17* regulates cell cycle progression and apoptosis, which may contribute to gastric cancer progression.

Silencing SNHG17 upregulated P15 and P16 expression and decreased CDK4 in gastric cancer cells

CKIs have been demonstrated to have functional diversity beyond cell cycle regulation, including regulation of cell proliferation, migration and apoptosis (17-19). To investigate the mechanism of how *SNHG17* regulates cell cycle progression, we studied the effects of *SNHG17* on the expression of INK4 family proteins and CDK4 (*Figure 4A*) in AGS and SGC7901 cells. As shown in *Figure 4B*, silencing *SNHG17* significantly increased *P15* and *P16* mRNA expression (P<0.01), but did not change *P18* and *P19* levels. Western blotting analysis indicated that silencing *SNHG17* upregulated *P15* and *P16* levels and decreased CDK4 (*Figure 4C*). Thus, it appears that *SNHG17* inhibits *P15* and *P16*, increasing proliferation and promoting gastric cancer progression.

Silencing SNHG17 inhibited gastric cancer cell tumorigenicity in vivo

Human gastric cancer cell xenografts were established in nude mice to verify the role of *SNHG17* in gastric cancer

Table 1 Correlation between *SNHG17* expression and clinicopathological features in gastric cancer patients

Clinicopathological features	N	*SNHG17* expression in tumor tissue		P value
		High cases (%)	Low cases (%)	
Age (years)				0.1646
<60	33	22 (66.7)	11 (33.3)	
≥60	47	24 (51.1)	23 (48.9)	
Gender				0.3130
Male	58	31 (53.4)	27 (46.6)	
Female	22	15 (68.2)	7 (31.8)	
Lauren classification				0.7818
Intestinal	48	27 (56.3)	21 (43.7)	
Diffuse and mixed	32	19 (59.4)	13(40.6)	
Tumor size (cm)				0.1408
≥3	54	28 (51.9)	26 (48.1)	
<3	26	18 (69.2)	8 (30.8)	
Tumor location				0.9519
Upper third	28	16 (57.1)	12 (42.9)	
Middle third	20	11 (55.0)	9 (45.0)	
Lower third	32	19 (59.4)	13 (40.6)	
Depth of tumor invasion				0.4805
Localized in subserosa	27	17 (63.0)	10 (37.0)	
Beyond subserosa	53	29 (54.7)	24 (45.3)	
Lymph node metastasis				0.0006
N0	25	7 (28.0)	18 (72.0)	
N1–N3	55	38 (69.1)	17 (30.9)	
pTNM stage				0.0061
I/II	33	13 (39.4)	20 (60.6)	
III/IV	47	33 (70.2)	14 (29.8)	
Lymphovascular invasion				0.0005
Absence	52	22 (42.3)	30 (57.7)	
Presence	28	24 (85.7)	4 (14.3)	

cell tumorigenicity. As shown in *Figure 5*, AGS cells transfected with si-*SNHG17*#1 (AGS-si-*SNHG17*#1) and AGS cells with control (AGS-si-NC) formed tumors after subcutaneously inoculation; however, tumor growth in the AGS-si-*SNHG17*#1 group was slower than in the AGS-si-NC group. Accordingly, reduced tumor weights were observed in the AGS-si-*SNHG17*#1 group compared with the control group after 40 d post-inoculation (*Figure 5A*; P<0.01). RT-qPCR assays of tumor tissues confirmed *SNHG17* expression in the two groups (*Figure 5B*; P<0.05), and indicated that *P15* and *P16* expression were increased (*Figure 5C*; P<0.05), and CDK4 expression was decreased significantly (*Figure 5D*; P<0.05) in AGS-si-*SNHG17*#1 tumor compared with in AGS-si-NC tumors.

