LncRNA SNHG16 Contributes to Tumor Progression via miR-302b-3p/SLC2A4 in Pancreatic Cancer

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Research

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Abstract

Background: It is reported that lncRNA SNHG16 is significantly highly expressed in pancreatic cancer (PC). However, the functions and mechanisms of SNHG16 are not clear. The aim of this study was to explore the effects of SNHG16 in PC.

Methods: qRT-PCR analyze was applied to detect the expression levels of SNHG16, miR-302b-3p and SLC2A4 in PC tissues and cells. CCK8 and EdU assays were used to determine the proliferation of PC cells. Transwell assay were used to measure the capacities of PC cells migration and invasion. Apoptosis were evaluated by flow cytometry, and the expression of apoptosis related proteins (including Bax, Bcl-2, cleaved caspase-3 and cleaved caspase-9), which were tested by western blot. The interactions between miR-302b-3p and SNHG16 or miR-302b-3p and SLC2A4 mRNA 3'UTR were clarified by Dual luciferase reporter assay and RNA immunoprecipitation.

Results: SNHG16 was significantly elevated in PC tissues and cell lines, which was associated with poor prognosis of PC patients. Knockdown of SNHG16 reduced PC cells proliferation, migration and invasion. SNHG16 acted as a sponge to regulate miR-302b-3p expression in PC cells. And miR-302b-3p targeted SLC2A4 directly.

Conclusions: SNHG16 promoted the progression of PC via miR-302b-3p/SLC2A4 axis and was expected to be a potential target for early diagnosis and treatment of PC.

Introduction

Pancreatic cancer (PC) is one of the most severe gastrointestinal malignancies, which is the fourth most common cause of cancer-related deaths[1]. PC is characterized by the rapid, atypical and advanced disease progression[2]. As a result of a biological process involving multiple steps, there is no clinically sensitive early diagnosis indicator or effective treatment point[3]. Therefore, the research for diagnostic markers and targeted therapies to gradually inhibit the progression of PC has become the focus of attention in the treatment of PC.

It has been proved that only 2% of the genome sequence is capable of coding proteins, whereas more than 95% transcripts are identified as non-coding RNA[4]. Long non-coding RNAs (lncRNAs) is a class of non-coding RNA which is featured with no protein coding ability and longer than 200 nt. Emerging evidences demonstrate that lncRNA involved in multifarious malignant cancers, including PC[5–7]. For example, lncRNA HOTTIP contributes to PC to enhance the Wnt/β-catenin pathway via binding to WDR5[8]. However, it was reported that LINC01197 was down-regulated in PC tissues, which inactivated Wnt/β-catenin pathway through interfering with β-catenin binding to TCF4 in PC cells[9].

Recently, there has gradually been discovered in the study of small nucleolar RNA host gene 16 (SNGH16) in tumorigenesis of many cancer types[10, 11]. And SNHG16 was also widely regarded as an essential

oncogene[12]. In hepatocellular carcinoma, SNHG16 was high expressed in HCC-resistant tissues and promoted HCC cells viability and autophagy, suppressed apoptosis via regulating miR-23b-3p/EGR1 pathway[13]. However, the exact roles of SNHG16 in PC have not been clarified yet.

In this study, we purposed to explore the roles of lncRNA SNHG16 functionally and mechanically impacting on PC. By detecting SNHG16 expression in paired PC tissues and cell lines, we found lncRNA SNHG1 was significantly overexpressed in PC. In addition, elevated SNHG1 exhibited an oncogenic role in PC cell proliferation, migration, invasion and apoptosis. Mechanistic investigations revealed that SNHG16 served as a sponge of miR-302b-3p, which reversed SNHG16-induced proliferation by targeting SLC2A4 in PC.

Materials And Methods

Tissue collection

PC samples and corresponding adjacent non-cancerous tissues are collected from PC patients who received a surgical operation at the First Hospital of Lanzhou University. The study was approved by the ethics committee of the First Hospital of Lanzhou University, and all included patients signed informed consent.

Cell culture

The human normal pancreatic duct epithelial cell line (HPY-Y5) and pancreatic cancer cell lines (including BxPC3, Panc-1, MIA Paca-2 and SW1990) are all bought in the Chinese Academy of Sciences, Shanghai, China. All PC cells are cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) containing with 10% fetal bovine serum (Gibco, USA) in a sterile incubator of 5% CO_2, 37 °C.

RNA isolation, reverse transcription and qRT-PCR assay

Total RNA was extracted from tumor tissues and cells by TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. cDNAs are produced by the reverse transcript kit from Takara (Dalian, China). SNHG16 and SLC2A4 expression was detected by using SYBR Green real-time PCR Kit (Qiagen, Germany). miR-302b-3p expression was analyzed by Hairpin-it TM miRNAs qPCR kit (GenePharma, China). The data of mRNA and miRNA are presented as the fold change in mRNA/miRNA abundance normalized against the β-actin or U6 snRNA. The primer sequences used in this study are presented in Table 1.

Table 1
Primer sequences for qRT-PCR

Genes		Primer sequences
SNHG16	F	5′-CAGAATGCCATGGTTTCCCC-3′
	R	5′-TGGCAAGAGACTTCCTGAGG-3′
miR-302b-3p	Loop	5'-GTCGTATCCAGTCCAGGGACCGAGGACTGGATACGACCTACTA-3'
	F	5'-GCGTAAGTGCTTCCATGTT-3'
	R	5'-TCCAGGGACCGAGGA-3'
SLC2A4	F	5'-TGGCTGGGTTCTCCAACTG-3'
	R	5'-CTGGAAACCCATGCCAATG-3'
β-actin	F	5'-ACCGAGCGCGGCTACAG-3'
	R	5'-CTTAATGTCACGCACGATTTCC-3'
U6 snRNA	F	5' -CTCGCTTCGGCAGCACA-3'
	R	5'-AACGCTTCACGAATTTGCGT-3'

Plasmids construction and cell transfection

The miR-302b-3p mimics, inhibitors, small hairpin RNA of SLC2A4 (sh-SLC2A4) and corresponding negative control (NC mimics or sh-NC) are purchased from RiboBio, Guangzhou, China. The inhibitors and shRNAs were transfected into BxPC3 and Panc-1 cells using Lipofectamine 3000 (Invitrogen, USA) according to the manufacturer's directions. The transfection efficiencies were detected at 48 hours post transfection by qRT-PCR. Transfected cells were converged for the subsequent experiments.

