

LINC01232 Promotes Metastasis and EMT by Regulating miR-506-5p/PAK1 Axis in Gastric Cancer

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Background: Long non-coding RNA LINC01232 plays an important role in the progression of metastasis in several cancers. However, the function of LINC01232 in gastric cancer is limited. Authors aimed to investigate the role and mechanism of LINC01232 in the metastasis of gastric cancer.

Methods: The expression levels and correlation of LINC01232, miR-506-5p, and PAK1 were analyzed by GEPIA or ENCORI, and the abundance of LINC01232 and miR-506-5p was measured in tissues and cells via qRT-PCR, the location of LINC01232 in gastric cells was analyzed by nuclear and cytoplasmic fractionation, while the protein levels of PAK1, E-cadherin and vimentin were additionally quantified by Western blotting. Interactions between LINC01232, miR-506-5p, and PAK1 were detected through luciferase reporter assays, qRT-PCR and Western blotting. Cellular viability was evaluated through CCK8 assays, migration ability was measured by transwell assays, invasion ability was tested by wound healing experiment.

Results: LINC01232 was overexpressed in gastric cancer tissues and cells, and mainly located in nucleus. The inhibition of LINC01232 could suppress migration, invasion and EMT of gastric cancer cells. MiR-506-5p was downregulated in gastric cancer tissues and cells. LINC01232 sponged miR-506-5p to accelerate migration and EMT. PAK1 was certified to be a target of miR-506-5p, inhibition of PAK1 could interrupt LINC01232 overexpression-induced migration of gastric cancer cells.

Conclusion: The LINC01232/miR-506-5p/PAK1 axis promotes metastasis of gastric cancer cells.

Keywords: gastric cancer, LINC01232, miR-506-5p, metastasis, PAK1

Introduction

Gastric cancer is the fifth most common tumor and the fourth death causing malignant tumor globally.¹ More than 1 million cases are diagnosed with gastric cancer, and there are nearly 800 thousand gastric cancer-related deaths each year. It is the most common diagnosed cancer in South Central Asia and Eastern Asia. The mortality rate of gastric cancer is the highest in South Central Asia.² Despite early detection, early therapy and the development of chemotherapy and molecular targeted therapy for gastric cancer, the prognosis remains not satisfied. Recurrence and metastasis are the main reasons for poor outcome of gastric cancer.³

Epithelial mesenchymal transition (EMT) is an important role for tumor invasion and metastasis. EMT is characterized by the inhibition of epithelial cell properties and behaviors, and the promotion of mesenchymal cell properties. A lot of researches demonstrate that loss of E-cadherin expression is a consequence of EMT.⁴ E-cadherin is a transmembrane protein, belongs to type I classical cadherin family. The main function of E-cadherin is to maintain cell-cell interactions.⁵ E-cadherin is involved in multiple cancer-related signaling pathways, such as EGFR pathway, Wnt/beta-catenin pathway, PI3K/Akt pathway, which promotes carcinogenesis in several cancers, including gastric cancer.⁶ The suppression of E-cadherin in gastric cancer could be regulated by epigenetic mechanisms, such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs), histone methylation and DNA methylation.⁷

LncRNAs, which are heterogeneous RNA transcripts longer than 200 nucleotides and with limited protein-coding potential, play crucial roles in cancer progression.⁸ They act as sponges for miRNAs and transcription factors to modulate gene expression, mRNA stability, RNA splicing and translation.^{9,10} LncRNAs not only act biomarkers for diagnosis and prognosis, but also affect chemoresistance, apoptosis, EMT, autophagy in gastric cancer.¹¹ A number of lncRNAs are identified to regulate EMT in gastric cancer. Liu et al revealed that lncRNA SNHG1 promoted EMT process in gastric cancer cells through regulation of the miR-15b/DCLK1/Notch1 axis.¹² Gong et al reported that lncRNA CHRF enhanced cell invasion and migration ability via EMT in gastric cancer.¹³ Overexpressed lncRNA GATA6-AS1 was found to suppress EMT via FZD4 through the Wnt/beta-catenin pathway in gastric cancer.¹²

LINC01232 is a 1002nt long lncRNA which is located in chromosome 13. Accumulating researches demonstrate that it is dysregulated and plays oncogenic role in several cancers. LINC01232 enhances the metastasis ability by activating A-Raf induced MAPK/ERK pathway in pancreatic cancer.^{14,15} Overexpression of LINC01232 is associated with poor prognosis in hepatocellular carcinoma.¹⁶ LINC01232 could sponge miR-204-5p to increase RAB22A expression and promote tumor progression in clear cell renal cell carcinoma.¹⁷ Recent study identified that LINC01232 promoted cell proliferation by interacting with EZH2 in gastric cancer.¹⁸ However, the function of LINC01232 on metastasis and the possible mechanism is still unclear.

In the current study, we planned to clarify the expression and function of LINC01232 in gastric cancer, and to explore the mechanism of LINC01232 in promoting progression in gastric cancer.

Materials and Methods

Tissue Specimens

Forty-six gastric adenocarcinoma frozen tissue samples and para-carcinoma tissue were collected from Hunan provincial people's hospital, between October 2013 and March 2015. The current study was performed with the approval of the Ethics Committee of the Faculty of Medicine, Hunan provincial people's hospital, and after obtaining informed consent from all patients. The experiments were carried out following the Declaration of Helsinki. Clinical parameters, including gender, age, tumor size, and metastasis, were collected. Patients included 15 females and 31 males, age ranging from 31 to 77 years; the mean and median ages were 57.2 and 61 years, respectively.

Cell Lines and Treatments

The following human gastric cancer cell lines obtained from Type Culture Collection of the Chinese Academy of Science (Shanghai, China) were used: gastric epithelium cell line GES-1, gastric cancer cell line AGS, BGC-823, HGC-27 and SGC-7901. GES-1 cells were cultured in RPMI1640 medium with 10% FBS. AGS cells were maintained in F12K medium (Sigma, USA) with 10% FCS (Gibco, Germany), and BGC823, HGC-27, SGC-7901 and 293T cells were maintained in RPMI-1640 with 10% FCS. Cells were cultured at 37°C in a humidified atmosphere of 5% CO₂ with 10% FCS.

Nuclear and Cytoplasmic Fractionation

The nuclear and cytoplasmic fractions of gastric cancer cells were extracted using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific) according to the manufacturer's protocol. The extracts were dissolved by TRIzol® reagent for RNA extract. The RNA was reverse transcribed and the expression of LINC01232 was analyzed by qRT-PCR.

Cloning of the Promoter, Plasmid Construction and Transfection

The whole sequences of LINC01232 were amplified from AGS cells, was digested by NdeI and BamHI. Then digested fragment was cloned to pcDNA3.1-EGFP to create vector pcDNA3.1-LINC01232 (LINC01232 OE). The upstream and downstream 100bp of predicted binding sites of LINC01232, and sequences containing the mutant of binding sites were synthesized from BGI Genomics (China), and then digested by HindIII and SpeI, and cloned to pMIR-report luciferase vector to construct pMIR-LINC01232-WT vector or pMIR-LINC01232-MUT vector. The 3' untranslated region (3'UTR)

of PAK1, and 3'UTR containing the mutant of binding sites were synthesized from BGI Genomics (China), and then digested by HindIII and SpeI, and cloned to pMIR-report luciferase vector to construct pMIR-PAK1-WT vector or pMIR-PAK1-MUT vector. The sequences of the cloned fragments were confirmed by sanger sequencing. Plasmids used for transfection were isolated and purified using the PURELINK PLASMID MINI 25 REACTIONS (Invitrogen–Life Technologies). The mimics or inhibitor of miR-506-5p was transfected using Lipofectamine 2000, scramble sequences were used as negative control. The binding of miR-506-5p and LINC01232, and the binding of miR-506-5p and PAK1 was tested by transient transfection in 293T cells using Lipofectamine 2000. For the luciferase-based assay, results were normalized against Renilla luciferase activity. At least three-independent assays were performed. The sequences of siRNA, negative control, miRNA-506-5p mimics and inhibitor were listed in [Supplementary Materials](#).

