European Review for Medical and Pharmacological Sciences 2018; 22: 4128-4137

Long non-coding RNA NEAT1 promotes viability and migration of gastric cancer cell lines through up-regulation of microRNA-17

C.-L. WANG[1], D. WANG[2], B.-Z. YAN[3], J.-W. FU[1], L. QIN[1]

[1]Department of Gastrointestinal Surgery, Jining No. 1 People's Hospital, Jining, Shandong, China
[2]Department of General Surgery, Liangshan County People's Hospital, Jining, Shandong, China
[3]Department of General Surgery, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China

Chunlin Wang and *Dong Wang* are co-first authors

Abstract. – OBJECTIVE: Gastric carcinoma (GC) is a common cancer with heavy mortality and poor outcome at advanced stages and metastasis. Long non-coding RNA nuclear-enriched abundant transcript 1 (NEAT1) has been reported to be an oncogene in GC recently. However, the underlying mechanism is far from understood. We aimed to explore the role of NEAT1 in GC as well as the underlying mechanisms.

PATIENTS AND METHODS: The expression of NEAT1 in clinical human GC tissues and GC cell lines were assessed by quantitative reverse transcription PCR. Then, NEAT1 was non-physiologically expressed in GC cells (SGC-7901 and MKN45 cells), followed by estimation of cell viability, migration, invasion, apoptosis, activation of the phosphatidylinositol-3-kinase (PI3K)/AKT and glycogen synthase kinase 3β (GSK3β) pathways, and microRNA (miR)-17 level. Moreover, the effects of miR-17 inhibition on cell viability, migration, and activation of the PI3K/AKT and GSK3β pathways in GC cells overexpressing NEAT1 were also explored.

RESULTS: NEAT1 was up-regulated in GC tissues and cell lines. Then, cell viability and migration of GC cells were markedly increased by NEAT1 overexpression, while the cell invasion and apoptosis were unchanged. The phosphorylated level of PI3K, AKT, and GSK3β were increased by NEAT1 overexpression. Subsequently, we found miR-17 level was positively correlated with NEAT1 expression, and NEAT1 functions through up-regulating miR-17.

CONCLUSIONS: NEAT1 was up-regulated in GC tissues and cell lines. Its overexpression enhanced cell viability and migration through up-regulating miR-17, along with activation of the PI3K/AKT and GSK3β pathways.

Key Words:
Gastric carcinoma, lncRNA NEAT1, Viability, Migration, miR-17, PI3K/AKT/GSK3β.

Introduction

As the most common gastrointestinal malignant tumor, gastric carcinoma (GC) is the second leading cause of cancer-related death worldwide, just following lung cancer[1]. In China, 400,000 new cases with GC are diagnosed annually, accounting for 42% of the total cases worldwide[2]. Currently, radical surgery is the unique effective therapy for GC, and the overall survival rate of curative resection after five years is approximately 45% in advanced GC[3]. However, the high recurrence and occurrence of lymph node or distal metastasis, which make the radical resection impractical, lead to poor prognosis[4,5]. Therefore, it is prominently needed to explore innovative and effective therapeutic strategies to improve outcome of GC. Long non-coding RNAs (lncRNAs) are transcripts of more than 200 nucleotides in length, which lack an extended open reading frame (ORF)[6]. Mounting evidence[7,8] has shown that lncRNAs are cell-type specific and play important roles in diagnose and progression of multiple cancers. The lncRNA nuclear-enriched abundant transcript 1 (NEAT1) was firstly identified[9] to be transcribed from the familial tumor syndrome multiple endocrine neoplasia (MEN) type 1, which locus on chromosome 11. Recently, NEAT1 has been reported to act as a diagnostic and prognostic biomarker, and an oncogene in diverse tumors, including glioma[10], colorectal cancer[11], and hepatocellular carcinoma[12]. For GC, to our knowledge, the related studies are limited. Fu et al[13] reported that NEAT1 is an unfavorable prognostic factor and promotes migration and

Corresponding Author: Lei Qin, MD; e-mail: leiqin0064@sina.com

invasion in GC. Likewise, Zhang et al[14] also reported the oncogenic role of NEAT1 in GC as well as the up-regulation. However, the underlying mechanism remains unclear. Generally speaking, lncRNAs may function through acting as scaffolds, guides or decoys for interactions with RNA, DNA, and protein[15]. MicroRNAs (miRNAs/miRs) are conserved, single-stranded non-coding RNA with about 22 nucleotides in length[16]. Commonly, miRNAs can post-transcriptionally regulate translation of downstream genes and thereby participate in substantial tumor development and progression[17]. miR-17 has been reported as an oncogene in hepatocellular carcinoma[18] and GC[19]. Thompson et al[20] have shown that up-regulated miR-17 could markedly increase expression of p53. Of note, NEAT1, as a p53 target, is regulated by p53 in response to DNA damage[21]. Thus, we suggested that there might be a potential interaction between NEAT1 and miR-17. In our study, the expression levels of NEAT1 in both clinical GC tissues and GC cell lines were explored, and the functional roles of NEAT1 in cell viability, migration, invasion, and apoptosis of GC cells were further tested. Moreover, the involved signaling cascades and the relationship between NEAT1 and miR-17 were also studied.

Patients and Methods

Clinical Specimens

Fresh-frozen GC tissues and the corresponding adjacent non-tumorous gastric tissues from 20 patients (10 male and 10 female, mean age 54.9 ± 8.0 years) were provided by the Jining No. 1 People's Hospital between January 2012 and July 2014. None of the patients received any treatments such as chemotherapy or radiotherapy before resection surgery. All the patients signed the written informed consents prior to surgery. The study was approved by the Ethics Committee of the Jining No. 1 People's Hospital.

Cell Culture

The human normal gastric epithelial cell line (GES-1), and four human gastric cancer cell lines (AGS, MKN28, MKN45, and SGC-7901) were obtained from the Cell Bank Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cells were maintained in high glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS, Gibco, Carlsbad, CA, USA). Cell culture was performed in a humidified incubator at 37°C with 5% CO_2.