Construction of stably transfected cells

The lentiviral particles of LV-shSNHG16 and LV-sh-NC were purchased from GenePharma, China. The lentivirus was used directly to infect BxPC3 and Panc-1 cells with the addition of 8 μg/ml polybrene (Sigma-Aldrich) to construct the stably transfected cells. Puromycin (Invitrogen, USA) was added to the media 48 h after infection and maintained for 2 weeks to select stably transfected cells (BxPC3/Panc-1-sh-SNHG16 or BxPC3/Panc-1-sh-NC).

EdU assay

The regents used in the EdU assay was purchased from Ribobio (Guangzhou, China). PC cells were sowed in confocal dishes at a density of 3×10^5. The 4% paraformaldehyde (Beyotime, China) was used to fix the cells for 10 min. The 1% triton (Beyotime, China) was used to transparent the cells for 5 min

after washing three times with PBS. Subsequently, the cells were incubated with dyeing agent for 30 min in the dark, following stained with DAPI (Olympus, Tokyo, Japan) and incubated for 5 min at 37 °C. The images were trapped using a microscopy with a magnification of 400×.

Wound healing assay

PC cells were seeded in 6-well plates and grown to 70-80% confluence. The wound was scraped by 100 μl pipette tips. The remaining cells were cultured in serum-free DMEM medium for 48 h. Images of migration were trapped at 0 and 48 h after scraping.

Migration and invasion assays

Cells were suspended in serum-free DMEM medium. 100 μl of the cell suspension was seeded on the upper part of a 24-well culture plate with an 8 μm transwell chamber (Corning, USA) for 48 h, and 500 μl of DMEM medium supplemented with 10% FBS was added to the lower chamber. The chambers uniformly covered with Matrigel (BD Biosciences) are used to invasion assay. The transwell chambers were fixed in 4% paraformaldehyde for 15 min and stained with 0.1% crystal violet for 30 min at room temperature. Then, cells above the chambers were wiped off by a cotton swab. And stained cells were washed with PBS and observed with a microscope. The capacities of cell migration and invasion were assessed with average stained cells in 5 areas.

Luciferase reporter assay

The full-length of SNHG16 (WT or Mut) is synthesized and cloned into the pGL3-promoter vectors (Promega, USA). Dual luciferase reporter activities are conducted in BxPC3 and PANC-1 cells co-transfected with pGL3-promoter-SNHG16 (WT or Mut) and NC mimics or miR-302b-3p mimics. The luciferase activity was analyzed by the dual Luciferase reporter assay system (Promega) according to the manufacturer's instruction.

Western Blot analysis

Total proteins were extracted from cells with RIPA (Beyotime, China). The concentration of total proteins was texted by BCA protein assay kit (Beyotime, China). 30 μg of total proteins was split by 6%, 8% or 10% SDS-PAGE gels and then transferred to PVDF membrane (Millipore, USA). After blocked with 5% skim milk for 2 h, the membrane was incubated overnight with a specific primary antibody at 4 ℃. The specific primary antibodies (including Bax, Bcl-2, Cleaved caspase-3, Cleaved caspase-9) were purchased from Proteintech Group and the concentration of these antibodies applied in this study was 1:500. The specific primary antibodies (including ICAM-1, VCAM-1 and MMP9) were purchased from Abcam, the concentration of these antibodies applied in this study was 1:1000. Next, the membrane was incubated with corresponding secondary antibodies for 2 h at room temperature. The signals at protein level are visualized with the ECL western blotting substrate (Tanon, Shanghai, China).

Statistical analysis

All statistical analyses were accomplished with SPSS 22.0 software (Chicago, USA) and Prism version 7.0 software (California, USA). Data are presented as the mean ± SEM. And each experiment was repeated at least three times independently. An unpaired *t*-test was performed to compare the mean of two groups. The one-way ANOVA and Bonferroni multiple comparison test were applied to analyze the differences between two or more groups. Spearman correlation analysis was performed to detect the statistical dependence between SNHG16, miR-302b-3p and SLC2A4 levels. Significant differences were defined as P 0.05.

Results

LncRNA SNHG16 is elevated in PC tissues and cell lines

In this study, the expression level of SNHG16 in human PC tissues was firstly evaluated by qRT-PCR. Compared with the pair-matched adjacent normal samples, SNHG16 expression was significantly increased in PC tissues (Fig. 1A). Moreover, the expression level of SNHG16 was up-regulated in four PC cell lines BxPC3, Panc-1, MIA Paca-2 and SW1990, compared with HPY-Y5 (Fig. 1B). In addition, Kaplan-Meier analysis indicated that PC patients with increased SNHG16 shown a shorter over-all survival than those with decreased SNHG16 (Fig. 1C). The above results indicate that elevated SNHG16 might be a critical role in the progression of PC.

SNHG16 affects PC cells proliferation and apoptosis

In order to explore the roles of SNHG16 in PC process, we established BxPC3 and Panc-1 cells that stably silenced SNHG16, which were named sh-SNHG16 (Fig. 2A). Next, CCK8 and EdU assays were employed to determinate the proliferation of PC cells, and it was revealed that knockdown of SNHG16 inhibited BxPC3 and Panc-1 cells proliferation (Fig. 2B and 2C). Consistently, the protein levels of cell proliferation markers, PCNA and Ki-67, were decreased as SNHG16-silencing (Fig. 2D). Then, flow cytometry cell apoptosis analysis demonstrated that SNHG16 knockdown increased the proportion of apoptotic PC cells (Fig. 2E), and western blot analysis revealed that SNHG16 knockdown promoted the expression of apoptosis-related proteins Bax, cleaved-caspase-3 and cleaved-caspase-9, while inhibited the expression of Bcl-2 (Fig. 2F). Above these, SNHG16 knockdown suppressed PC cells proliferation and promoted apoptosis.

Knockdown of SNHG16 suppresses PC cells migration and invasion

Subsequently, we examined the roles of SNHG16 in migration and invasion. Wound healing assay indicated that the motility of PC cells that stably silenced SNHG16 was significantly decreased (Fig. 3A). Transwell assay was implemented to evaluate the abilities of PC cells migration and invasion, and it is observed the reduced migration and invasion abilities of PC cells that stably silenced SNHG16 (Fig. 3B). The results of western blot demonstrated that reduced SNHG16 inhibited the expression of ICAM-1, VCAM-1 and MMP-9 (Fig. 3C). In general, SNHG16 knockdown suppressed PC cells migration and invasion.