Cell Growth Assay

A number of 1×10^4 AGS and SGC-7901 cells were planted to each well in 96-well plate, and were transfected with siRNA, miRNA mimics or control sequences, overexpression vector or empty vector plasmid. Every group was duplicated for six wells. After incubated for 24, 48 and 72 hours, cell growth was detected using Cell Counting Kit-8 (Beyotime, China) in triplicate.

Wound Healing Assays

AGS cells were planted in 6-well plate and were transfected with Lipofectamine 2000 plus siRNA, miRNA mimics or control sequences, overexpression vector or empty vector plasmid. Cells were scratched using tips after transfected for 6 hours and the distance between two sides was assessed on 24 hours later. For each field, the distance of the wound borders was compared to the control. The ratio of cell migration was calculated.

Transwell Assays

The cell migration abilities of AGS and SGC-7901 was investigated using Transwell Chamber (8.0 μ m, Millipore, USA). Cells were seeded at 24-well upper uncoated chambers with serum-free medium for migration analysis. After incubation for 48h, the un-migrated cells in the upper chamber were wiped with a cotton swab. Cells were fixed with 4% paraformaldehyde, followed by staining with 1% Crystal Violet. The number of migrated cells was counted under a light microscope (Leica, Germany) ($\times 50$ magnification). At least three-independent assays were performed.

Quantitative Real-Time PCR Analysis (qRT-PCR)

The total RNA from the cells or tissues was extracted with TRIzol reagent (Life Technology). Reverse transcription was carried out using and qRT-PCR were conducted using SYBR green mix (Takara, China). The threshold cycle (CT) values were calculated, GAPDH was used as internal control for PAK1, U6 was used as internal control for LINC01232 and miR-506-5p. The qRT-PCR detection was using Bio-Rad CTF100 Real-Time PCR Detection System (USA). Amplification reactions were performed using the following profile: 95°C for 10 min, followed by 40 cycles at 95°C for 30s, 60°C for 30s and 72°C for 1 min. The expression level was measured using the formula $2^{-\Delta\Delta C_t}$. The sequences of primers are listed in [Supplementary Table](#).

Western Blotting

Cell lysates were prepared by homogenization of 5×10^5 cells in 100 μ L lysis buffer containing 1% phenylmethanesulfonyl fluoride (Beyotime, China) and 1:25 cocktail (Roche). Thirty microgram proteins and 7.5 μ L 4 $\times$ loading buffer were separated by 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes (GE Healthcare Biosciences, USA). The membranes were blocked in 10% TBST-milk buffer at room temperature for 1 hour, and incubated with antibodies against E-cadherin (#3195, Cell Signaling Technology, Inc), Vimentin (#5741, Cell Signaling Technology, Inc), PAK1 at the dilution of 1:1000. Then the membranes were incubated with anti-rabbit secondary antibodies (BA1054, Boster, China) at the dilution of 1:3000. Chemiluminescence was detected using an ECL Plus immunoblotting detection system (GE Healthcare Biosciences). GAPDH (#5174, Cell Signaling Technology, Inc) was the internal control.

Luciferase Reporter Assay

For luciferase reporter assay, 293T cells were plated in a 24-well plate and then co-transfected with 20 ng of either pMIR-WT, pMIR-MUT or control vector plasmids, and 2 ng of pRL-TK (Promega). Cells were collected 48 h after transfection and analyzed using the Dual-Luciferase Reporter Assay System (Promega). Luciferase activity was detected by M200 microplate fluorescence reader (Tecan, Beijing, China). Transfection experiments were performed in duplicates and repeated at least thrice in independent experiments.

Bioinformatics Analysis

The expression of LINC01232 in gastric cancer tissues and normal gastric mucosa TCGA dataset, the genes correlated with LINC01232 was screened, and the correlation between LINC01232 and PAK1 was analyzed by online software GEPIA (<http://gepia2.cancer-pku.cn/#index>). Then binding of LINC01232 to miRNAs was predicted using the online database ENCORI (<https://starbase.sysu.edu.cn/>). The correlation between LINC01232 and miR-506-5p expression was analyzed using ENCORI. Target genes of miR-506-5p was predicted using TargetScanHuman 8.0 (http://www.targetscan.org/vert_80/).

Statistical Analysis

All experiments were conducted in triplicate. Unless otherwise indicated, the data were reported as mean $\pm$ SD. Statistical significance of expression between two groups for independent unpaired samples were analyzed using Student's *t*-test. Categorical variables were analyzed by the χ^2 or Fisher exact tests. All statistical tests were two-sided. If P value is less than 0.05, there was statistically significant. The SPSS19.0 software was used. Kyoto Encyclopedia of Genes and Genomes (KEGG) was analyzed using KOBAS (<http://kobas.cbi.pku.edu.cn/genelist/>).

Results

LINC01232 is Overexpressed in Gastric Cancer Tissues and Cell Lines

The expression of LINC01232 in 408 gastric adenocarcinoma samples and 211 normal gastric mucosa from TCGA dataset was analyzed by online software GEPIA. LINC01232 was upregulated in gastric adenocarcinoma samples (Figure 1A). Then we detected the expression of LINC01232 in 46 gastric adenocarcinoma tissue samples and paired para-cancer tissues. The result was shown that the level of LINC01232 in gastric adenocarcinoma tissues was higher than the matched para-cancer tissues (Figure 1B). The expression of LINC01232 in gastric cancer cell lines was analyzed by qRT-PCR. Compared to GES-1 cells, the expression of LINC01232 was enhanced in all of the four selected gastric cancer cell lines (Figure 1C). In order to explore the function of LINC01232, we detect the location of LINC01232 in gastric cancer cells. The data showed that LINC01232 was major located in nucleus (Figure 1D).

Knockdown of LINC01232 Suppresses Cell Proliferation, Invasion, Migration and Epithelial Mesenchymal Transition (EMT)

In order to investigate the effects of LINC01232 on gastric cancer cells, we transfected AGS and SGC-7901 cells with LINC01232 siRNA and overexpressed vector plasmids (Figure 2A). Knockdown of LINC01232 could suppress cells proliferation, and overexpression of LINC01232 could promote cells proliferation in AGS and SGC-7901 cells detected by CCK8 assays (Figure 2B). After transfected with LINC01232 siRNA for 24h, the invasion ability of AGS and SGC-7901 cells was inhibited (Figure 2C, $P < 0.05$) analyzed by wound healing assays, while the migration of AGS and SGC-7901 cells was suppressed detected by transwell assays (Figure 2D, $P < 0.01$). When the expression of LINC01232 was inhibited, the expression of E-cadherin was downregulated, on the other side, the expression of Vimentin was upregulated in AGS and SGC-7901 cells (Figure 2E, $P < 0.05$). These data indicated that silencing of LINC01232 could suppress the proliferation, invasion, migration and EMT.