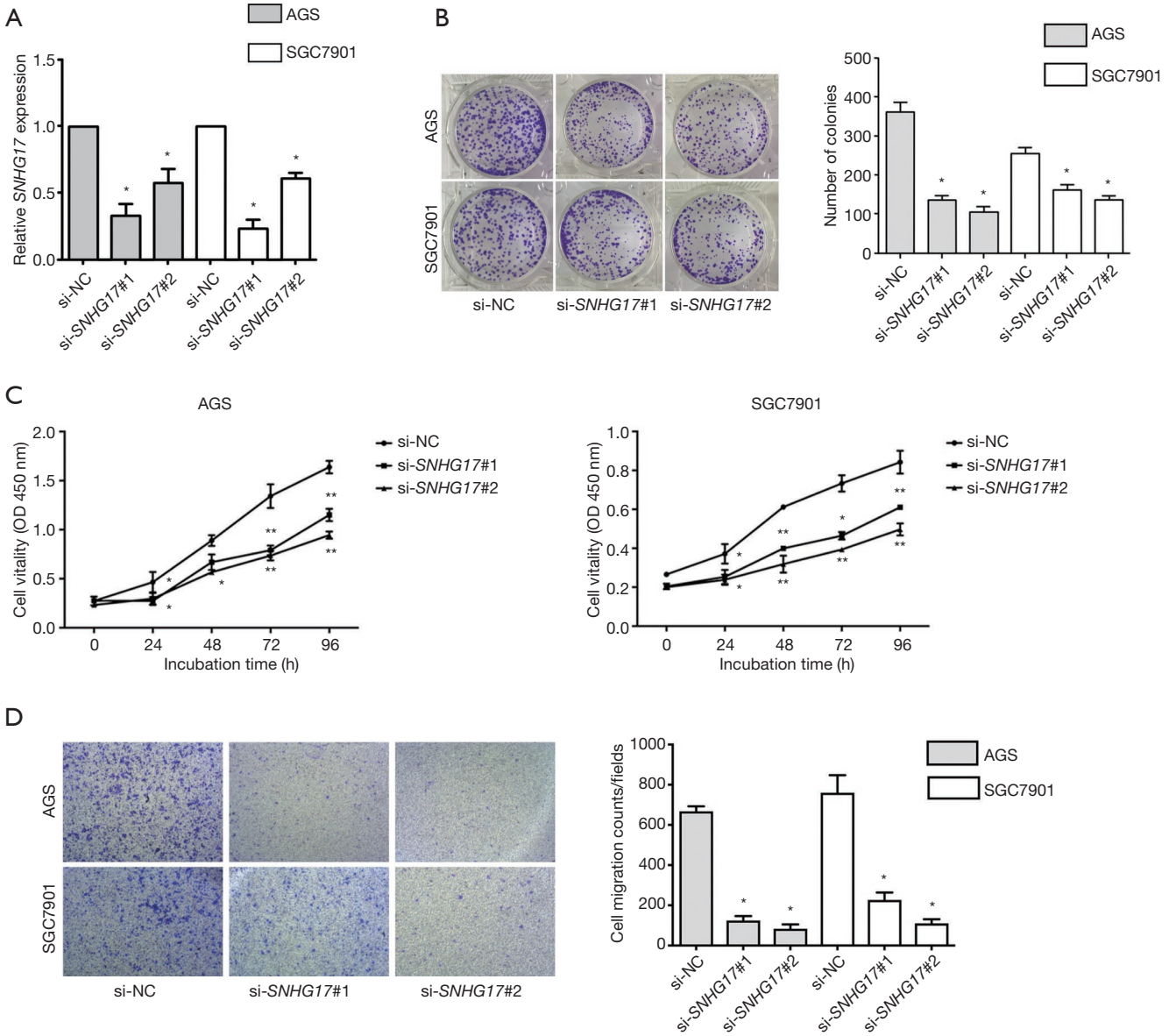

Figure 2 Silencing *SNHG17* inhibited gastric cancer cell proliferation and migration. (A) Both siRNA-*SNHG17*#1 and siRNA-*SNHG17*#2 silenced *SNHG17* in AGS and SGC7901 cells (*, P<0.01 *vs.* si-NC); (B) silencing *SNHG17* inhibited the colony formation ability of AGS and SGC7901 cells (*, P<0.01 *vs.* si-NC); (C) cell counting Kit-8 assays showed that silencing *SNHG17* significantly reduced cell proliferation rates (*, P<0.05 and **, P<0.01 *vs.* si-NC); (D) transwell assays showed that the migration of AGS and SGC7901 cells decreased significantly after silencing *SNHG17* (*, P<0.01 *vs.* si-NC).