Generation of Stably and Transiently Transfected Cells

Short-hairpin RNA directed against human NEAT1 or a non-targeting sequence was inserted into the pGPU6/Neo plasmid (GenePharma, Shanghai, China), and the recombined plasmids were referred to sh-NEAT1 or shNC. Full-length human NEAT1 sequences were sub-cloned into the pEX-2 plasmid (GenePharma), and the recombined plasmids were referred to as pEX-NEAT1. All the recombined plasmids were confirmed by sequencing (Sangon Biotechnology, Shanghai, China). Then, shNC, sh-NEAT1, pEX-2 or pEX-NEAT1 was transfected into SGC-7901 and MKN45 cells by using the Lipofectamine™ 3000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Complete culture medium supplemented with 0.5 mg/mL G418 (Sigma-Aldrich, St. Louis, MO, USA) was used for selection of stably transfected cells. G418-resistant cell clones were constructed after about 4 weeks' selection. For transient transfection, miR-17 inhibitor and its negative control (NC), synthesized by GenePharma, were respectively transfected into SGC-7901 and MKN45 cells as described above, and the cells were harvested at 72 h post-transfection for the following experiments.

Cell Counting Kit-8 (CCK-8) Assay

Cell viability of transfected SGC-7901 and MKN45 cells was assessed by using the CCK-8 assay. Briefly, cells were seeded in 96-well plates with 5×10^3 cells per well and maintained at 37°C. After treatments and maintaining at 37°C for 48 h, 10 μL of CCK-8 solution (Dojindo Molecular Technologies, Gaithersburg, MD, USA) was added to each well, followed by additional 1 h of incubation at 37°C. Then, the absorbance was measured by a Microplate Reader at 450 nm (Bio-Rad, Hercules, CA, USA).

Migration and Invasion Assay

Cell migration was assessed by using BD Falcon™ Cell Culture Inserts (pore size, 8-μm; BD Bioscience, Bedford, MA, USA) in a 24-well culture plate. Cell invasion was tested by using BD BioCoat Matrigel Invasion Chambers (BD Biosciences, Franklin Lakes, NJ, USA). The procedures of cell migration and invasion were

the same. Briefly, 1×10^5 transfected SGC-7901 and MKN45 cells in 200 µL FBS-free medium were plated on the upper chamber. Meanwhile, 600 µL of complete culture medium was added to the lower chamber. After 24 h, non-migrated or non-invasive cells on the upper surface of the inserts were removed with a cotton swab. After being fixed with methanol and staining with Diff-Quick (BD Biosciences), the cells on the bottom of the inserts were counted using an IX71 inverted microscope (Olympus, Tokyo, Japan).

Flow-Cytometric Analysis of Apoptosis

Double-staining with Annexin V-fluorescein isothiocynate (FITC) and propidium iodide (PI) was used for assessments of cell apoptosis. Briefly, transfected SGC-7901 and MKN45 cells (1×10^5 cells) were collected and washed with phosphate-buffered saline (PBS). After resuspension in binding buffer, cells were treated with Annexin V-FITC and PI (both 5 µL; FITC Annexin V apoptosis detection kit, BD Biosciences) according to the recommendation of the supplier. Then, cells were detected by using a FACS can (Beckman Coulter, Fullerton, CA, USA), and the data were analyzed by using FlowJo software (Tree Star, San Carlos, CA, USA).

Quantitative Reverse Transcription-PCR (qRT-PCR)

Total RNA of tissues and cells were extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's recommendation. The expression level of NEAT1 was estimated by using the One Step SYBR® PrimeScript™ PLUS RT-RNA PCR Kit (TaKaRa Biotechnology, Dalian, China) according to the manufacturer's protocol. For the estimation of miR-17 level, the TaqMan MicroRNA Reverse Transcription Kit and TaqMan Universal Master Mix II (both from Applied Biosystems, Foster City, CA, USA) were used for reverse transcription and quantitative PCR. The reaction condition of reverse transcription was 16°C for 30 min, 42°C for 30 min followed by 85°C for 5 min. The thermal cycling parameters of quantitative PCR were 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s, and 60°C for 1 min. The relative expression was calculated on the basis of the $2^{-\Delta\Delta Ct}$ method[22]. GAPDH and U6 were acted as the internal control of NEAT1 and miR-17.

Western Blot Analysis

Cell lysates were lysed in radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) containing a cocktail of protease inhibitors (Roche, Basel, Switzerland). After centrifugation at 12000 × g for 20 min, the supernatant was collected and the protein concentration was quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Then, proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. After blockage with 0.5% non-fat milk, the membranes were probed with primary antibody against mammalian B cell lymphoma-2 (Bcl-2; ab194583), Bcl-2-associated X protein (Bax; ab32503), pro caspase-3 (ab44976), cleaved caspase-3 (ab2302), pro caspase-9 (ab2013), cleaved caspase-9 (ab2324), phosphatidylinositol-3-kinase (PI3K; ab135952), phospho-PI3K (p-PI3K; ab182651), glycogen synthase kinase 3β (GSK3β; ab93926), phospho-GSK3β (p-GSK3β; ab75745), GAPDH (ab181603) (Abcam, Cambridge, MA, USA), AKT (9272) or phospho-AKT (p-AKT; 9271) (all from Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. After rinsing, the membranes were incubated with secondary antibodies marked by horseradish peroxidase (ab97051 and ab205719, Abcam) at room temperature for 1 h, followed by rinsing again. The bands in the membranes were visualized using a SuperSignal West Pico chemiluminescence ECL kit (Pierce, Appleton, WI, USA).

Statistical Analysis

All experiments had a minimum of three determinations. The results were presented as the mean ± standard deviation (SD). Statistical analyses were performed using SPSS 19.0 statistical software (IBM, Armonk, NY, USA). The *p*-values were calculated using the one-way analysis of variance (ANOVA) with Bonferroni's correction or unpaired two-tailed *t*-test. $p < 0.05$ was considered as statistically significant.