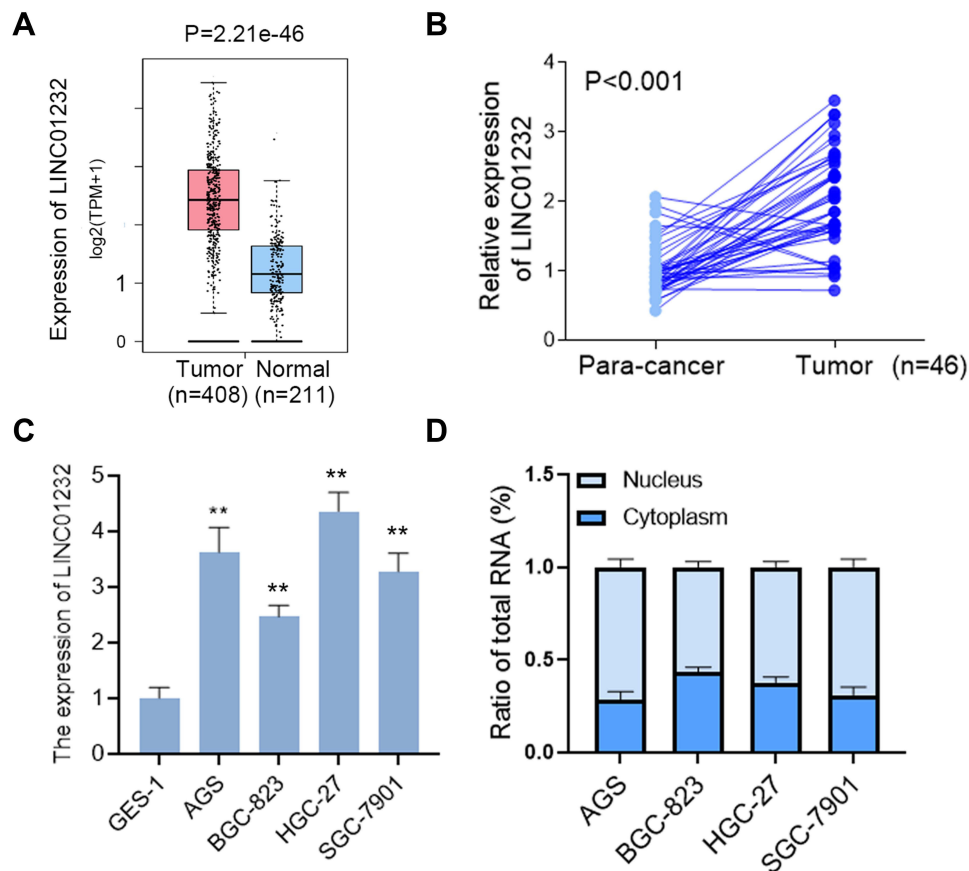


Figure 1 LINC01232 was overexpressed in gastric cancer: **(A)** The expression of LINC01232 in gastric cancer was analyzed using GEPIA. **(B)** The expression of LINC01232 in gastric cancer tissues and matched adjacent gastric mucosa was detected using qRT-PCR. **(C)** The expression of LINC01232 in normal gastric epithelial cells GES-1 and gastric cancer cell lines AGS, BGC-823, HGC-27 and SGC-7901 was detected using qRT-PCR, ** $P < 0.01$. **(D)** The expression of LINC01232 in cytoplasm and nucleus of gastric cancer cells was analyzed using nuclear and cytoplasmic fractionation combining with qRT-PCR.

LINC01232 Acts as a Sponge of miR-506-5p in Gastric Cancer Cells

As lncRNAs function as sponges of miRNAs, we predicted miRNAs which could bind to LINC01232 using online database ENCORI. MiR-506-5p was predicted to bind to LINC01232 (Figure 3A). In order to verify their binding, we mutated the binding sites to construct mutant luciferase vector (Figure 3A). Compared to the control group, the luciferase activity was decreased in LINC01232 wildtype group ($P < 0.05$); but it was not changed in LINC01232 mutant group (Figure 3B). The expression of miR-506-5p was significantly decreased in gastric cancer tissues compared to matched adjacent cancer tissues tested by qRT-PCR (Figure 3C). Compared to GES-1 cells, the expression of miR-506-5p was also decreased in AGS, BGC-823, HGC-27 and SGC-7901 cells (Figure 3D). Then the expression of LINC01232 and miR-506-5p was identified to be negatively correlated in gastric cancer tissues ($R^2 = 0.0139$, $P = 0.0108$) (Figure 3E). MiR-506-5p mimics was transfected to AGS and SGC-7901 cells to overexpress miR-506-5p (Figure 3F). When miR-506-5p was overexpressed, the expression of LINC01232 was remarkably decreased in AGS and SGC-7901 cells (Figure 3G, $P < 0.05$). However, when LINC01232 was inhibited, the expression of miR-506-5p was not affected in gastric cancer cells (Figure 3H).

LINC01232 Promotes Cell Migration and EMT Through Sponging miR-506-5p in Gastric Cancer Cells

The cell proliferation was significantly inhibited when miR-506-5p mimics was transfected with AGS and SGC-7901 cells on 48h and 72h (Figure 4A and B). In order to verify whether LINC01232 promote the progression of gastric cancer by sponging miR-506-5p, we constructed LINC01232 overexpressed vector, and transfected with AGS and SGC-7901