Discussion

Gastric cancer, which is an ongoing serious public health threat, represents the second leading cause of cancer mortality worldwide (26). Early detection, diagnosis, and effective prognostic indicators are necessary to improve the survival of gastric cancer patients. However, the lack of early diagnostic biomarkers, effective prognostic indicators, and therapeutic targets accounts for the current poor prognosis of gastric cancer patients. Thus, searching for novel molecular biomarkers is imperative.

There has been increasing interest in the epigenetic regulation of human cancers. Setting aside the Central

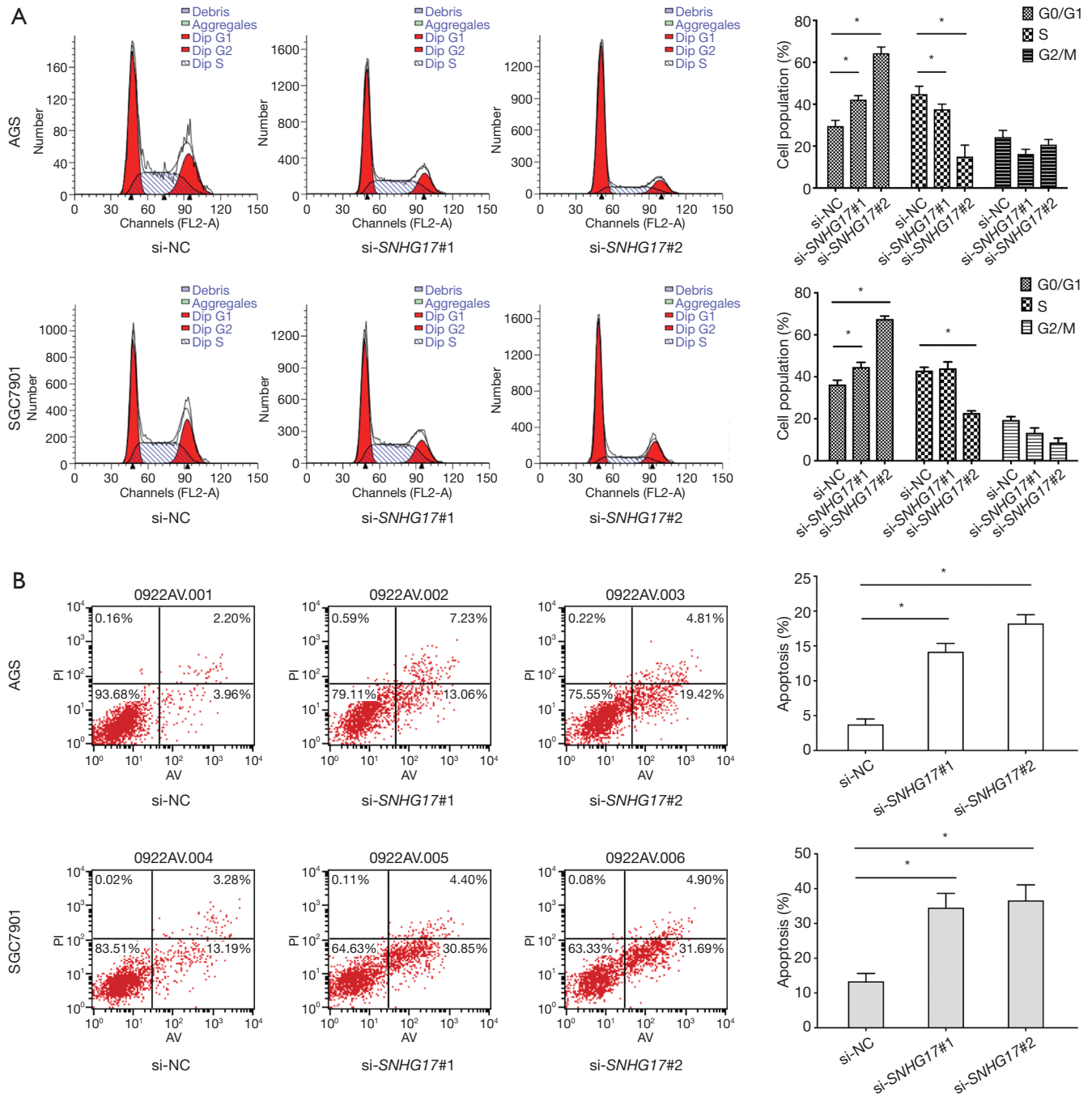

Figure 3 Silencing *SNHG17* resulted in G0/G1 cell cycle arrest and increased apoptosis in stomach carcinoma cells. (A) Treatment with siRNA-*SNHG17*#1 or siRNA-*SNHG17*#2 led to a cell cycle arrest at the G0/G1 phase in AGS and SGC7901 cells (*, P<0.05); (B) flow cytometry indicated that silencing *SNHG17* increased the percentage of apoptotic AGS and SGC7901 cells (*, P<0.01).

Dogma, both miRNAs and lncRNAs are epigenetic regulators of human cancers (8,27). LncRNAs fold into various conformations that allow interactions with RNA, DNA and proteins to regulate nuclear, cytoplasmic and mitochondrial functions (28,29). LncRNAs regulate biological processes including transcription, chromatin dynamics, chromatin looping, histone modifications, telomere biology, protein complex assembly, RNA splicing,