SNHG16 acts as a sponge to regulate miR-302b-3p expression in PC cells

It is well-known that the commonly molecular mechanism of lncRNAs is acted as molecular sponges of miRNAs. Previous researches confirmed that SNHG16 regulated miRNA expression[14]. Therefore, we hypothesized that SNHG16 might exert functions via interacting with miRNAs in PC. The online software ENCORI was used for predicting the miRNAs which might be regulated by SNHG16, and qRT-PCR was performed to determine the expression levels of these predicted miRNAs in PC cells. It was found that only miR-302b-3p was downregulated in PC cells (Fig. 4A), and the biding site of miR-302b-3p on SNHG16 were displayed in Fig. 4B. The expression of miR-302b-3p was increased in SNHG16-knockdown PC cells (Fig. 4C). Dual luciferase reporter assays and RIP were performed to verify whether miR-302b-3p binds to SNHG16 directly. The luciferase activity in PC cells co-transfected with SNHG16-WT and miR-302b-3p mimics were weaker than these in cells co-transfected with SNHG16-WT and NC mimics, or co-transfected with SNHG16-Mut and miR-302b-3p (Fig. 4D). The results of RIP revealed that SNHG16 and miR-302b-3p were highly enriched in anti-Ago2 beads compared with IgG beads (Fig. 4E). In addition, miR-302b-3p was reduced both in PC tissues and cell lines (Fig. 4F and 4G). Taken together, above results proved that SNHG16 acts as a molecular sponge to regulate miR-302b-3p expression in PC cells.

Overexpression of miR-302b-3p inhibits PC cells proliferation, migration and invasion, and promotes apoptosis

The effects of miR-302b-3p on biological phenotypes of PC cells were further explored in PC cells transfected with miR-302b-3p mimics or negative controls. The transfection efficiency of miR-302b-3p was detected by qRT-PCR (Fig. 5A). The results of CCK8 and EdU assays indicated that overexpressed miR-302b-3p inhibited the proliferation of PC cells (Fig. 5B and 5C). Apoptosis experiment revealed that miR-302b-3p overexpression induces PC cells apoptosis (Fig. 5D). And miR-302b-3p overexpression attenuated the capacities of PC cells migration and invasion (Fig. 5E).

MiR-302b-3p targets SLC2A4 and inhibits its expression directly

Three online tools were used to screen seven targets of miR-302b-3p, including SLC2A4, RSBN1, ELK4, NFIA, CELF1, WEE1 and NTN4. Among them, SLC2A4 (Solute carrier family 2 member 4) was the most significantly elevated gene in PC cells (Fig. 6A). The putative binding site between miR-302b-3p and SLC2A4 was indicated in Fig. 6B. Dual luciferase reporter assay verified that the target binding of miR-302b-3p to SLC2A4 mRNA 3'UTR (Fig. 6C). Moreover, transfection of miR-302b-3p mimics inhibited the mRNA level of SLC2A4, as well as protein level (Fig. 6D and 6E). In addition, elevated SLC2A4 was observed in PC tissues and cell lines (Fig. 6F and 6G). These data proved that miR-302b-3p targets SLC2A4 and inhibits its expression directly.

SNHG16 promotes PC progression partly through miR-302b-3p/SLC2A4 axis

To confirm the correlation of SNHG16/miR-302b-3p/SLC2A4 axis in PC progression, PC cells that stably silenced SNHG16 were transfected with miR-302b-3p inhibitor, miR-302b-3p inhibitor plus sh-SLC2A4, or

NC inhibitor, and biological phenotypes of PC cells were analyzed. Firstly, after transfected with sh-SNH616 in Bx-PC3 and Panc-1 cells, miR-302b-3p inhibitors significantly inhibited miR-302b-3p expression, and sh-SLC2A4 decreased the expression of SLC2A4 (Fig. 7A). CCK-8 and EdU assays revealed that SLC2A4 knockdown reversed the promotion of PC cells proliferation increased by miR-302b-3p inhibitor (Fig. 7B and 7C). The inhibition of apoptosis by miR-302b-3p inhibitor was also abolished in PC cells co-transfected with miR-302b-3p inhibitor and sh-SLC2A4 (Fig. 7D). Meanwhile, the increased migration and invasion abilities of PC cells with miR-302b-3p knockdown were suppressed by sh-SLC2A4 (Fig. 7E). These results confirmed that SNHG16 participated in the progression of PC via targeting miR-302b-3p/SLC2A4 axis.

Discussion

PC is a highly aggressive solid tumor that frequently causes local invasion and early metastasis, which induces more than 300,000 deaths each year. The prognosis of PC patients is very poor, and the overall 5-year survival rate is less than 5%[15]. Therefore, it is important to identify the promising diagnostic markers or targeted therapies to gradually inhibit the progression of PC. With the development of sequencing technology, more and more non-coding RNA have been discovered. Among them, lncRNAs have been getting more and more attention due to their wide range of functions.

Small nucleolar RNA host gene 16 (SNHG16) has been reported as an oncogenic lncRNA in multiple cancers such as colorectal cancer[16], non-small cell lung cancer[17], breast cancer[18], clear cell renal cell carcinoma[19]. A further research indicated that SNHG16 served as a novel prognostic marker which promote tumor formation and metastasis in vivo and vitro by sponging miR-146a further inducing MUC5AC in NSCLC[17]. Here, we investigated novel biological effects of SNHG16 in PC.

The pivotal conclusion of this study is that SNHG16 plays a critical role in PC. The results demonstrated that SNHG16 was significantly upregulated in human PC tissues and PC cells, and that increasing SNHG16 was correlated with poor prognosis of PC patients. Functional experiments indicated the inhibition effects of silenced SNHG16 on PC cell proliferation, migration and invasion, whereas promotion of apoptosis.

Mechanistically, the target gene, miR-302b-3p, was predicted and identified by luciferase reporter assay and RIP. MiR-302b-3p was highly expressed both in PC tissues and cells. Following functional experiments confirmed that miR-302b-3p promoted the proliferation, migration and invasion of PC cells, which was opposite with SNHG16. These data implied that SNHG16 acts as a sponge to negatively regulate miR-302b-3p expression in PC cells. Moreover, we investigated that miR-302b-3p can directly target 3’UTR of SLC2A4 and inhibited SLC2A4 expression in PC cells.

SLC2A4 is a gene codified the insulin-sensitive glucose transporter GLUT4, which is an insulin-sensitive glucose transporter that plays a key role in glucose homeostasis[20]. SLC2A4 is an efficient glucose transporter, which is located in the cytoplasmic vesicles and can be transferred to the plasma membrane

to take up glucose when stimulated by insulin[21]. It has been shown that SLC2A4 was elevated in cancer progression[22, 23], which is in conformity with our results. SLC2A4 inhibition abolished the biological effects of PC cells in proliferation, migration and invasion induced by miR-302b-3p downregulation. Thus, we suggested that SNHG16 was an oncogene in PC progression via targeting miR-302b-3p/SLC2A4 axis and expected to be a potential target for early diagnosis and treatment of PC.