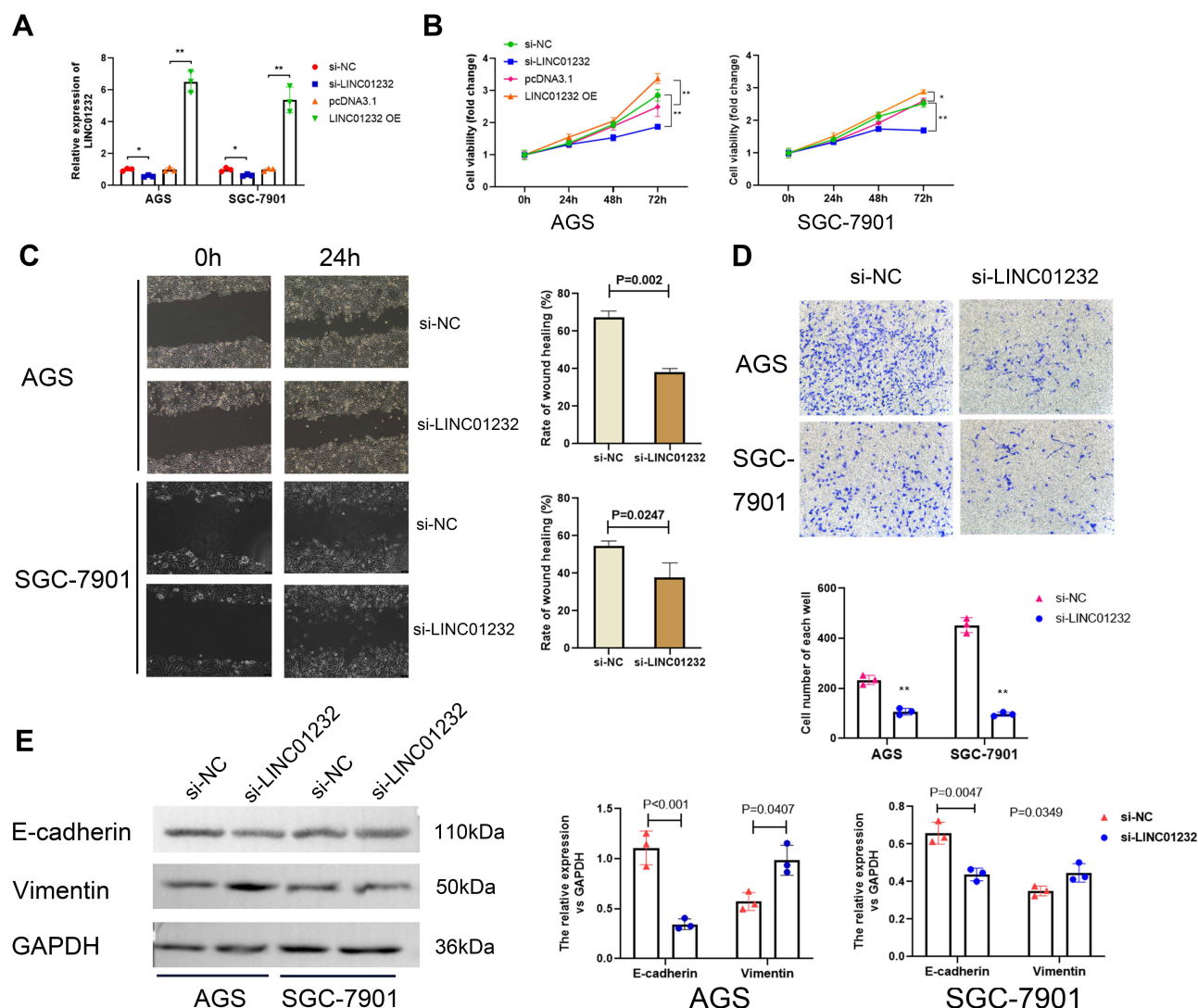


Figure 2 The effects of LINC01232 on gastric cancer cells. **(A)** The expression of LINC01232 was detected by qRT-PCR after gastric cells transfected with LINC01232 siRNA and overexpressing vector plasmids, si-NC and pcDNA3.1 plasmids were used as the control, * $P<0.05$, ** $P<0.01$. **(B)** The cell proliferation of AGS and SGC-7901 was analyzed using CCK8 assays, * $P<0.05$, ** $P<0.01$. **(C)** The invasion ability of AGS and SGC-7901 cells after treated with LINC01232 siRNA or overexpressing vector plasmids for 24h and 48h was tested by wound healing assays. **(D)** The migration ability of AGS and SGC-7901 cells after treated with LINC01232 siRNA or overexpressing vector plasmids for 24h and 48h was tested by transwell assays, ** $P<0.01$. **(E)** The protein levels of E-cadherin and vimentin in AGS and SGC-7901 cells transfected with LINC01232 siRNA or overexpressing vector plasmids was analyzed using Western blotting, GAPDH used as the internal control.

cells (Figure 4C). As the results of transwell assays showing, LINC01232 overexpression could promote cell migration, miR-506-6p mimics could suppress the migration induced by LINC01232 in gastric cancers cells ($P<0.05$, Figure 4D). MiR-506-6p could upregulated the expression of E-cadherin and downregulated the expression of Vimentin. Moreover, miR-506-5p could recover the expression of E-cadherin reduced by LINC01232 and decrease the expression of Vimentin induced by LINC01232 (Figure 4E).

MiR-506-6p Inhibits Gastric Cancer Cells by Targeting PAK I

For the sake of exploring the mechanism of LINC01232 inhibiting gastric cancer cells, we selected 1111 genes correlated with LINC01232 expression using GEPIA and predicted 4240 target genes of miR-506-5p using online software TargeScanHuman, then obtained the intersection of these two genes gathers which included 858 genes (Figure 5A). The selected genes were found to take part in several cancers related pathways using KEGG analysis. The cancer-related pathways included PI3K-Akt signaling pathway, pathways in cancer, focal adhesion (Figure 5B). PAK1 was predicted to

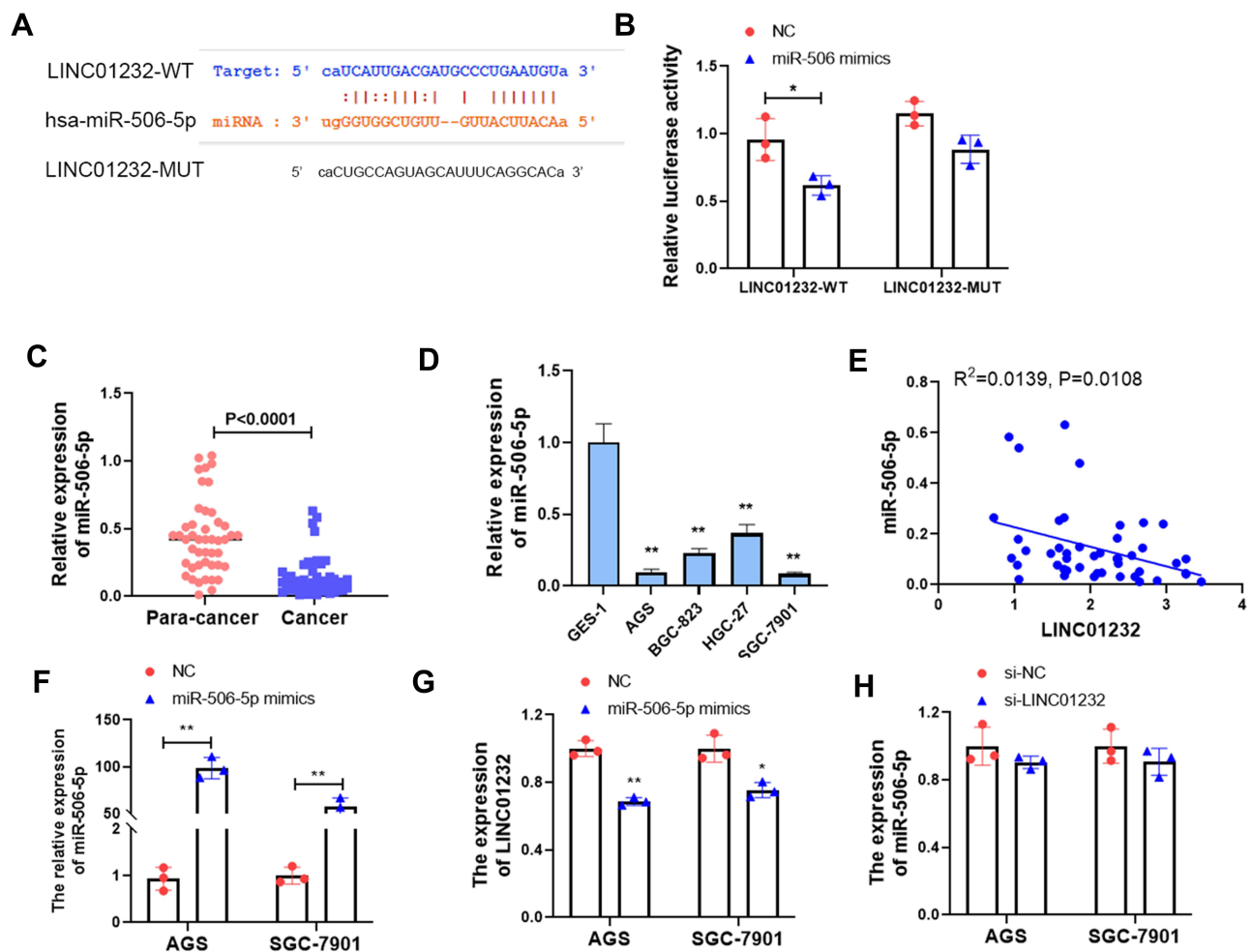


Figure 3 LINC01232 sponges miR-506-5p in gastric cancer cells. (A) The binding of LINC01232 and miR-506-5p was predicted by ENCORI, and the mutant of binding sites was listed. (B) The luciferase activity of 293T cells after transfected with miR-506-5p mimics and LINC01232 wildtype or mutant luciferase reporter vector plasmids was analyzed by luciferase reporter assays, $*P<0.05$. (C) The expression of miR-506-5p of gastric cancer tissues and matched para-cancer tissues was detected by qRT-PCR. (D) The expression of miR-506-5p in normal gastric epithelial cells GES-1 and gastric cancer cell lines AGS, BGC-823, HGC-27 and SGC-7901 was detected using qRT-PCR, $**P<0.01$. (E) The correlation between LINC01232 and miR-506-5p was analyzed using ENCORI. (F) The expression of miR-506-5p in AGS and SGC-7901 cells after transfected with miR-506-5p mimics was tested by qRT-PCR, NC was negative control, $**P<0.01$. (G) The expression of LINC01232 in AGS and SGC-7901 cells after transfected with miR-506-5p mimics was tested by qRT-PCR, NC was negative control, $*P<0.05$, $**P<0.01$. (H) The expression of miR-506-5p in AGS and SGC-7901 cells after transfected with LINC01232 siRNA was tested by qRT-PCR, si-NC was negative control.

be a miR-506-5p target by Targetscan (Figure 5C). The luciferase activity was decreased in wildtype group, but no effect was found in mutant group (Figure 5D). The expression of PAK1 mRNA (Figure 5E) and protein (Figure 5F) was downregulated by miR-506-5p mimics in AGS and SGC-7901 cells.