Results

LncRNA NEAT1 is Up-Regulated in Both GC Tissues and Cell Lines

The expression of NEAT1 in clinical GC tissues and adjacent non-tumorous tissues was shown in the Figure 1A. Of note, the expression of NEAT1 in tumor tissues was significant-

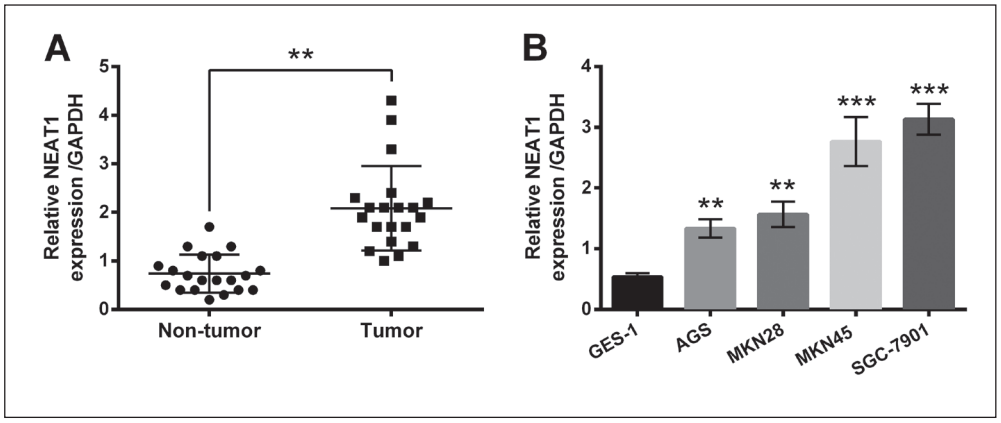

Figure 1.NEAT1 was up-regulated in gastric carcinoma tissues and cell lines. **A,** Expression of NEAT1 in clinical tissues. **B,** Expression of NEAT1 in normal gastric epithelial GES-1 cells and 4 gastric cancer cell lines. Data are presented as the mean ± SD. **, $p < 0.01$; ***, $p < 0.001$. NEAT1, long non-coding RNA nuclear enriched abundant transcript 1.

ly higher than that in adjacent non-tumorous tissues ($p < 0.01$). Likewise, the expression of NEAT1 in 4 GC cell lines, including AGS, MKN28, MKN45, and SGC-7901, was markedly higher than that in normal GES-1 cells ($p < 0.01$ or $p < 0.001$, Figure 1B). Those results illustrated that NEAT1 was up-regulated in GC, indicating the potential role of NEAT1 in the progression of GC.

Cell Viability and Migration Are Promoted by NEAT1 Overexpression Whereas Cell Invasion and Apoptosis Are Not Changed

Then, recombined plasmids were transfected into SGC-7901 cells and MKN45 cells, respectively, to construct stably transfected cells. As evidenced from Figure 2A, transfection with sh-NEAT1 remarkably down-regulated NEAT1 as compared to the shNC group, whereas transfection with pEX-NEAT1 significantly up-regulated NEAT1 as compared to the pEX-2 group, in both SGC-7901 and MKN45 cells (all $p < 0.01$). Data suggested that NEAT1 was non-physiologically expressed after stable transfection. Then, the cell viability, migration, invasion, and apoptosis of transfected cells were assessed. When compared to respective controls, cell viability (Figure 2B) and migration (Figure 2C) of both SGC-7901 and MKN45 cells were significantly reduced by NEAT1 suppression while were markedly enhanced by NEAT1 overexpression (all $p < 0.05$). However, the cell invasion (Figure 2D) and percentage of apoptotic cells (Figure 2E) were unchangeable in cells abnormally expressing NEAT1. Meanwhile, the expression levels of apoptosis-related proteins were stable in transfected cells, consolidating the unchanged cell apoptosis (Figure 2F). Results stated that NEAT1 promoted cell viability and migration but had no influence on cell invasion and apoptosis in GC cells.

NEAT1 Activates the PI3K/AKT and GSK3β Pathways

To explore the underlying mechanisms of NEAT1 modulation, phosphorylation of key kinases in the PI3K/AKT and GSK3β pathways was determined. In Figure 3A and 3B, expression of p-PI3K, p-AKT, and p-GSK3β in both SGC-7901 and MKN45 cells was significantly down-regulated by NEAT1 knockdown while were up-regulated by NEAT1 overexpression. Results proved that NEAT1 could activate the PI3K/AKT and GSK3β pathways in GC cells.

miR-17 is Up-Regulated by NEAT1 Overexpression

The interaction between miR-17 and NEAT1 was investigated by using qRT-PCR. In both SGC-7901 and MKN45 cells, the expression of miR-17 was significantly down-regulated in cells with NEAT1 silence compared with the shNC group (both $p < 0.05$, Figure 4). However, miR-17 expression was markedly up-regulated in cells overexpressing NEAT1 compared to the pEX-2 group (both $p < 0.05$, Figure 4). Data illustrated that miR-17 was positively correlated with NEAT1 expression in GC cells.