Conclusion

1. LncRNA SNHG16 is elevated in PC tissues and cell lines
2. Knockdown of SNHG16 suppresses PC cells migration and invasion
3. SNHG16 acts as a sponge to regulate miR-302b-3p expression in PC cells
4. Overexpression of miR-302b-3p inhibits PC cells proliferation, migration and invasion, and promotes apoptosis
5. MiR-302b-3p targets SLC2A4 and inhibits its expression directly
6. SNHG16 promoted the progression of PC via miR-302b-3p/SLC2A4 axis
7. SNHG16 was expected to be a potential target for early diagnosis and treatment of PC

List Of Abbreviations

Abbreviation	Full name
PC	pancreatic cancer
lncRNAs	Long non-coding RNAs
SNGH16	small nucleolar RNA host gene 16
DMEM	Dulbecco's modified Eagle's medium

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of the First Hospital of Lanzhou University, and all included patients signed informed consent.

Consent for publication

The authors agrees to publication in the Journal.

Authors' contributions

Wence Zhou conceived and designed the study. Chen Bo and Xin Miao performed the literature search. Xin Li and Haofei performed data extraction. Hao Xu drafted the manuscript. All authors read and approved the final manuscript.

Availability of data and material

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

Competing interests

The authors declare that they have no conflicts of interest.

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Figures

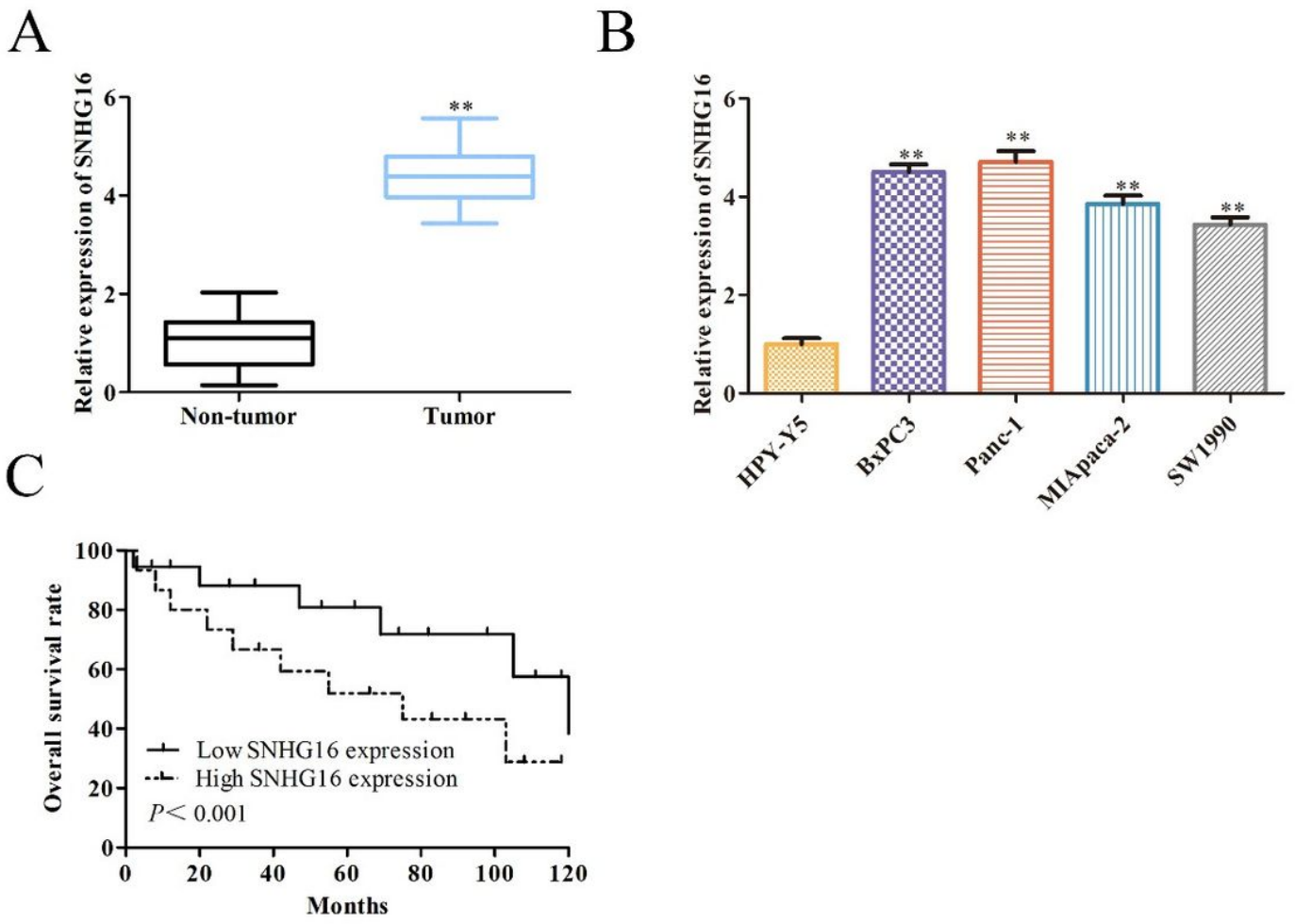

Figure 1

LncRNA SNHG16 is elevated in PC tissues and cell lines. (A) The expression level of SNHG16 in PC tissues and adjacent normal tissues was detected by qRT-PCR assay. (B) The expression of SNHG16 in the normal pancreatic duct epithelial cell line HPY-Y5 and pancreatic cancer cell lines (BxPC3, Panc-1, MIA Paca-2 and SW1990). (C) Kaplan-Meier overall survival analysis of SNHG16 expression and the survival time of PC patients. **P□0.01.