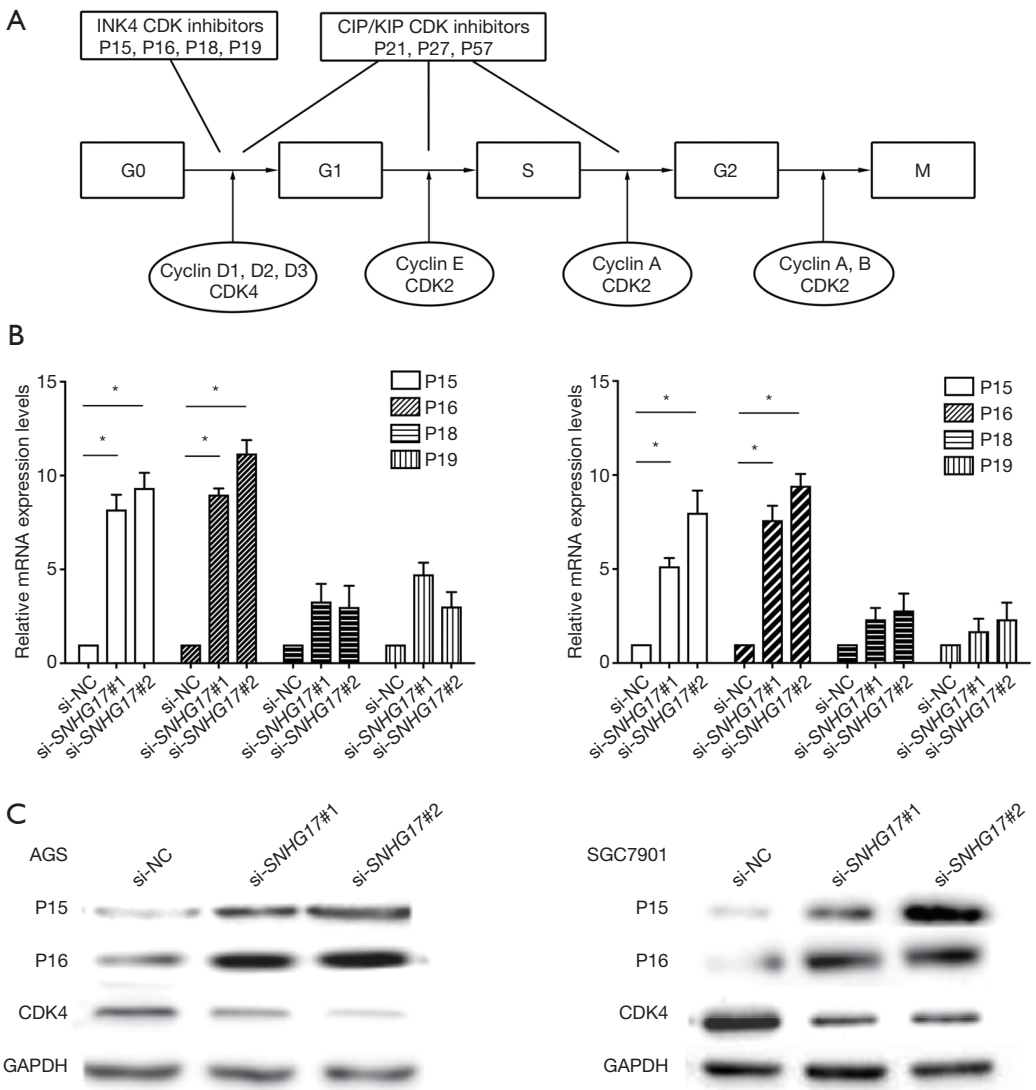

Figure 4 Silencing *SNHG17* upregulated *P15* and *P16* expression and decreased CDK4 in gastric cancer cells. (A) The INK4 family proteins play a competitive role with CKD4 in the G0/G1 phase; (B) RT-qPCR showed that silencing *SNHG17* significantly increased *P15* and *P16* expression (*, P<0.01 *vs.* si-NC), but had no effect on *P18* and *P19* expression in AGS and SGC7901 cells; (C) Western blotting indicated that silencing *SNHG17* significantly increased *P15* and *P16* levels and decreased CDK4 levels. RT-qPCR, real-time quantitative polymerase chain reaction; CDK4, cyclin dependent kinases-4.

and translation (30). Many lncRNAs have been found to be dysregulated in cancers, and lncRNAs are thought to play indispensable roles in each of the hallmarks of cancer (31). The regulatory patterns of lncRNAs make cancer pathogenesis more intricate and complicated; however, the multi-functional and tissue-specific properties of lncRNAs provide new avenues for developing novel diagnostic, prognostic, and therapeutic biomarkers for cancers (11).

A myriad of evidence has indicated that lncRNAs are involved in gastric cancer development. Liu *et al.* (32) reported that upregulation of the lncRNA *HOTAIR* is related to larger tumor size, advanced pathological stage, extensive metastasis, and dismal OS in gastric cancer patients. The lncRNA *ATB* contributes to gastric cancer growth through a MiR-141-3p/TGFβ2 feedback loop (33), and *FEZF1-AS1* is associated with adverse prognosis in stomach cancer and promotes tumorigenesis via activating the Wnt pathway (34). Currently, upregulated lncRNAs in