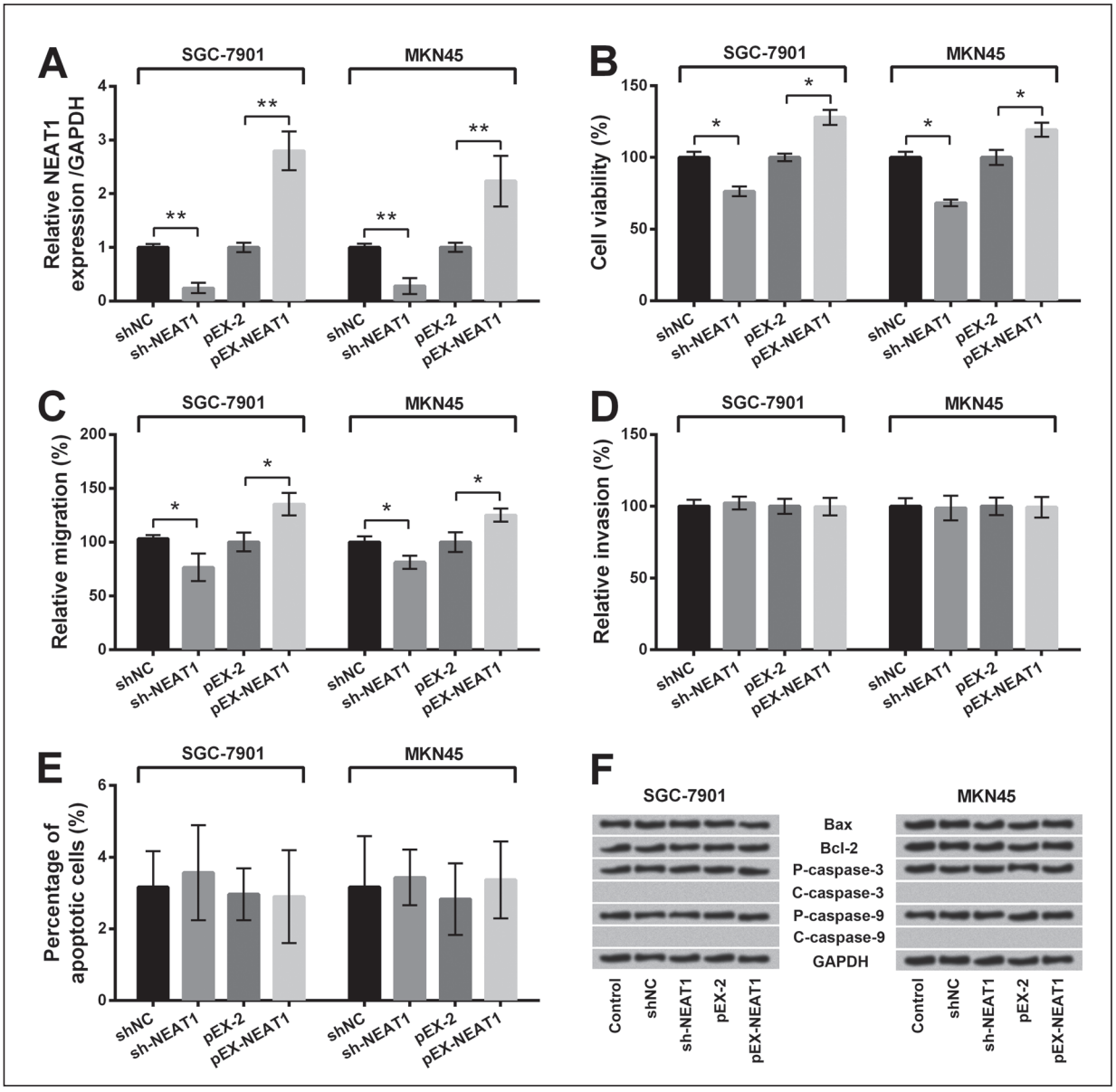

Figure 2. NEAT1 overexpression promoted cell viability and migration but had no influence on cell invasion and apoptosis in gastric cancer cells. All the experiments were performed in both SGC-7901 and MKN45 cells. **A,** Expression of NEAT1 by qRT-PCR. **B,** Cell viability by Cell Counting Kit-8 assay. Cell migration **(C)** and invasion **(D)** by transwell assays. **E,** Percentage of apoptotic cells by flow cytometry. **F,** Expression of apoptosis-associated proteins by Western blot analysis. Data are presented as the mean ± SD. *, $p < 0.05$; **, $p < 0.01$. NEAT1, long non-coding RNA nuclear enriched abundant transcript 1; sh-NEAT1, pGPU6/Neo plasmid carrying a short-hairpin RNA directed against NEAT1; shNC, pGPU6/Neo plasmid carrying a non-targeting sequence; pEX-NEAT1, pEX-2 plasmid containing full-length NEAT1; P-, pro; C-, cleaved.

NEAT1 Promotes Cell Viability and Migration by Up-Regulating miR-17

We further tested the effects of miR-17 silence on cell viability and migration of SGC-7901 and MKN45 cells overexpressing NEAT1. As evidenced from Figure 5A, expression of miR-17 in cells transfected with miR-17 inhibitor was significantly lower than that in the NC group ($p < 0.01$ or $p < 0.001$). Results showed the miR-17 inhibitor could successfully down-regulate miR-17 expression. Then, cells were co-transfected with plasmids and miRNAs, followed by assays of cell viability and migration. Data showed that the increases of cell viability (Figure 5B) and migration (Figure 5C) induced by NEAT1 overexpression were significantly reversed by miR-17 inhibition