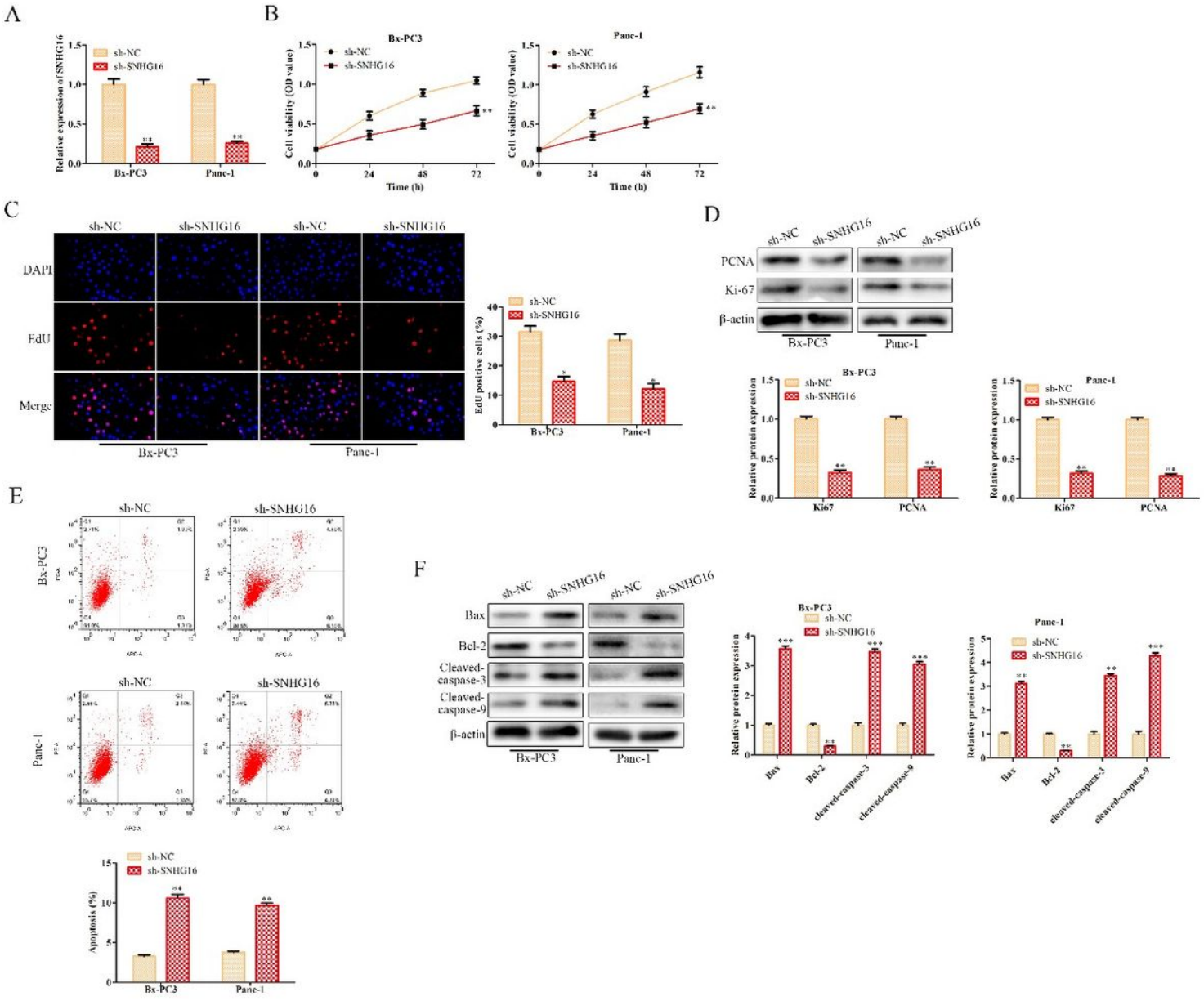

Figure 2

SNHG16 affects PC cells proliferation and apoptosis. PC cells, BxPC3 and Panc-1 were transfected with sh-SNHG16 or sh-NC respectively. (A) Relative expression of SNHG16 in PC cells were detected by qRT-PCR. (B) Cell viability of PC cells was analyzed by CCK8 assay. (C) The proliferation ability of PC cells was measured by EdU assay. (D) The cell proliferation markers, PCNA and Ki-67 were detected by Western blot. (E) Apoptosis rate of PC cells was analyzed by flow cytometry. (F) Western blot analysis of apoptosis-related proteins Bax, Bcl-2, Cleaved caspase-3 and Cleaved caspase-9. β-actin was used as the internal control. *P□0.05, **P□0.01, ***P□0.001.