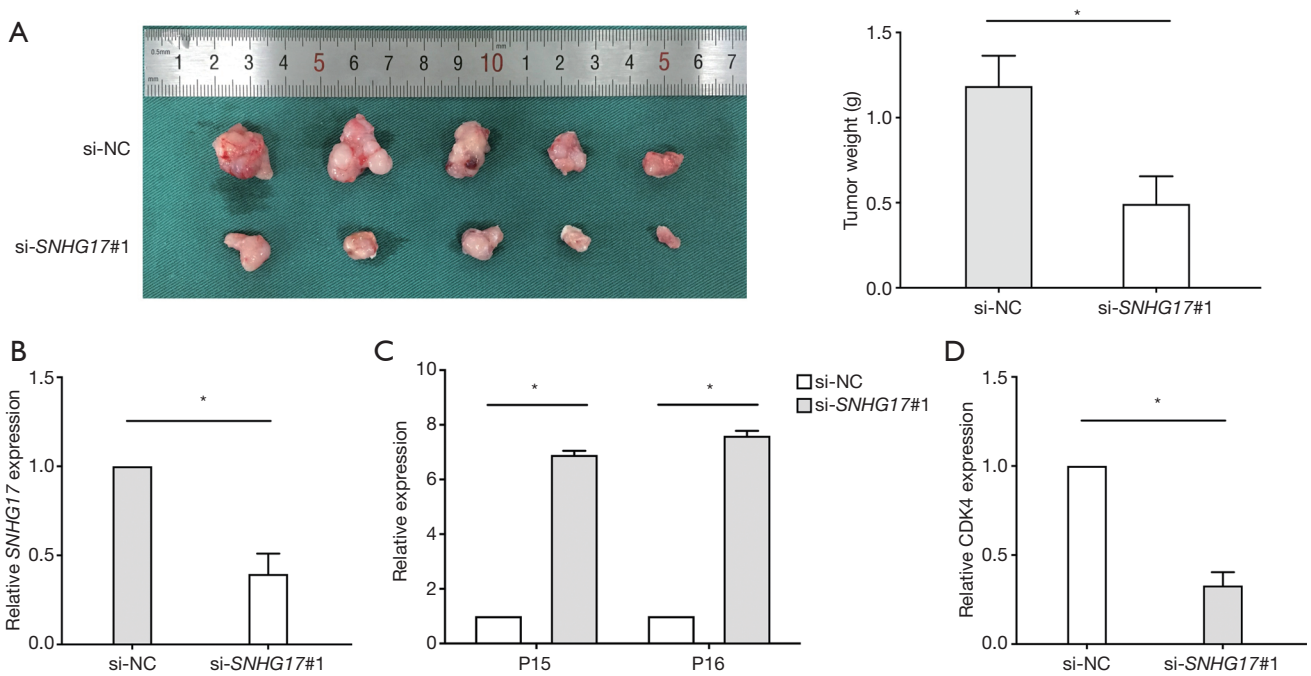

Figure 5 Silencing *SNHG17* inhibited gastric cancer cell tumorigenicity *in vivo*. (A) Xenografts in nude mice showed that tumor growth in the AGS-si-*SNHG17*#1 group was slower than in the AGS-si-NC group. Tumor weights from the AGS-si-*SNHG17*#1 group were remarkably lower than in the AGS-si-NC group (*, P<0.01); (B) RT-qPCR confirmed *SNHG17* expression in the two groups (*, P<0.05); (C) RT-qPCR indicated that P15 and P16 expression were increased, and (D) CDK4 expression was decreased significantly in the AGS-si-*SNHG17*#1 tumors compared with the AGS-si-NC tumors (*, P<0.05). RT-qPCR, real-time quantitative polymerase chain reaction; CDK4, cyclin dependent kinases-4.

cancer are thought to function as oncogenes the expedite the acquisition of the malignant cancer phenotypes, whereas the downregulated lncRNAs may possess tumor suppressive activities (11). For example, the lncRNA *AC138128.1* is significantly repressed in gastric tumor tissues compared with adjacent noncancerous mucosae, and thus, is thought to be a potential biomarker for gastric cancer (35).

Non-coding multiple *SNHGs* have been suggested to play critical roles in regulating cell behaviors and carcinogenesis. Li *et al.* (22) reported that *SNHG6* was overexpressed in human gastric cancer tissues and *SNHG6* knockdown inhibited gastric cancer development by upregulating *P21*, which was dependent on the activation of the JNK pathway and suppression of enhancer of zeste homolog 2 (EZH2). *SNHG1* expression is significantly higher in gastric tumor tissues than in adjacent normal mucosae, and *SNHG1* accelerated the proliferation of gastric cancer cells and increased DNMT1 expression (36). *SNHG14* overexpression contributes