LINC01232 Sponge miR-506-5p to Release PAK1 in Gastric Cancer Cells

In order to investigate the effect of LINC01232 on PAK1, the expression correlation was analyzed using GEPIA database. The data was shown that the expression of LINC01232 and PAK1 was positively related in gastric cancer tissues ($R=0.29$, $P<0.001$) (Figure 6A). The expression of PAK1 mRNA (Figure 6B) and protein (Figure 6C) was increased by LINC01232 overexpression in AGS and SGC-7901 cells. In LINC01232 overexpressed gastric cells, inhibition of PAK1 could significantly suppress cell migration (Figure 6D and E).

Discussion

Increasing evidence shows that a class of lncRNAs are dysregulated in gastric cancer, and directly regulate EMT and metastasis process in gastric cancer. In this study, we analyzed LINC01232 expression using GEPIA online database, and

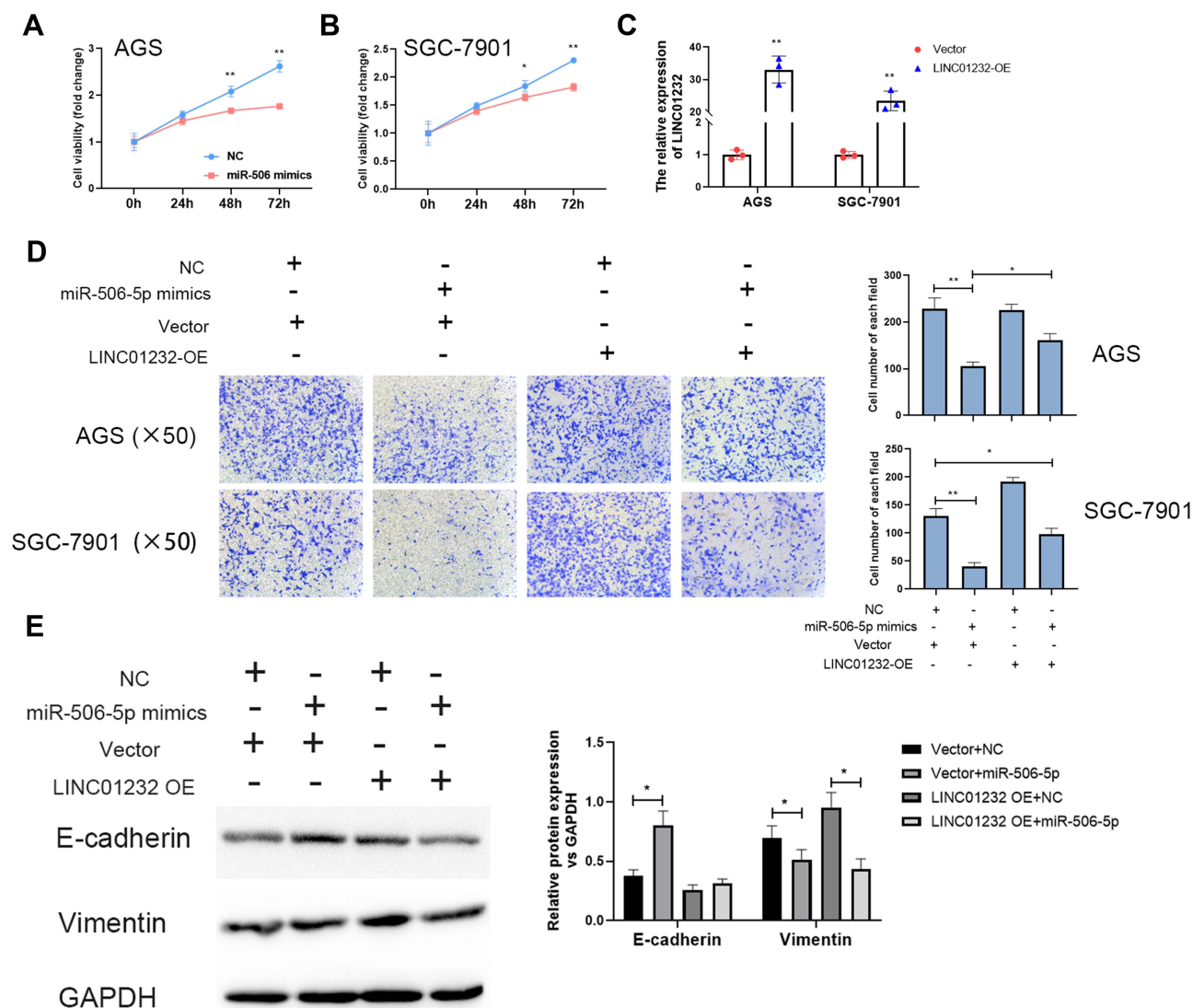


Figure 4 LINC01232 promotes proliferation, migration and EMT by sponging miR-506-5p in gastric cancer cells. **(A)** The effect of miR-506-5p on the proliferation of AGS cells was analyzed using CCK8 assay, $^{**}P<0.01$. **(B)** The effect of miR-506-5p on the proliferation of SGC-7901 cells was analyzed using CCK8 assay, $^{*}P<0.05$, $^{**}P<0.01$. **(C)** The expression of LINC01232 in AGS and SGC-7901 cells after transfected with LINC01232 overexpressing vector plasmids was tested by qRT-PCR, $^{**}P<0.01$. **(D)** The effect of LINC01232 and miR-506-5p on the migration ability of AGS and SGC-7901 cells was detected by transwell assays, $^{*}P<0.05$, $^{**}P<0.01$. **(E)** The effect of LINC01232 and miR-506-5p on the expression of E-cadherin and vimentin of AGS and SGC-7901 cells was detected by Western blotting, $^{*}P<0.05$, $^{**}P<0.01$.

detected its expression in gastric cancer tissues and para-cancer tissues using qRT-PCR. The results found that not only in data from TCGA but also in our selected samples, LINC01232 was upregulated in gastric cancer tissues compared to para-cancer tissues. These results were in accordance with reported studies by Liu et al.¹⁸

LINC01232 is revealed to act as an oncogene in several cancers. It can promote metastasis of pancreatic cancer,¹⁵ clear cell renal cell carcinoma¹⁷ and esophageal squamous cell carcinoma.¹⁹ However, the function of LINC01232 on the metastasis of gastric cancer has not been reported. In this study, we verified that silencing of LINC01232 suppressed invasion, migration and EMT ability in gastric cancer cells. That suggested that LINC01232 plays an important role in metastasis in multiple cancers including gastric cancer.