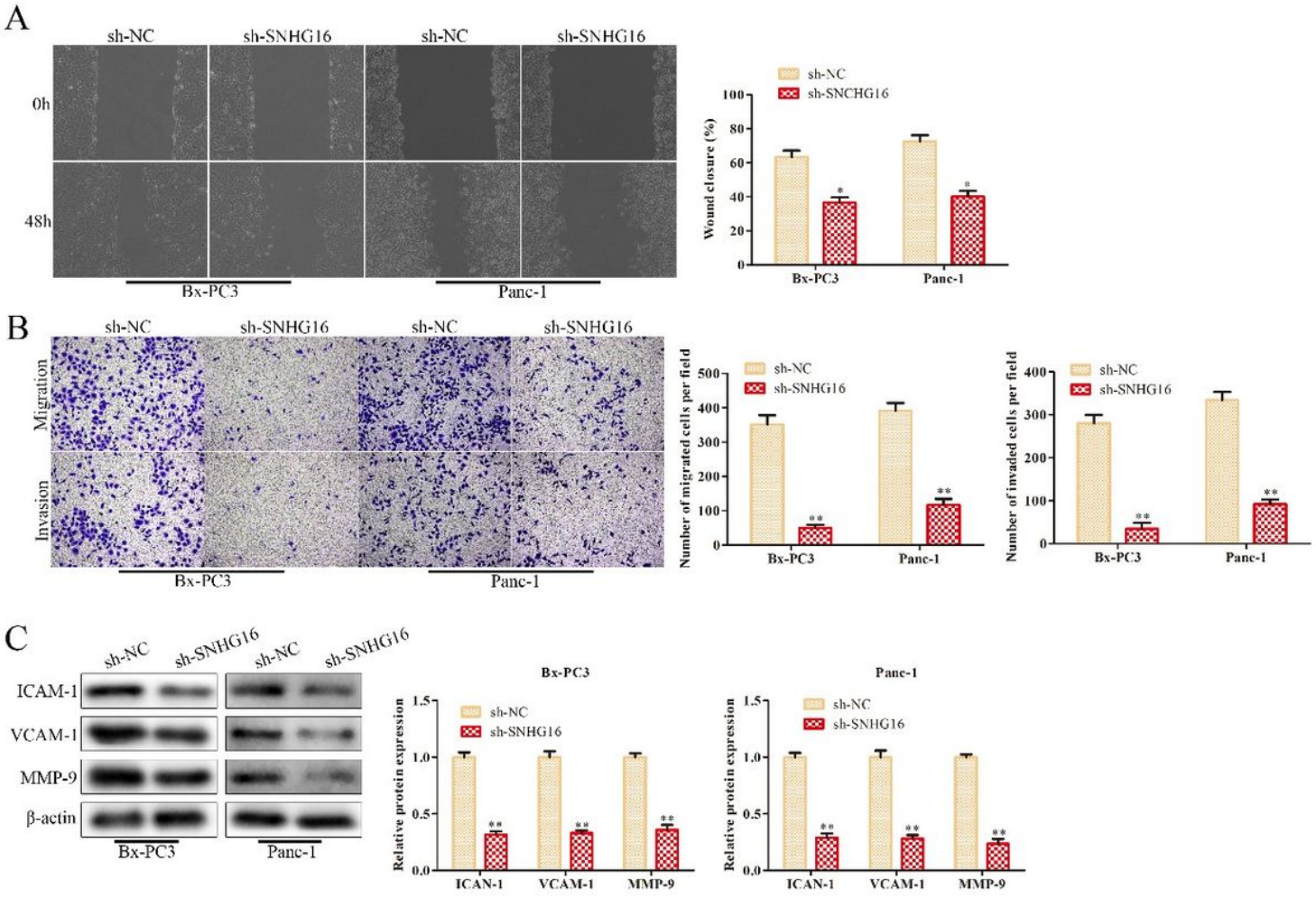

Figure 3

Knockdown of SNHG16 suppresses migration and invasion of PC cells. (A) The migration ability of PC cells transfected with sh-SNHG16 or sh-NC was assessed by wound healing assay. (B) The migration and invasion abilities of PC cells transfected with sh-SNHG16 or sh-NC were measured by transwell assay. (C) Western blot analysis of ICAM-1, VCAM-1 and MMP-9. β-actin was used as the internal control. *P□0.05, **P□0.01.

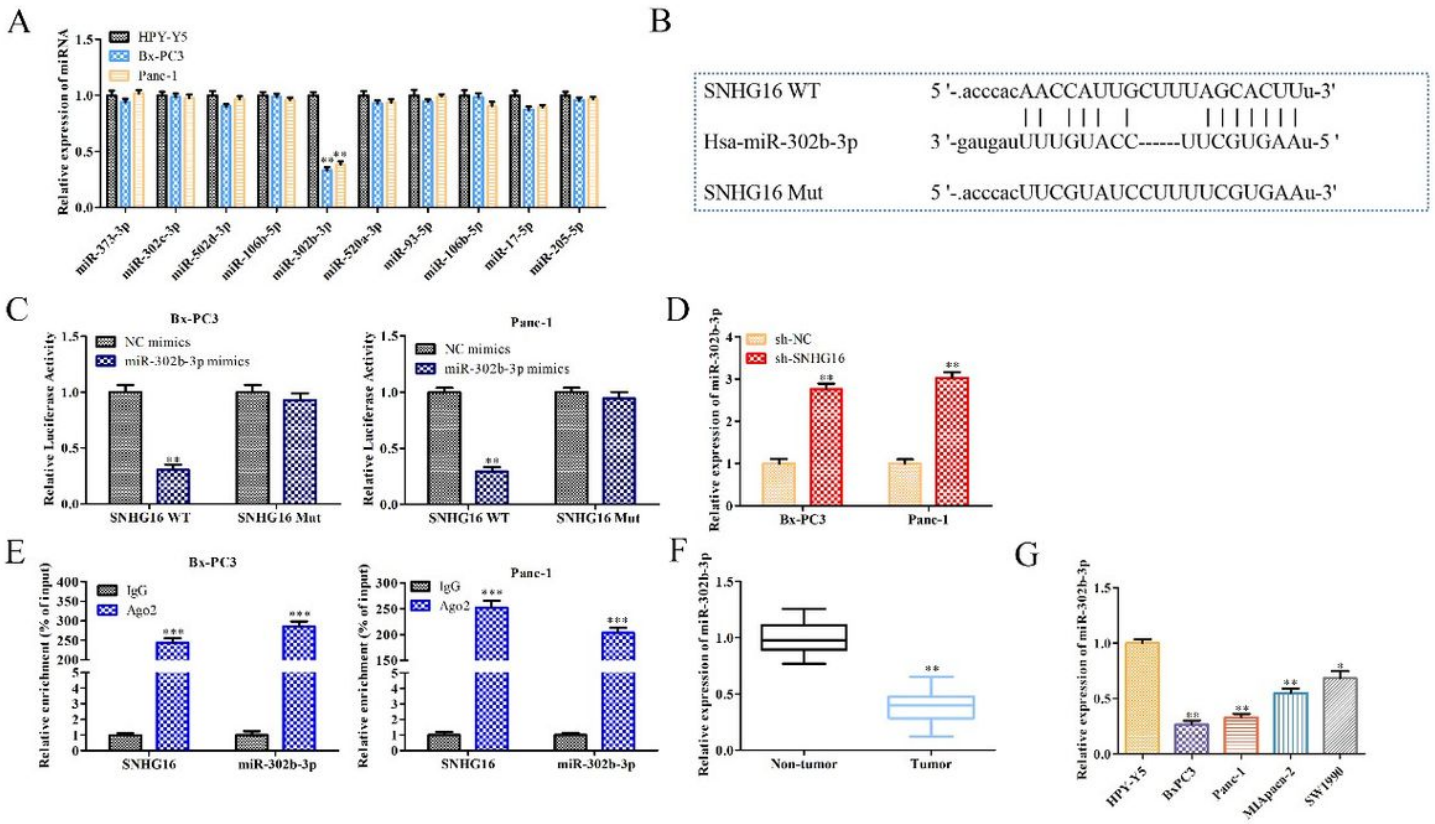

Figure 4

SNHG16 acts as a sponge to regulate miR-302b-3p expression in PC cells. (A) qRT-PCR analysis of the target miRNAs of SNHG16 in HPY-Y5, BxPC3 and Panc-1 cells. (B) Schematic diagram of binding sites between miR-302b-3p and SNHG16 (WT or Mut). (C) Luciferase activity of SNHG16 WT or Mut in BxPC3 and Panc-1 cells co-transfected with miR-302b-3p mimics or NC mimics, respectively. (D) Relative expression of miR-302b-3p in BxPC3 and Panc-1 cells within SNHG16 silencing. (E) RIP analysis of the enrichment of SNHG16 and miR-302b-3p pulled down from the Ago2 protein in BxPC3 and Panc-1 cells. IgG was used as the control. (F and G) qRT-PCR assay of miR-302b-3p expression in PC tissues and cell lines. *P□0.05, **P□0.01, ***P□0.001.