to gastric cancer development via targeting the miR-145/SOX9, which is involved in the PI3K/AKT/mTOR pathway (37). Conversely, *SNHG5* is remarkably downregulated in stomach tumor tissues, as it suppresses gastric carcinoma cell proliferation, migration, and invasion by trapping MTA2 in the cytosol (25). Computationally, Zhao *et al.* (25) found that *SNHG17* was upregulated in human gastric tumor tissues compared with normal mucosa tissues, and *SNHG17* has been shown to be an unfavorable prognostic factor in colorectal cancer that promotes proliferation by epigenetically silencing P57 (38). Recently, Zhang *et al.* (39) reported that *SNHG17* promotes gastric cancer progression by silencing *P15* and *P57*.

Analysis of TCGA database revealed that *SNHG17* was significantly overexpressed in stomach cancer tissues compared with noncancerous mucosae, and we verified this result in human gastric cancer specimens and cell lines. Furthermore, *SNHG17* levels in tumor tissues were related to lymph node metastasis, pTNM stage, and lymphovascular invasion, but were not related to OS. Loss-of-function analyses indicated that *SNHG17* promoted stomach carcinoma cell proliferation *in vitro* and *in vivo*, and that *SNHG17* enhanced gastric cancer cell migration. Further assays demonstrated that *SNHG17* inhibited *P15* and *P16*,

and enhances CDK4 expression, resulting in cell cycle arrest at G0/G1 phase, which was consistent with previous reports (39). Our study also showed that *SNHG17* inhibited cell apoptosis. Thus, we inferred that *SNHG17* promotes gastric cancer progression *via* inhibiting *P15* and *P16*. However, how *SNHG17* acts on *P15* and *P16* and whether other factors are involved in the process need to be further studied.