LncRNAs have been recommended to exhibit various functions including transcriptional regulation in *cis* or *trans*, and regulation of gene expression according to their location in cells.²⁰ Numerous evidence has shown that lncRNAs can interact with miRNAs. There are four mechanisms of interaction between lncRNAs and miRNAs. On one hand, lncRNAs act as miRNA sponges and competitive endogenous RNAs (ceRNAs) of miRNAs to hinder the binding of miRNAs to

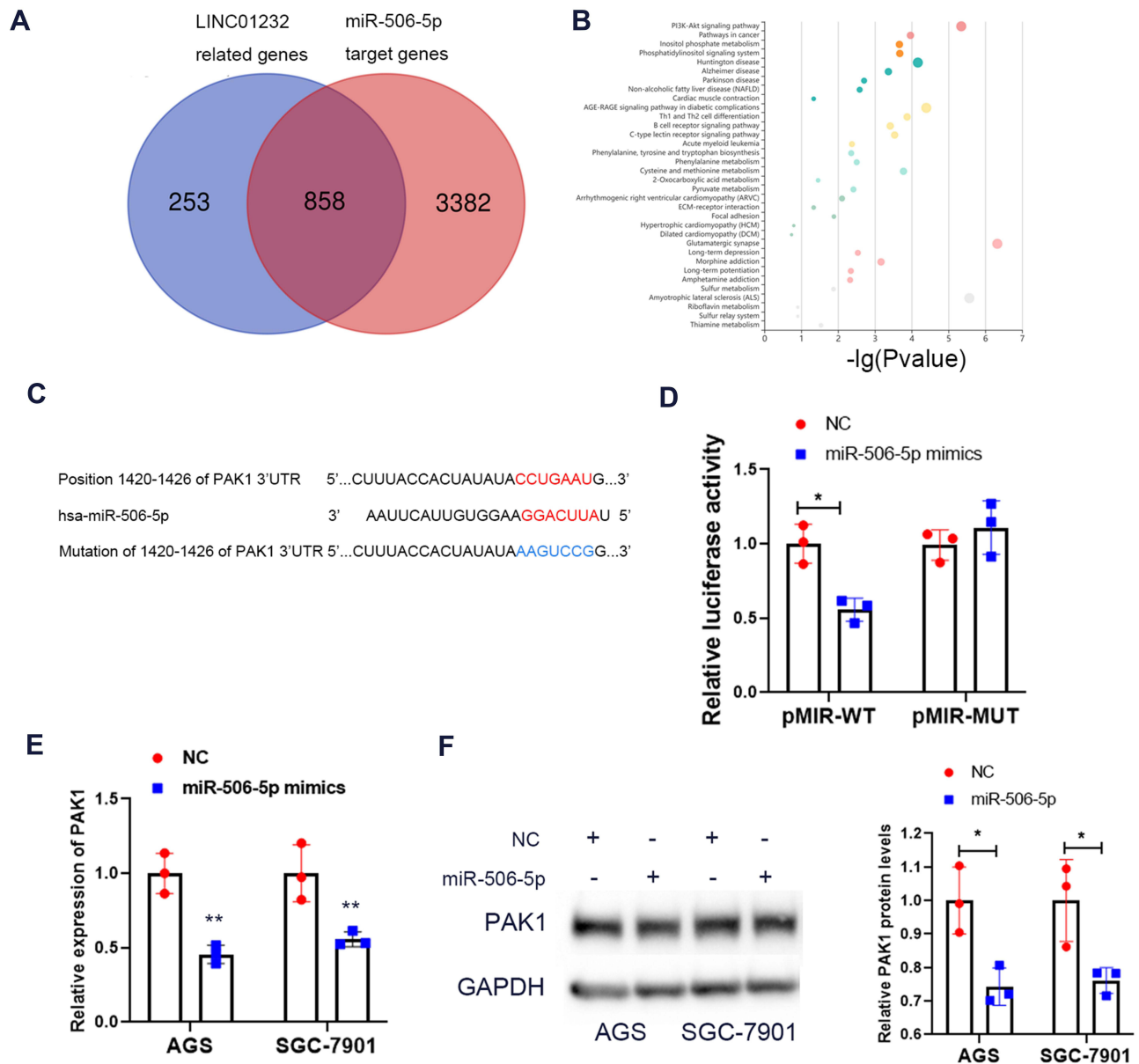


Figure 5 PAK1 is a target gene of miR-506-5p in gastric cancer cells. **(A)** The intersection of LINC01232 related genes and miR-506-5p target genes was selected by GEPIA and Targetscan. **(B)** The genes of intersection from results **(A)** was used to perform KEGG analysis using KOBAS. **(C)** The binding sites were predicted using TargetScan and was mutated. **(D)** The luciferase activity of 293T cells transfected with miR-506-5p mimics and pMIR-PAK1-VT vector or pMIR-PAK1-MUT was analyzed by luciferase reporter assays, * $P < 0.05$. **(E)** The effect of miR-506-5p mimics on the expression of PAK1 mRNA was detected using qRT-PCR, ** $P < 0.01$. **(F)** The effect of miR-506-5p mimics on the expression of PAK1 protein was detected using Western blotting, * $P < 0.05$.

target genes.²¹ LncRNA HAGLR sponges miR-338-3p to accelerate 5-Fu resistance in gastric cancer via targeting the LDHA-glycolysis pathway.²² The ceRNA network formed by lncRNA-miRNA-mRNA plays a crucial part in several cancers including gastric cancer.²³ LINC00240 promotes gastric cancer progression through regulating the miR-338-5p/METTL3 axis.²⁴ In gastric cancer cells, LINC01050 is activated by c-Myc and facilitate metastasis by sponging miR-7161-3p to upregulate SPZ1.²⁵ LncRNA SLCO4A1-AS1 accelerates metastasis of gastric cancer through modulating the miR-149/XIAP axis.²⁶ RNF144A-AS1 promotes metastasis in gastric cancer by targeting the miR-30c-2-3p/LOX axis.²⁷ On the other hand, lncRNAs could directly compete with miRNAs to bind with target mRNAs. DICER-AS1 acts as ceRNA to target CSR1 by sponging microRNA-650 to obstruct gastric cancer progression.²⁸ Still, miRNAs decrease the stability of lncRNAs.²⁹ A previous study reported that overexpression of let-7b facilitate the degradation of RNA,