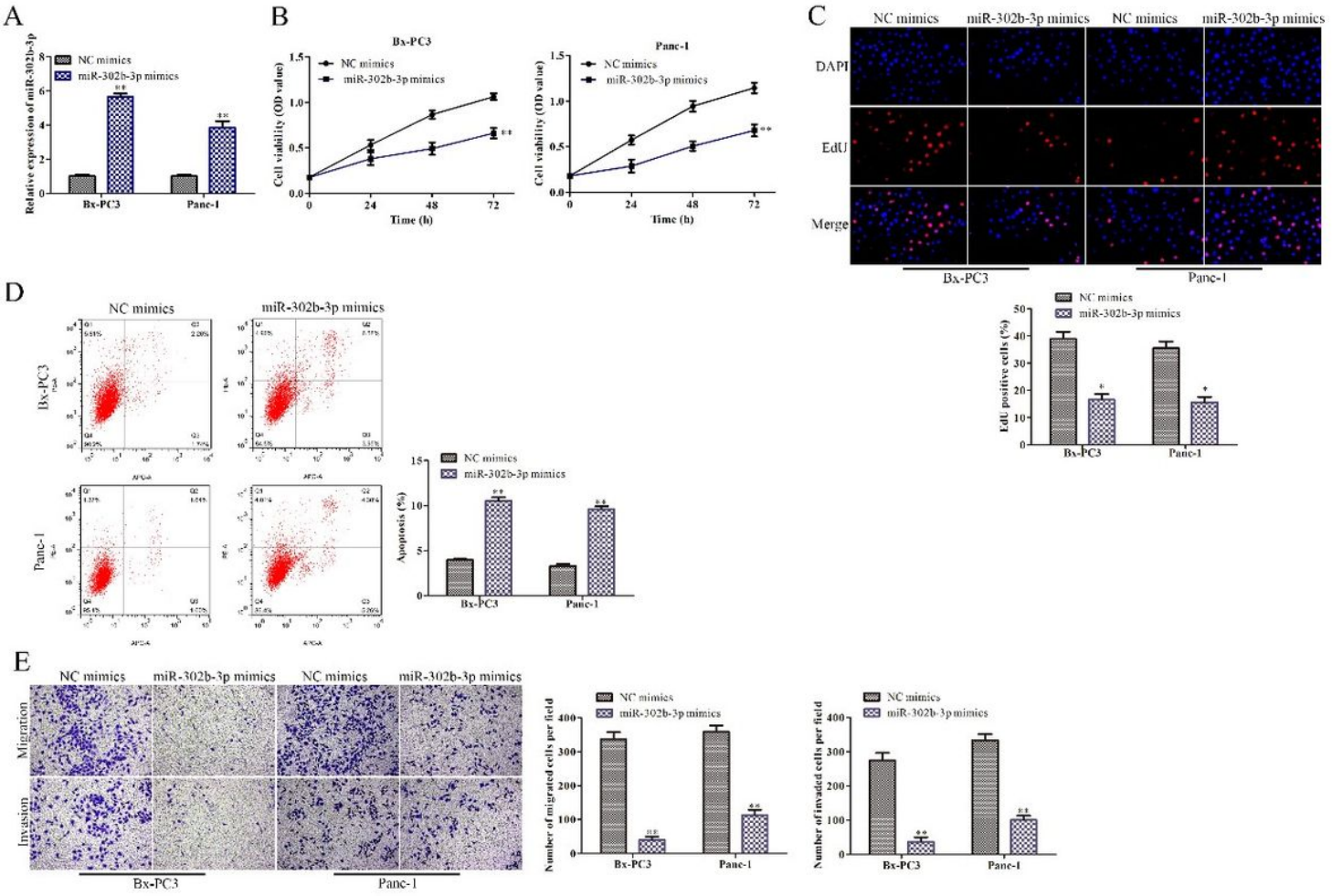

Figure 5

MiR-302b-3p inhibits metastasis and induces apoptosis in PC cells. Pancreatic cancer cells, BxPC3 and Panc-1 were transfected with miR-302b-3p mimics or NC mimics. (A) The transfection efficiency of miR-302b-3p was analyzed by qRT-PCR. (B and C) The effects of miR-302b-3p on PC cells proliferation were determined by CCK8 and EdU assays. (D) The apoptosis of PC cells was analyzed by flow cytometry. (E) The migration and invasion abilities of PC cells were assessed by transwell assays. *P□0.05, **P□0.01.