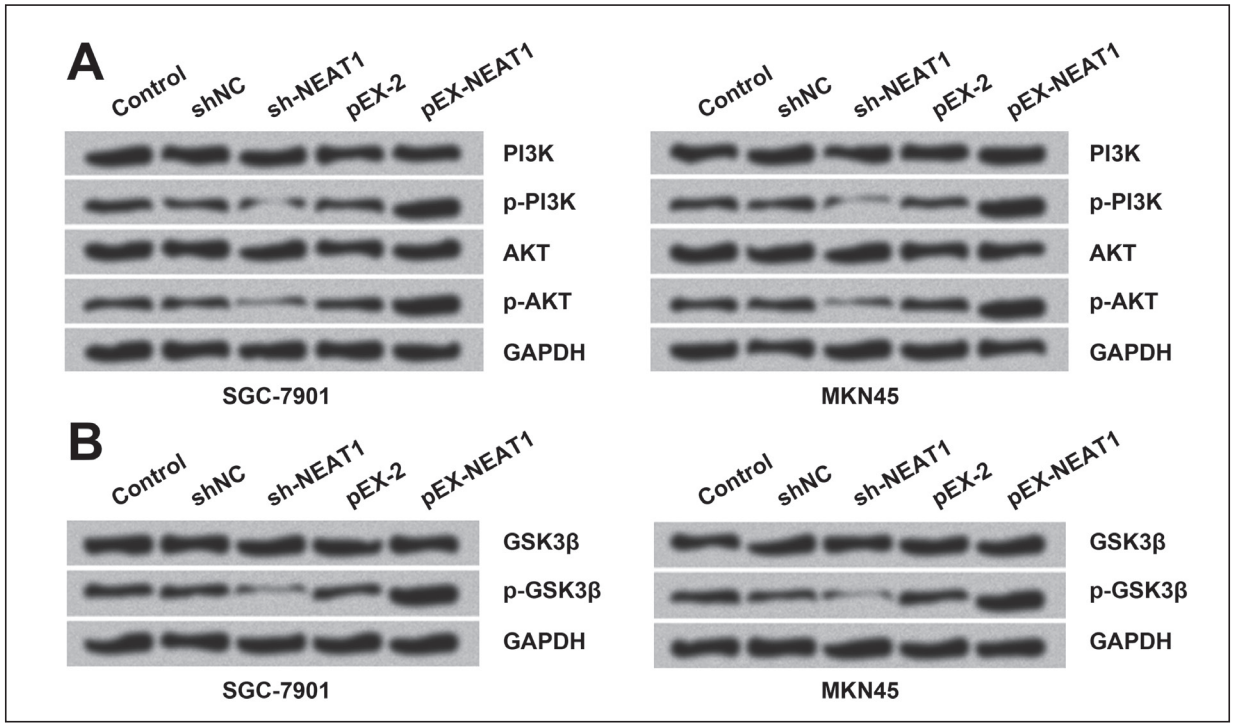

Figure 3. NEAT1 activated the PI3K/AKT and GSK3β pathways in gastric cancer cells. Expression of key kinases in the PI3K/AKT **(A)** and GSK3β **(B)** pathways in SGC-7901 and MKN45 cells was measured by Western blot analysis. NEAT1, long non-coding RNA nuclear enriched abundant transcript 1; PI3K, phosphatidylinositol-3-kinase; GSK3β, glycogen synthase kinase 3β; p-, phospho; sh-NEAT1, pGPU6/Neo plasmid carrying a short-hairpin RNA directed against NEAT1; shNC, pGPU6/Neo plasmid carrying a non-targeting sequence; pEX-NEAT1, pEX-2 plasmid containing full-length NEAT1.

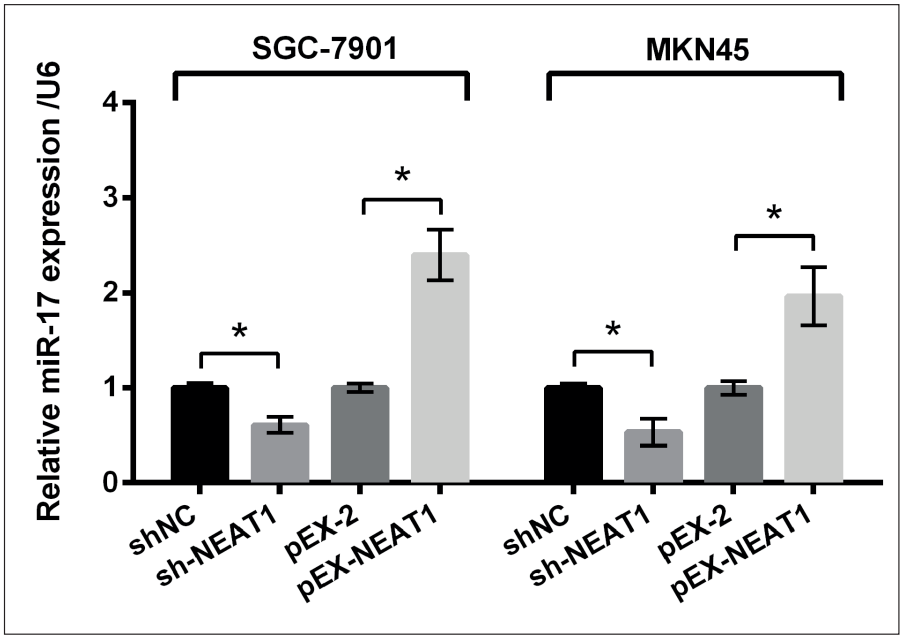

Figure 4. MicroRNA (miR)-17 was positively correlated with NEAT1 expression in gastric cancer cells. Expression of miR-17 in SGC-7901 and MKN45 cells was measured by qRT-PCR. Data are presented as the mean ± SD. *, $p < 0.05$. NEAT1, long non-coding RNA nuclear enriched abundant transcript 1; sh-NEAT1, pGPU6/Neo plasmid carrying a short-hairpin RNA directed against NEAT1; shNC, pGPU6/Neo plasmid carrying a non-targeting sequence; pEX-NEAT1, pEX-2 plasmid containing full-length NEAT1.

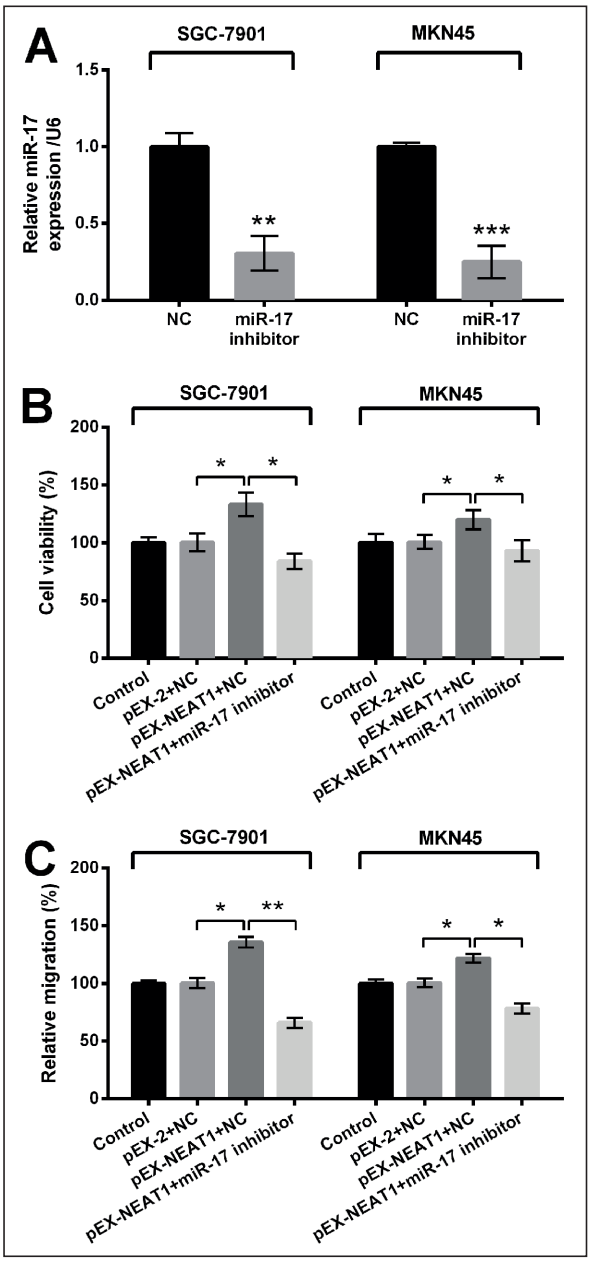

Figure 5. NEAT1 promoted cell viability and migration through up-regulating microRNA (miR)-17 in gastric cancer cells. All the experiments were performed in both SGC-7901 and MKN45 cells. **A,** Expression of miR-17 by qRT-PCR. **B,** Cell viability by Cell Counting Kit-8 assay. **C,** Cell migration by transwell assay. Data are presented as the mean ± SD. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. NEAT1, long non-coding RNA nuclear enriched abundant transcript 1; pEX-NEAT1, pEX-2 plasmid containing full-length NEAT1; NC, negative control of miR-17 inhibitor.

as compared to the pEX-NEAT1 + NC group ($p < 0.05$ or $p < 0.01$). The results described above illustrated that NEAT1 promoted cell viability and migration by up-regulating miR-17 in GC cells.