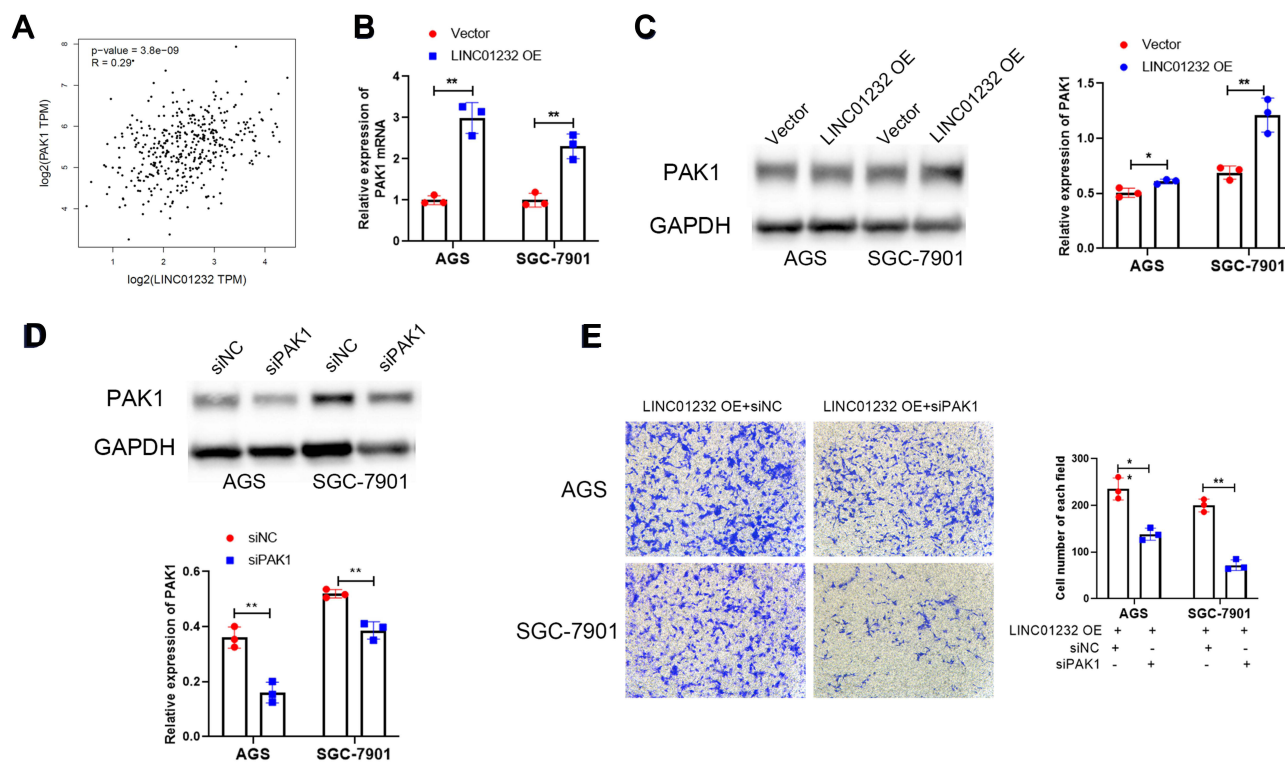


Figure 6 LINC01232 promotes migration of gastric cancer cells by inducing PAK1 expression. **(A)** The correlation between LINC01232 and PAK1 in gastric cancer tissues was analyzed using GEPIA. **(B)** The effect of LINC01232 on PAK1 mRNA expression in AGS and SGC-7901 cells was detected by qRT-PCR, ** $P < 0.01$. **(C)** The effect of LINC01232 on PAK1 protein levels in AGS and SGC-7901 cells was detected by qRT-PCR, * $P < 0.05$, ** $P < 0.01$. **(D)** The effect of PAK1 siRNA on PAK1 protein levels in AGS and SGC-7901 cells was detected by qRT-PCR, ** $P < 0.01$. **(E)** The effect of PAK1 siRNA on cell migration ability of LINC01232 overexpressed AGS and SGC-7901 cells was analyzed using transwell assays, ** $P < 0.01$.

inducing the instability of lncRNA-p21.³⁰ In addition, lncRNAs could generate miRNAs. It has been verified that lncRNA H19 could generate miR-675.³⁰ To achieve a better understanding of the molecular mechanisms of LINC01232-mediated network in the metastasis of gastric cancer, we predicted miRNAs which may bind to LINC01232, and selected miR-506-5p as a potential interactive miRNA. Then we used luciferase reporter assay and qRT-PCR to certify that miR-506-5p could bind to LINC01232 and suppress LINC01232 expression. However, we did not discover any effect of LINC01232 on miR-506-5p expression. Our data displayed that LINC01232 could bind to miR-506-5p and act as a ceRNA.

Limited evidence showed that miR-506-5p act as a tumor suppressor and interact with lncRNAs in gastric cancer³¹ and glioma.³² For instance, lncRNA SNHG15 promotes gastric cancer tumorigenesis by impairing miR-506-5p expression.³¹ FOXD2-AS1 facilitates cell proliferation, metastasis and EMT in glioma by sponging miR-506-5p.³² In this study, we demonstrated that overexpression of miR-506-5p could inhibit the proliferation, migration and EMT of gastric cancer cells, while enhanced LINC01232 abundance could interrupt the tumor suppressor effect of miR-506-5p. These results further confirmed that LINC01232 act as a ceRNA in gastric cancer cells.

For the sake of determining targets of LINC01232/miR-506-5p axis, we analyzed LINC01232 expression-related genes using GEPIA and predicted miR-506-5p target genes using TargetScan, then intersected these two groups. After that, the intersection was performed KEGG analysis. Genes associated with focal adhesion were concerned. P21-activated kinase-1 (PAK1) is a serine/threonine kinase. Accumulated studies reported that PAK1 played key role in cell motility, cytoskeletal remodeling and apoptosis, it also participated in progression of cancers, especially in metastasis and EMT. Pak1 phosphorylates the serine 62 residue of ATF2 and blocks its translocation into the nucleus, then decreases the expression of miR-132, resulting in hematogenous metastasis of gastric cancer cells.³³ PAK1 facilitates EMT through upregulating Snail in hepatocellular carcinoma.³⁴ Several studies reported that PAK1 was regulated by tumor suppressor miRNAs in cancers. MiR-331-3p suppresses EMT by downregulating ErbB2 and VAV2 via Rac1/PAK1/beta-catenin axis

in non-small cell lung cancer.³⁵ MiR-485-5p reverses EMT and induces cisplatin-induced cell death by inhibiting PAK1 in oral tongue squamous cell carcinoma.³⁶ Furthermore, PAK1 is modulated by lncRNA. LncRNA-H19 promotes migration and invasion by targeting miR-15b to activate CDC42/PAK1 axis in hepatocellular carcinoma.³⁷ In our study, PAK1 was certified as a target of miR-506-5p, its expression was positively related to LINC01232 in gastric cancer tissues. LINC01232 could enhance the expression of PAK1, suppression of PAK1 could obstructed LINC01232-induced migration in gastric cancer cells.

Our study first discovered that LINC01232 was an important promoter for migration, EMT, and its overexpression could sponge miR-506-5p to relieve PAK1 to facilitate metastasis in gastric cancer cells. But in our study, the binding sites of LINC01232 to miR-506-5p was not discovered, and the signaling pathways mediated by LINC01232 and PAK1 was not clarified. These questions should be studied in the future. Taken together, our studies establish a novel lncRNA-miRNA-mRNA pathway and supply new potential target for therapeutic intervention of gastric cancer.

Conclusion

In summary, LINC01232 was overexpressed in gastric cancer tissues and cells, and acted as a promoter of migration, invasion and EMT. It sponged miR-506-5p to recover the expression of PAK1 to facilitate migration in gastric cancer cells.

Disclosure

The authors declare no conflicts of interest in this work.