Currently, the concept of precision medicine and translational medicine has been well accepted (40), and certain dysregulated lncRNAs are promising candidate molecular biomarkers for cancer. Our preliminary findings highlight the role of *SNHG17* in gastric cancer, and suggest that it may serve as a novel biomarker or therapeutic target for diagnosing and treating stomach carcinoma. However, further studies are needed to clarify the underlying mechanisms in detail.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.04.14). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Nanjing Medical University Institutional Review Board. All the patients provided the written informed consent for their information and samples to be stored in the hospital database and used for researches. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. Charalampakis N, Economopoulou P, Kotsantis I, et al. Medical management of gastric cancer: a 2017 update. Cancer Med 2018;7:123-33.
2. Salati M, Di Emidio K, Tarantino V, et al. Second-line treatments: moving towards an opportunity to improve survival in advanced gastric cancer. ESMO Open 2017;2:e000206.
3. Van Cutsem E, Sagaert X, Topal B, et al. Gastric cancer. Lancet 2016;388:2654-64.
4. Nemtsova MV, Strelnikov VV, Tanas AS, et al. Implication of gastric cancer molecular genetic markers in surgical practice. Curr Genomics 2017;18:408-15.
5. Sitarz R, Skierucha M, Mielko J, et al. Gastric cancer: epidemiology, prevention, classification, and treatment. Cancer Manag Res 2018;10:239-48.
6. Awan HM, Shah A, Rashid F, et al. Primate-specific long non-coding RNAs and microRNAs. Genomics Proteomics Bioinformatics 2017;15:187-95.
7. Esteller M. Non-coding RNAs in human disease. Nat Rev Genet 2011;12:861-74.
8. Klinge CM. Non-coding RNAs: long non-coding RNAs and microRNAs in endocrine-related cancers. Endocr Relat Cancer 2018;25:R259-82.
9. Ma L, Bajic VB, Zhang Z. On the classification of long non-coding RNAs. RNA Biol 2013;10:925-33.
10. Bhan A, Mandal SS. Long noncoding RNAs: emerging stars in gene regulation, epigenetics and human disease. ChemMedChem 2014;9:1932-56.
11. Zhao J, Liu Y, Huang G, et al. Long non-coding RNAs in gastric cancer: versatile mechanisms and potential for clinical translation. Am J Cancer Res 2015;5:907-27.
12. Yang G, Lu X, Yuan L. LncRNA: a link between RNA and cancer. Biochim Biophys Acta 2014;1839:1097-109.
13. Zhao W, Geng D, Li S, et al. LncRNA HOTAIR influences cell growth, migration, invasion, and apoptosis