NEAT1 Activates the PI3K/AKT and GSK3β Pathways by Up-Regulating miR-17

Also, the influence of miR-17 silence on the PI3K/AKT and GSK3β pathways in SGC-7901 and MKN45 cells overexpressing NEAT1 was further explored. In Figure 6A-6B, the up-regulation of p-PI3K, p-AKT, and p-GSK3β, which was induced by NEAT1 overexpression, was observably down-regulated by miR-17 knockdown. We drew the conclusion that NEAT1 could activate the PI3K/AKT and GSK3β pathways by up-regulating miR-17 in GC cells.

Discussion

GC is a common cancer with heavy mortality and poor outcome at advanced stages and metastasis[23]. NEAT1 has been reported to be an oncogene in GC recently. However, the underlying mechanism is far from understood. In our work, NEAT1 was identified to be up-regulated in clinical GC tissues and four GC cell lines. Then, in SGC-7901 and MKN45 cells, cell viability and migration were significantly enhanced by NEAT1 overexpression, whereas cell invasion and apoptosis remained stable when the NEAT1 was abnormally expressed. The PI3K/AKT and GSK3β pathways were activated by NEAT1 overexpression. The expression of miR-17 was positively modulated by NEAT1 expression, and further experiments proved that NEAT1 functioned through up-regulating miR-17 in both SGC-7901 and MKN45 cells.

Accumulating evidence has reported that NEAT1 expression in clinical tumor tissues is significantly higher than that in adjacent non-tumorous tissues, for instance, in pancreatic cancer[24] and non-small cell lung cancer (NSCLC)[25]. These observations made NEAT1 become a biomarker for diagnose and prognostic prediction. In our study, the NEAT1 level was identified to be up-regulated in GC tissues as well as in GC cell lines (AGS, MKN28, MKN45, and SGC-7901 cells). Results of our study were consistent with the researches described above. The ectopic expression of NEAT1 suggested the potential involvements of NEAT1 in GC.

Previous studies[25,26] have proved NEAT1 could promote cell survival, migration, invasion but inhibit cell apoptosis in NSCLC cells. Meanwhile, in GC, NEAT1 has been reported to act as an oncogene[14]. To verify the specific roles of NEAT1

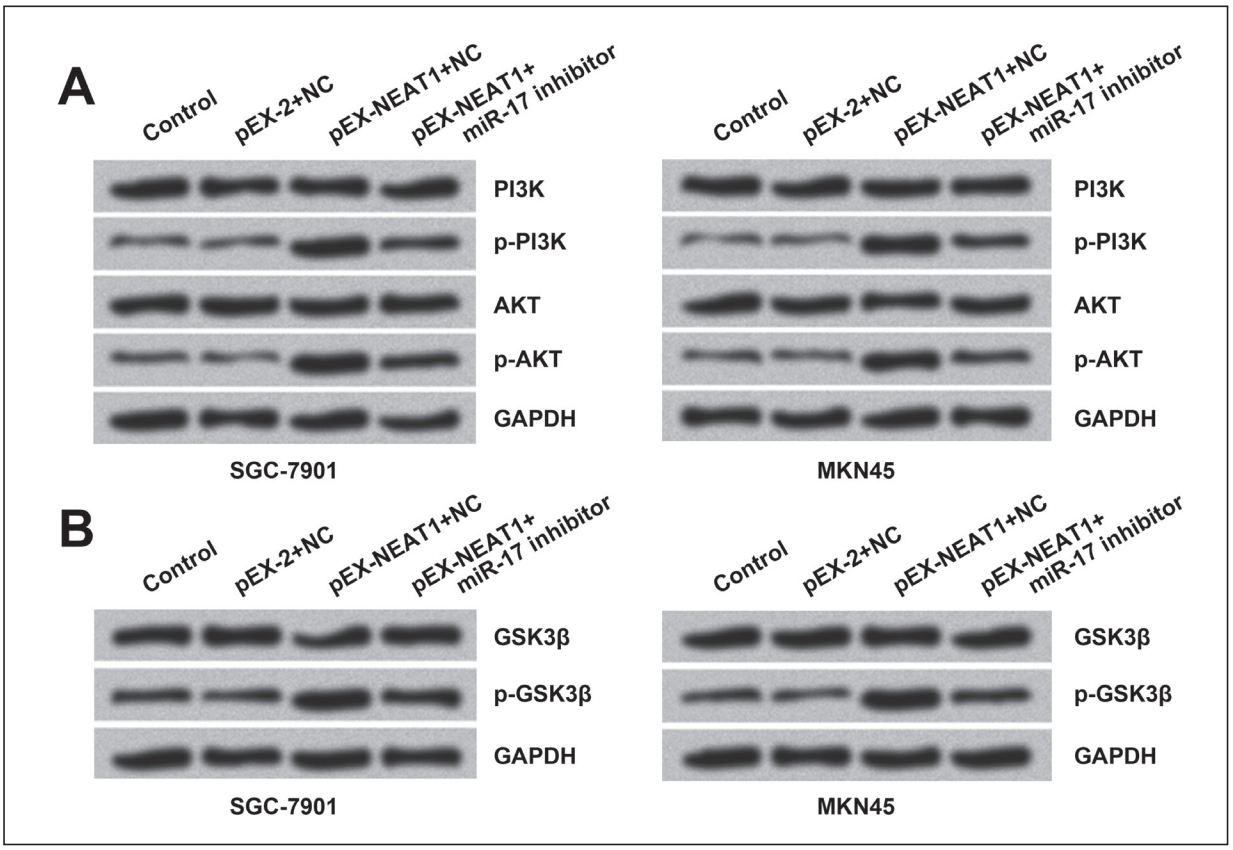

Figure 6. NEAT1 activated the PI3K/AKT and GSK3β pathways through up-regulating microRNA (miR)-17 in gastric cancer cells. Expression of key kinases in the PI3K/AKT **(A)** and GSK3β **(B)** pathways in SGC-7901 and MKN45 cells was measured by Western blot analysis. NEAT1, long non-coding RNA nuclear enriched abundant transcript 1; PI3K, phosphatidylinositol-3-kinase; GSK3β, glycogen synthase kinase 3β; p-, phospho; pEX-NEAT1, pEX-2 plasmid containing full-length NEAT1; NC, negative control of miR-17 inhibitor.