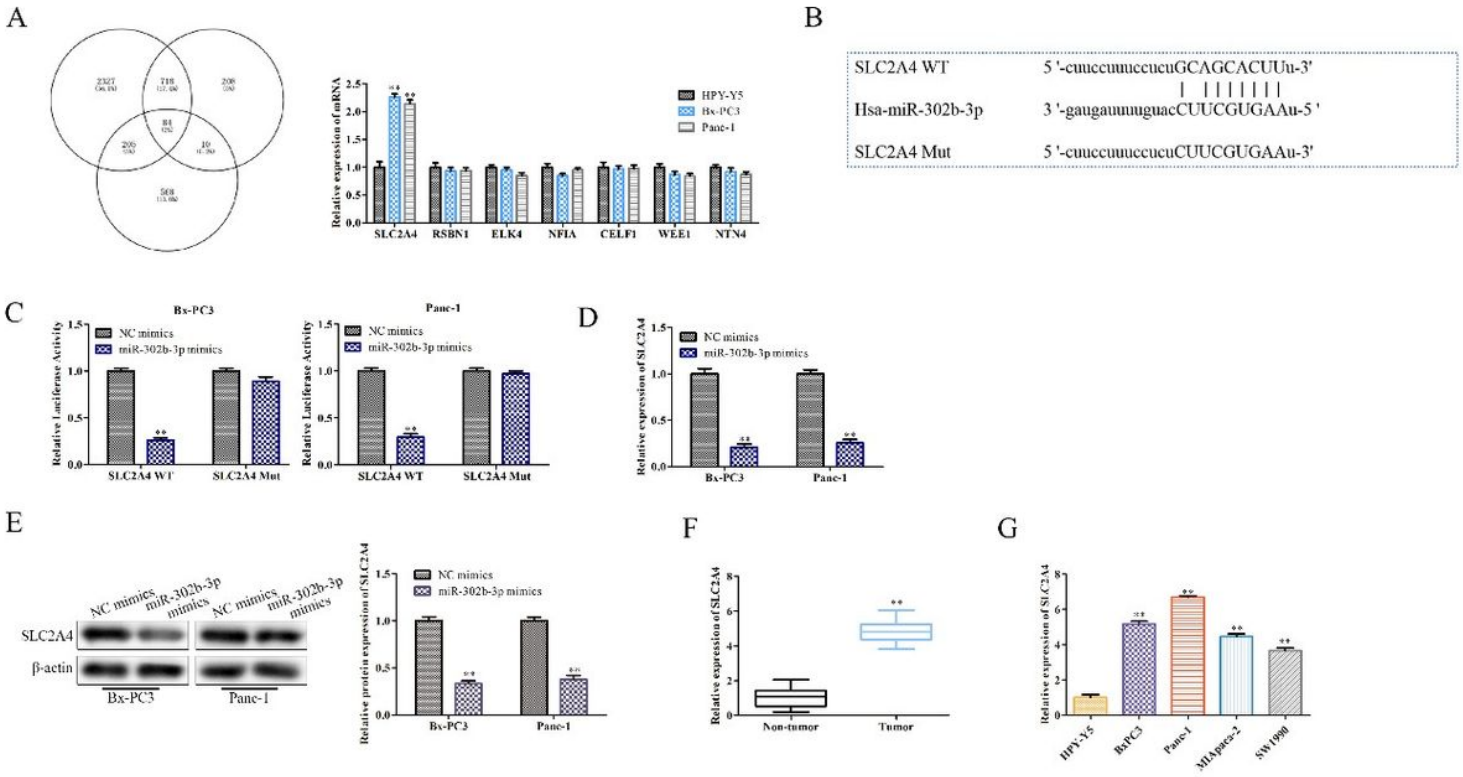

Figure 6

MiR-302b-3p targets SLC2A4 and inhibits its expression directly. (A) Identification of the potential targets of miR-302b-3p using online prediction databases and qRT-PCR analysis. (B) The predicted binding site of miR-302b-3p within SLC2A4 3'UTR. (C) The binding interaction betweenSNHG16 and miR-302b-3p was confirmed by luciferase reporter assay. (D and E) qRT-PCR and western blot analyses of the effect of miR-302b-3p on SLC2A4 expression. (F and G) Relative expression of SLC2A4 in PC tissues and cell lines. *P 0.05, **P0.01, ***P0.001.

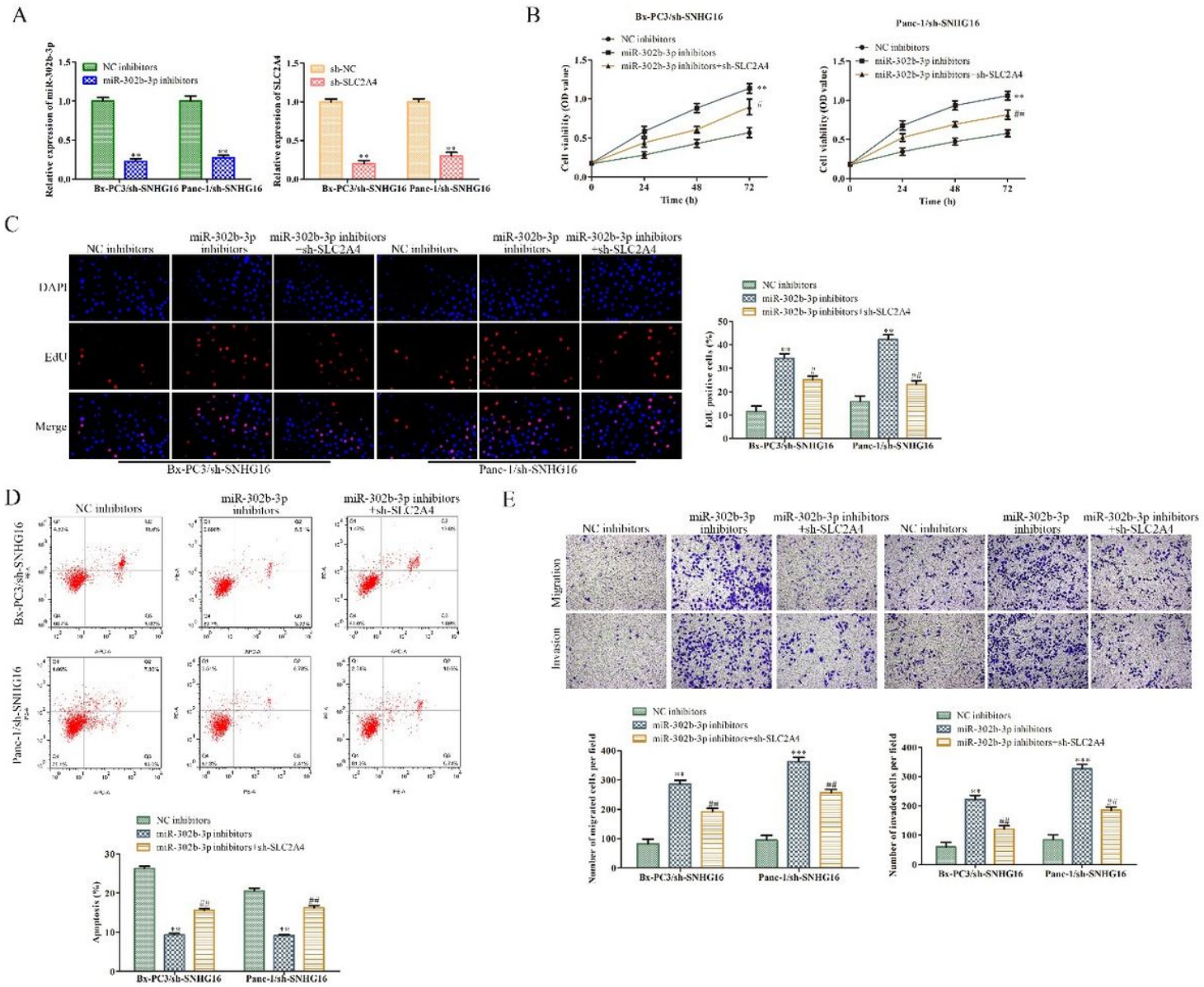

Figure 7

SNHG16 promotes PC progression partly via miR-302b-3p/SLC2A4 axis. BxPC3 or Panc-1 cells with SNHG16 knockdown were transfected with miR-302b -3p inhibitor, sh-SLC2A4, miR-302b -3p inhibitor plus sh-SLC2A4 or their respective negative control. (A) The transfection efficiencies were detected by qRT-PCR. (B and C) Cell proliferation was measured by CCK8 and EdU assays. (D) The apoptosis of BxPC3 or Panc-1 cell was detected by flow cytometry. (E) The migration and invasion abilities were detected by transwell assay. **P□0.01 vs NC inhibitors. #P□0.05, ##P□0.01 vs miR-302b-3p inhibitors.