via the miR-20a-5p/HMGA2 axis in breast cancer. Cancer Med 2018;7:842-55.
14. Mens MMJ, Ghanbari M. Cell cycle regulation of stem cells by microRNAs. Stem Cell Rev 2018;14:309-22.
15. Boeynaems S, Tompa P, Van Den Bosch L. Phasing in on the cell cycle. Cell Div 2018;13:1.
16. Saha P, Datta K. Multi-functional, multicompartmental hyaluronan-binding protein 1 (HABP1/p32/gC1qR): implication in cancer progression and metastasis. Oncotarget 2018;9:10784-807.
17. Lim S, Kaldis P. Cdks, cyclins and CKIs: roles beyond cell cycle regulation. Development 2013;140:3079-93.
18. Kumar A, Gopalswamy M, Wolf A, et al. Phosphorylation-induced unfolding regulates p19INK4d during the human cell cycle. Proc Natl Acad Sci U S A 2018;115:3344-9.
19. Grimmler M, Wang Y, Mund T, et al. Cdk-inhibitory activity and stability of p27Kip1 are directly regulated by oncogenic tyrosine kinases. Cell 2007;128:269-80.
20. Cheng CW, Leong KW, Ng YM, et al. The peptidyl-prolyl isomerase PIN1 relieves cyclin-dependent kinase 2 (CDK2) inhibition by the CDK inhibitor p27. J Biol Chem 2017;292:21431-41.
21. Georgakilas AG, Martin OA, Bonner WM. p21: a two-faced genome guardian. Trends Mol Med 2017;23:310-9.
22. Li Y, Li D, Zhao M, et al. Long noncoding RNA SNHG6 regulates p21 expression via activation of the JNK pathway and regulation of EZH2 in gastric cancer cells. Life Sci 2018;208:295-304.
23. Su J, Zhang E, Han L, et al. Long noncoding RNA BLACAT1 indicates a poor prognosis of colorectal cancer and affects cell proliferation by epigenetically silencing of p15. Cell Death Dis 2017;8:e2665.
24. Zhang F, Peng H. LncRNA-ANCR regulates the cell growth of osteosarcoma by interacting with EZH2 and affecting the expression of p21 and p27. J Orthop Surg Res 2017;12:103.
25. Zhao L, Guo H, Zhou B, et al. Long non-coding RNA SNHG5 suppresses gastric cancer progression by trapping MTA2 in the cytosol. Oncogene 2016;35:5770-80.
26. Wen J, Wang Y, Gao C, et al. Helicobacter pylori infection promotes Aquaporin 3 expression via the ROS-HIF-1α-AQP3-ROS loop in stomach mucosa: a potential novel mechanism for cancer pathogenesis. Oncogene 2018;37:3549-61.
27. Liz J, Esteller M. LncRNAs and microRNAs with a role in cancer development. Biochim Biophys Acta 2016;1859:169-76.
28. Delás MJ, Hannon GJ. LncRNAs in development and disease: from functions to mechanisms. Open Biol 2017;7:170121.
29. De Paepe B, Lefever S, Mestdagh P. How long noncoding RNAs enforce their will on mitochondrial activity: regulation of mitochondrial respiration, reactive oxygen species production, apoptosis, and metabolic reprogramming in cancer. Curr Genet 2018;64:163-72.
30. Mercer TR, Mattick JS. Structure and function of long noncoding RNAs in epigenetic regulation. Nat Struct Mol Biol 2013;20:300-7.
31. Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. RNA Biol 2012;9:703-19.
32. Liu XH, Sun M, Nie FQ, et al. Lnc RNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p in gastric cancer. Mol Cancer 2014;13:92.
33. Lei K, Liang X, Gao Y, et al. Lnc-ATB contributes to gastric cancer growth through a MiR-141-3p/TGFβ2 feedback loop. Biochem Biophys Res Commun 2017;484:514-21.
34. Wu X, Zhang P, Zhu H, et al. Long noncoding RNA FEZF1-AS1 indicates a poor prognosis of gastric cancer and promotes tumorigenesis via activation of Wnt signaling pathway. Biomed Pharmacother 2017;96:1103-8.
35. Chen X, Sun J, Song Y, et al. The novel long noncoding RNA AC138128.1 may be a predictive biomarker in gastric cancer. Med Oncol 2014;31:262.
36. Hu Y, Ma Z, He Y, et al. LncRNA-SNHG1 contributes to gastric cancer cell proliferation by regulating DNMT1. Biochem Biophys Res Commun 2017;491:926-31.
37. Liu Z, Yan Y, Cao S, et al. Long non-coding RNA SNHG14 contributes to gastric cancer development through targeting miR-145/SOX9 axis. J Cell Biochem 2018;119:6905-13.
38. Ma Z, Gu S, Song M, et al. Long non-coding RNA SNHG17 is an unfavourable prognostic factor and promotes cell proliferation by epigenetically silencing P57 in colorectal cancer. Mol Biosyst 2017;13:2350-61.
39. Zhang G, Xu Y, Wang S, et al. LncRNA SNHG17 promotes gastric cancer progression by epigenetically silencing of p15 and p57. J Cell Physiol 2019;234:5163-74.
40. Wu JQ, Zhai J, Li CY, et al. Patient-derived xenograft in zebrafish embryos: a new platform for translational research in gastric cancer. J Exp Clin Cancer Res 2017;36:160.

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