in GC cells, NEAT1 was abnormally expressed in two GC cell lines (SGC-7901 and MKN45 cells) after stable transfection. Then, cell viability, migration, invasion, and apoptosis were all tested. Results showed NEAT1 acted as an oncogene in GC cells through increasing cell viability and migration, whereas the cell invasion and apoptosis were unchangeable. Besides, the totally stable expression of proteins associated with apoptosis consolidated the unchanged cell apoptosis after aberrant expression of NEAT1. These results were partially consistent with previous studies. However, the reason of the unchanged cell invasion and apoptosis should be well studied in the future.

The PI3K/AKT signaling pathway is critical for malignant transformation of tumors as well as the common manifestation of tumors, including increased cell growth, proliferation, migration, and invasion, and decreased cell apoptosis[27,28]. A previous study[29] stated NEAT1 could affect cell proliferation and apoptosis through activating the AKT signaling cascade in colorectal cancer. GSK3β is a crucial component that correlates with tumorigenesis, especially for cell migration[30]. Meanwhile, activated AKT can bound to GSK3β in the presence of Dvl, and cell migration of GC cells can be promoted via the PI3K/AKT/GSK3β pathways[31]. In our research, after abnormal expression of NEAT1, PI3K/AKT and GSK3β pathways were inhibited by NEAT1 suppression while were activated by NEAT1 overexpression. Therefore, we supposed that NEAT1 might act as an oncogene through activating the PI3K/AKT and GSK3β pathways, which is waiting for further verification.

Increasing literature[32,33] has proven that lncRNAs functions through modulating miRNAs. In our work, we interestingly found expression of miR-17 was positively regulated by NEAT1.

Moreover, the increased cell viability and migration as well as activation of the PI3K/AKT and GSK3β pathways, induced by NEAT1 overexpression, were reversed by miR-17 inhibition. Results in our study firstly indicated that NEAT1 functioned through positively modulating miR-17. A previous study[21] showed the presence of p53 induced the expression of NEAT1. Wang et al[34] showed the miR-17 could bind to the 3'-untranslated region of the tumor protein p53-induced nuclear protein 1 (TP53INP1) and thereby promoted the progression of GC. Therefore, there is still a query about whether the interaction between NEAT1 and miR-17 was associated with p53, which needs more experiments to clarify. Besides, the downstream target gene of miR-17 involved in the functional modulation of NEAT1 in GC will be further investigated.

Conclusions

We found the lncRNA NEAT1 was up-regulated in GC tissues and cell lines, acting as an oncogene through increasing cell viability and migration. The PI3K/AKT and GSK3β pathways were activated by NEAT1. Moreover, we interestingly found NEAT1 functioned through positively regulating miR-17 for the first time. This study deeply investigated the role of NEAT1 in GC as well as its potential mechanisms, providing innovative therapeutic targets for GC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Xin Q, Zhang N, Yu HB, Zhang Q, Cui YF, Zhang CS, Ma Z, Yang Y, Liu W. CXCR7/CXCL12 axis is involved in lymph node and liver metastasis of gastric carcinoma. World J Gastroenterol 2017; 23: 3053-3065.
2) Yang L. Incidence and mortality of gastric cancer in China. World J Gastroenterol 2006; 12: 17-20.
3) Xu CY, Guo JL, Jiang ZN, Xie SD, Shen JG, Shen JY, Wang LB. Prognostic role of estrogen receptor alpha and estrogen receptor beta in gastric cancer. Ann Surg Oncol 2010; 17: 2503-2509.
4) Min H, Yoon S. Got target? Computational methods for microRNA target prediction and their extension. Exp Mol Med 2010; 42: 233-244.
5) Jin X, Yu N. MicroRNA-421 Gene Polymorphism in Gastric Carcinoma. Med Sci Monit 2016; 22: 1467-1471.
6) Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigo R. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 2012; 22: 1775-1789.
7) Nötzold L, Frank L, Gandhi M, Polycarpou-Schwarz M, Gross M, Gunkel M, Beil N, Erfle H, Harder N, Rohr K, Trendel J, Krijgsveld J, Longerich T, Schirmacher P, Boutros M, Erhardt S, Diederichs S. The long non-coding RNA LINC00152 is essential for cell cycle progression through mitosis in HeLa cells. Sci Rep 2017; 7: 2265.
8) Ma Y, Yang Y, Wang F, Moyer MP, Wei Q, Zhang P, Yang Z, Liu W, Zhang H, Chen N, Wang H, Wang H, Qin H. Long non-coding RNA CCAL regulates colorectal cancer progression by activating Wnt/beta-catenin signalling pathway via suppression of activator protein 2alpha. Gut 2016; 65: 1494-1504.
9) Guru SC, Agarwal SK, Manickam P, Olufemi SE, Crabtree JS, Weisemann JM, Kester MB, Kim YS, Wang Y, Emmert-Buck MR, Liotta LA, Spiegel AM, Boguski MS, Roe BA, Collins FS, Marx SJ, Burns L, Chandrasekharappa SC. A transcript map for the 2.8-Mb region containing the multiple endocrine neoplasia type 1 locus. Genome Res 1997; 7: 725-735.
10) Zhen L, Yun-hui L, Hong-yu D, Jun M, Yi-long Y. Long noncoding RNA NEAT1 promotes glioma pathogenesis by regulating miR-449b-5p/c-Met axis. Tumor Biol 2016; 37: 673-683.
11) Wu Y, Yang L, Zhao J, Li C, Nie J, Liu F, Zhuo C, Zheng Y, Li B, Wang Z, Xu Y. Nuclear-enriched abundant transcript 1 as a diagnostic and prognostic biomarker in colorectal cancer. Mol Cancer 2015; 14: 191.
12) Wang Z, Zou Q, Song M, Chen J. NEAT1 promotes cell proliferation and invasion in hepatocellular carcinoma by negative regulating miR-613 expression. Biomed Pharmacother 2017; 94: 612-618.
13) Fu J-w, Kong Y, Sun X. Long noncoding RNA NEAT1 is an unfavorable prognostic factor and regulates migration and invasion in gastric cancer. J Cancer Res Clin 2016; 142: 1571-1579.
14) Zhang J, Zhao B, Chen X, Wang Z, Xu H, Huang B. Silence of long noncoding RNA NEAT1 inhibits malignant biological behaviors and chemotherapy resistance in gastric cancer. Pathol Oncol Res 2018; 24: 109-113.
15) Song YX, Sun JX, Zhao JH, Yang YC, Shi JX, Wu ZH, Chen XW, Gao P, Miao ZF, Wang ZN. Non-coding RNAs participate in the regulatory network of CLDN4 via ceRNA mediated miRNA evasion. Nat Commun 2017; 8: 289.