References

1. Wei L, Sun J, Zhang N, et al. Noncoding RNAs in gastric cancer: implications for drug resistance. *Mol Cancer*. 2020;19(1):62. doi:10.1186/s12943-020-01185-7
2. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660
3. Wei Z, Chen L, Meng L, Han W, Huang L, Xu A. LncRNA HOTAIR promotes the growth and metastasis of gastric cancer by sponging miR-1277-5p and upregulating COL5A1. *Gastric Cancer*. 2020;23(6):1018–1032. doi:10.1007/s10120-020-01091-3
4. Nieto MA, Huang RY, Jackson RA, Thiery JP. EMT: 2016. *Cell*. 2016;166(1):21–45. doi:10.1016/j.cell.2016.06.028
5. Bure IV, Nemtsova MV, Zaletaev DV. Roles of E-cadherin and noncoding RNAs in the epithelial-mesenchymal transition and progression in gastric cancer. *Int J Mol Sci*. 2019;20(12):2870. doi:10.3390/ijms20122870
6. Xu E, Xia X, Jiang C, et al. GPER1 silencing suppresses the proliferation, migration, and invasion of gastric cancer cells by inhibiting PI3K/AKT-Mediated EMT. *Front Cell Dev Biol*. 2020;8:591239. doi:10.3389/fcell.2020.591239
7. Cui H, Hu Y, Guo D, et al. DNA methyltransferase 3A isoform b contributes to repressing E-cadherin through cooperation of DNA methylation and H3K27/H3K9 methylation in EMT-related metastasis of gastric cancer. *Oncogene*. 2018;37(32):4358–4371. doi:10.1038/s41388-018-0285-1
8. Fabbri M, Girmila L, Varani G, Calin GA. Decrypting noncoding RNA interactions, structures, and functional networks. *Genome Res*. 2019;29(9):1377–1388. doi:10.1101/gr.247239.118
9. Yao RW, Wang Y, Chen LL. Cellular functions of long noncoding RNAs. *Nat Cell Biol*. 2019;21(5):542–551. doi:10.1038/s41556-019-0311-8
10. Chen D, Ping S, Xu Y, et al. Non-coding RNAs in gastric cancer: from malignant hallmarks to clinical applications. *Front Cell Dev Biol*. 2021;9:732036. doi:10.3389/fcell.2021.732036
11. Yuan L, Xu ZY, Ruan SM, Mo S, Qin JJ, Cheng XD. Long non-coding RNAs towards precision medicine in gastric cancer: early diagnosis, treatment, and drug resistance. *Mol Cancer*. 2020;19(1):96. doi:10.1186/s12943-020-01219-0
12. Liu ZQ, He WF, Wu YJ, et al. LncRNA SNHG1 promotes EMT process in gastric cancer cells through regulation of the miR-15b/DCLK1/Notch1 axis. *BMC Gastroenterol*. 2020;20(1):156. doi:10.1186/s12876-020-01272-5
13. Gong J, Wang Y, Shu C. LncRNA CHRF promotes cell invasion and migration via EMT in gastric cancer. *Eur Rev Med Pharmacol Sci*. 2020;24(3):1168–1176. doi:10.26355/eurrev_202002_20168
14. Li Q, Lei C, Lu C, Wang J, Gao M, Gao W. LINC01232 exerts oncogenic activities in pancreatic adenocarcinoma via regulation of TM9SF2. *Cell Death Dis*. 2019;10(10):698. doi:10.1038/s41419-019-1896-3
15. Meng LD, Shi GD, Ge WL, et al. Linc01232 promotes the metastasis of pancreatic cancer by suppressing the ubiquitin-mediated degradation of HNRNPA2B1 and activating the A-Raf-induced MAPK/ERK signaling pathway. *Cancer Lett*. 2020;494:107–120. doi:10.1016/j.canlet.2020.08.001
16. Guo L, Gao S, Sun W, Wang Y, Zhao J. Elevated LINC01232 is associated with poor prognosis and HBV infection in hepatocellular carcinoma patients and contributes to tumor progression in vitro. *Clin Res Hepatol Gastroenterol*. 2021;46:101813. doi:10.1016/j.clinre.2021.101813
17. Liu Q, Lei C. LINC01232 serves as a novel biomarker and promotes tumour progression by sponging miR-204-5p and upregulating RAB22A in clear cell renal cell carcinoma. *Ann Med*. 2021;53(1):2153–2164. doi:10.1080/07853890.2021.2001563
18. Liu J, Li Z, Yu G, Wang T, Qu G, Wang Y. LINC01232 promotes gastric cancer proliferation through interacting with EZH2 to inhibit the transcription of KLF2. *J Microbiol Biotechnol*. 2021;31(10):1358–1365. doi:10.4014/jmb.2106.06041
19. Zhao M, Cui H, Zhao B, Li M, Man H. Long intergenic noncoding RNA LINC01232 contributes to esophageal squamous cell carcinoma progression by sequestering microRNA6543p and consequently promoting hepatoma derived growth factor expression. *Int J Mol Med*. 2020;46(6):2007–2018. doi:10.3892/ijmm.2020.4750

20. Kopp F, Mendell JT. Functional classification and experimental dissection of long noncoding RNAs. *Cell*. 2018;172(3):393–407. doi:10.1016/j.cell.2018.01.011
21. Xiong G, Pan S, Jin J, et al. Long noncoding competing endogenous RNA networks in pancreatic cancer. *Front Oncol*. 2021;11:765216. doi:10.3389/fonc.2021.765216
22. Hu J, Huang L, Ding Q, Lv J, Chen Z. Long noncoding RNA HAGLR sponges miR-338-3p to promote 5-Fu resistance in gastric cancer through targeting the LDHA-glycolysis pathway. *Cell Biol Int*. 2021;46(2):173–184.
23. Landeros N, Santoro PM, Carrasco-Avino G, Corvalan AH. Competing endogenous RNA networks in the epithelial to mesenchymal transition in diffuse-type of gastric cancer. *Cancers*. 2020;12(10):2741. doi:10.3390/cancers12102741
24. Wang G, Zhang Z, Xia C. Long non-coding RNA LINC00240 promotes gastric cancer progression via modulating miR-338-5p/METTL3 axis. *Bioengineered*. 2021;12(2):9678–9691. doi:10.1080/21655979.2021.1983276
25. Ji Z, Tang T, Chen M, et al. C-Myc-activated long non-coding RNA LINC01050 promotes gastric cancer growth and metastasis by sponging miR-7161-3p to regulate SPZ1 expression. *J Exp Clin Cancer Res*. 2021;40(1):351. doi:10.1186/s13046-021-02155-7
26. Fang Y, Sun B, Gao J, Huang Y, Wang C. LncRNA SLCO4A1-AS1 accelerates growth and metastasis of gastric cancer via regulation of the miR-149/XIAP Axis. *Front Oncol*. 2021;11:683256. doi:10.3389/fonc.2021.683256
27. Li Z, Shi L, Li X, Wang X, Wang H, Liu Y. RNF144A-AS1, a TGF-beta1- and hypoxia-inducible gene that promotes tumor metastasis and proliferation via targeting the miR-30c-2-3p/LOX axis in gastric cancer. *Cell Biosci*. 2021;11(1):177. doi:10.1186/s13578-021-00689-z
28. An J, Guo X, Yan B. DICER-AS1 functions as competing endogenous RNA that targets CSR1 by sponging microRNA-650 and suppresses gastric cancer progression. *J Int Med Res*. 2021;49(9):3000605211041466. doi:10.1177/03000605211041466
29. Zheng YL, Song G, Guo JB, et al. Interactions among lncRNA/circRNA, miRNA, and mRNA in musculoskeletal degenerative diseases. *Front Cell Dev Biol*. 2021;9:753931. doi:10.3389/fcell.2021.753931
30. Deng K, Wang H, Guo X, Xia J. The cross talk between long, non-coding RNAs and microRNAs in gastric cancer. *Acta Biochim Biophys Sin*. 2016;48(2):111–116. doi:10.1093/abbs/gmv120
31. Chen Z, Zhong T, Li T, et al. LncRNA SNHG15 modulates gastric cancer tumorigenesis by impairing miR-506-5p expression. *Biosci Rep*. 2021;41(7). doi:10.1042/BSR20204177
32. Zhao J, Zeng XB, Zhang HY, Xiang JW, Liu YS. Long non-coding RNA FOXD2-AS1 promotes cell proliferation, metastasis and EMT in glioma by sponging miR-506-5p. *Open Med*. 2020;15(1):921–931. doi:10.1515/med-2020-0175
33. Liu F, Cheng Z, Li X, et al. A novel Pak1/ATF2/miR-132 signaling axis is involved in the hematogenous metastasis of gastric cancer cells. *Mol Ther Nucleic Acids*. 2017;8:370–382. doi:10.1016/j.omtn.2017.07.005
34. Cao F, Yin LX. PAK1 promotes proliferation, migration and invasion of hepatocellular carcinoma by facilitating EMT via directly up-regulating Snail. *Genomics*. 2020;112(1):694–702. doi:10.1016/j.ygeno.2019.05.002
35. Li X, Zhu J, Liu Y, Duan C, Chang R, Zhang C. MicroRNA-331-3p inhibits epithelial-mesenchymal transition by targeting ErbB2 and VAV2 through the Rac1/PAK1/beta-catenin axis in non-small-cell lung cancer. *Cancer Sci*. 2019;110(6):1883–1896. doi:10.1111/cas.14014
36. Lin XJ, He CL, Sun T, Duan XJ, Sun Y, Xiong SJ. hsa-miR-485-5p reverses epithelial to mesenchymal transition and promotes cisplatin-induced cell death by targeting PAK1 in oral tongue squamous cell carcinoma. *Int J Mol Med*. 2017;40(1):83–89. doi:10.3892/ijmm.2017.2992
37. Zhou Y, Fan RG, Qin CL, Jia J, Wu XD, Zha WZ. LncRNA-H19 activates CDC42/PAK1 pathway to promote cell proliferation, migration and invasion by targeting miR-15b in hepatocellular carcinoma. *Genomics*. 2019;111(6):1862–1872. doi:10.1016/j.ygeno.2018.12.009

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