16) Roberts TC. The microRNA biology of the mammalian nucleus. Mol Ther Nucleic Acids 2014; 3: e188.

17) Ling H, Fabbri M, Calin GA. MicroRNAs and other non-coding RNAs as targets for anticancer drug development. Nat Rev Drug Discov 2013; 12: 847-865.

18) Shan SW, Fang L, Shatseva T, Rutnam ZJ, Yang X, Du W, Lu W-Y, Xuan JW, Deng Z, Yang BB. Mature miR-17-5p and passenger miR-17-3p induce hepatocellular carcinoma by targeting PTEN, GalNT7 and vimentin in different signal pathways. J Cell Sci 2013; 126: 1517-1530.

19) Wu Q, Luo G, Yang Z, Zhu F, An Y, Shi Y, Fan D. miR-17-5p promotes proliferation by targeting SOCS6 in gastric cancer cells. FEBS Lett 2014; 588: 2055-2062.

20) Thompson MA, Edmonds MD, Liang S, McClintock-Treep S, Wang X, Li S, Eischen CM. miR-31 and miR-17-5p levels change during transformation of follicular lymphoma. Hum Pathol 2016; 50: 118-126.

21) Blume CJ, Hotz-Wagenblatt A, Hullein J, Sellner L, Jethwa A, Stolz T, Slabicki M, Lee K, Sharathchandra A, Benner A, Dietrich S, Oakes CC, Dreger P, te Raa D, Kater AP, Jauch A, Merkel O, Oren M, Hielscher T, Zenz T. p53-dependent non-coding RNA networks in chronic lymphocytic leukemia. Leukemia 2015; 29: 2015-2023.

22) Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001; 25: 402-408.

23) Shen J, Zhao DS, Li MZ. TGF-beta1 promotes human gastric carcinoma SGC7901 cells invasion by inducing autophagy. Eur Rev Med Pharmacol Sci 2017; 21: 1013-1019.

24) Cao J, Zhang Y, Yang J, He S, Li M, Yan S, Chen Y, Qu C, Xu L. NEAT1 regulates pancreatic cancer cell growth, invasion and migration though mircroRNA-335-5p/c-met axis. Am J Cancer Res 2016; 6: 2361-2374.

25) Sun C, Li S, Zhang F, Xi Y, Wang L, Bi Y, Li D. Long non-coding RNA NEAT1 promotes non-small cell lung cancer progression through regulation of miR-377-3p-E2F3 pathway. Oncotarget 2016; 7: 51784-51814.

26) Sun SJ, Lin Q, Ma JX, Shi WW, Yang B, Li F. Long non-coding RNA NEAT1 acts as oncogene in NSCLC by regulating the Wnt signaling pathway. Eur Rev Med Pharmacol Sci 2017; 21: 504-510.

27) Mabuchi S, Kuroda H, Takahashi R, Sasano T. The PI3K/AKT/mTOR pathway as a therapeutic target in ovarian cancer. Gynecol Oncol 2015; 137: 173-179.

28) Fu JH, Yang S, Nan CJ, Zhou CC, Lu DQ, Li S, Mu HQ. MiR-182 affects renal cancer cell proliferation, apoptosis, and invasion by regulating PI3K/AKT/mTOR signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 351-357.

29) Peng W, Wang Z, Fan H. LncRNA NEAT1 Impacts cell proliferation and apoptosis of colorectal cancer via regulation of Akt signaling. Pathol Oncol Res 2017; 23: 651-656.

30) Bie Q, Sun C, Gong A, Li C, Su Z, Zheng D, Ji X, Wu Y, Guo Q, Wang S, Xu H. Non-tumor tissue derived interleukin-17B activates IL-17RB/AKT/beta-catenin pathway to enhance the stemness of gastric cancer. Sci Rep 2016; 6: 25447.

31) Liu J, Zhang Y, Xu R, Du J, Hu Z, Yang L, Chen Y, Zhu Y, Gu L. PI3K/Akt-dependent phosphorylation of GSK3β and activation of RhoA regulate Wnt5a-induced gastric cancer cell migration. Cell Signal 2013; 25: 447-456.

32) Wang P, Wu T, Zhou H, Jin Q, He G, Yu H, Xuan L, Wang X, Tian L, Sun Y, Liu M, Qu L. Long noncoding RNA NEAT1 promotes laryngeal squamous cell cancer through regulating miR-107/CDK6 pathway. J Exp Clin Cancer Res 2016; 35: 22.

33) Gong W, Zheng J, Liu X, Ma J, Liu Y, Xue Y. Knockdown of NEAT1 restrained the malignant progression of glioma stem cells by activating microRNA let-7e. Oncotarget 2016; 7: 62208-62223.

34) Wang M, Gu H, Qian H, Zhu W, Zhao C, Zhang X, Tao Y, Zhang L, Xu W. miR-17-5p/20a are important markers for gastric cancer and murine double minute 2 participates in their functional regulation. Eur J Cancer 2013; 49: 2010-2021.