

Knockdown of lncRNA ABHD11-AS1 Suppresses the Tumorigenesis of Pancreatic Cancer via Sponging miR-1231

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Background: Pancreatic cancer ranks first among the most aggressive malignancies. Long non-coding RNA (lncRNA) ABHD11-AS1 is known to be upregulated in pancreatic cancer. However, the mechanism by which ABHD11-AS1 mediates the tumorigenesis of pancreatic cancer remains unclear.

Methods: Gene and protein expressions in pancreatic cancer cells were detected by qRT-PCR and Western blot, respectively. Cell viability was measured by CCK-8 assay. Cell apoptosis and cycle were tested by flow cytometry. In addition, cell migration and invasion were tested by wound healing and transwell assay, respectively. The correlation between ABHD11-AS1, miR-1231 and cyclin E1 was confirmed by dual-luciferase report and RNA pull-down. Finally, xenograft mice model was established to investigate the role of ABHD11-AS1 in pancreatic cancer in vivo.

Results: ABHD11-AS1 was found to be negatively correlated with the survival rate of patients with pancreatic cancer. ABHD11-AS1 silencing significantly inhibited the proliferation and induced the apoptosis of pancreatic cancer cells. Additionally, knockdown of ABHD11-AS1 greatly inhibited the migration and invasion of pancreatic cancer cells. Meanwhile, ABHD11-AS1 bound to miR-1231 and cyclin E1 was found to be the target of miR-1231. Moreover, ABHD11-AS1 knockdown-induced G1 arrest in pancreatic cancer cells was reversed by miR-1231 antagomir. Finally, knockdown of ABHD11-AS1 obviously inhibited the tumor growth of pancreatic cancer in vivo.

Conclusion: ABHD11-AS1 silencing significantly inhibited the tumorigenesis of pancreatic cancer in vitro and in vivo. Thus, ABHD11-AS1 may serve as a potential target for the treatment of pancreatic cancer.

Keywords: pancreatic cancer, ABHD11-AS1, miR-1231, cyclin E1

Introduction

Pancreatic cancer is one of the gastrointestinal cancers with poor prognosis, which ranks first among the cancerous causes of death all over the world.¹ It has been revealed that the incidence and mortality rate of pancreatic cancer is increasing in China.² According to the report of GLOBOCAN 2012, there are about 19% of over 33,000 cases of newly diagnosed pancreatic cancer and 20% of 330,391 deaths from pancreatic malignant tumor in China.³ Currently, the main treatment methods of pancreatic cancer are surgery and chemotherapy; however, overall 5-year survival rates of patients only reach 20%.⁴ Although many efforts have been made to improve the outcome of pancreatic cancer treatment, its high malignant behavior

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causes the poor development in prognosis of this malignancy.⁵ Therefore, it is urgent to find a new strategy for the treatment of pancreatic cancer.

Long non-coding RNAs (lncRNAs) are a group of non-coding RNA transcripts with a length of 200 nucleotides.⁶ In addition, lncRNAs are key mediators that are notably participated in the progression of multiple diseases.^{7,8} Recent studies have shown a close correlation between lncRNAs and cancer progression.^{9,10} For example, knock-down of LINC00324 could suppress the progression of liver cancer through upregulation of PU box binding protein.¹¹ Additionally, some lncRNAs have been confirmed to play key roles in the progression of pancreatic cancer.^{12,13} Meanwhile, Liu Y et al found that ABHD11-AS1 was notably upregulated in pancreatic cancer, compared with pancreatitis.¹⁴ However, the mechanism by which ABHD11-AS1 regulates the progression of pancreatic cancer remains unclear.

In this study, we sought to investigate the role of ABHD11-AS1 in pancreatic cancer. We hope this finding will provide a new strategy for the treatment of pancreatic cancer.

Materials and Methods

Cell Culture

The pancreatic cancer cell lines (MiaPaCa-1 and PANC-1) and 293T cell line were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). All cell lines were cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco) and penicillin (100 U/mL). In addition, cells were cultured at 37°C in the presence of 5% CO₂.

Bioinformatics Analysis

The gene expression data of pancreatic cancer and adjacent normal tissue were obtained from the Cancer Genome Atlas (TCGA) with online GEPIA.¹⁵ The survival curve was calculated based on the data from TCGA.¹⁵

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from pancreatic cancer cells using TRIzol reagent (TaKaRa, Tokyo, Japan) according to the manufacturer's protocol. cDNA was synthesized using the reverse transcription kit (TaKaRa, Ver.3.0) according to the manufacturer's protocol. Real-Time qPCRs were

performed in triplicate under the following protocol: 2 minutes at 94°C, followed by 35 cycles (30 s at 94°C and 45 s at 55°C). The primer for lncRNA ABHD11-AS1, miR-1231, cyclin E1, β -actin and U6 were obtained from GenePharma (Shanghai, China). ABHD11-AS1: forward, 5'-CTCTCCACCTGACAGCAACATC-3' and reverse 5'-TACTCTGGGCTGTGTGCGCAG-3'. MiR-1231: forward, 5'-CCTCAACTGAATTGCCGACTC-3' and reverse 5'-CTCAACTGGTGTCTGCGGAGTC-3'. Cyclin E1: forward, 5'-TAGAGAGGAAGTCTGGAAAATCATG-3' and reverse 5'-ATATACCGGTCAAAGAAATCTTGTG-3'. β -actin: forward, 5'-GTCCACCGCAAATGCTTCTA-3' and reverse 5'-TGCTGTACCTTCACCGTTC-3'. U6: forward, 5'-CTCGCTTCGGCAGCACAT-3' and reverse 5'-AACGCTTCACGAATTTGCGT-3'. The $2^{-\Delta\Delta C_t}$ method was used to quantify the results. The internal reference gene (U6 or β -actin) was used for normalization.

Cell Transfection

Lentiviral expressing short-hairpin RNA (shRNA1 or shRNA2) directed target ABHD11-AS1 and one nontargeting sequence (negative control) were obtained from GenePharma (Shanghai, China). Next, ABHD11-AS1 shRNA1 or shRNA2 was packaged into lentiviruses. Then, the lentiviral vector DNAs were then transfected into 293T cells including lenti-ABHD11-AS1 shRNAs and negative control (NC). After transfection, the cells were incubated at 37°C, and then the supernatant was collected. After that, supernatants of two ABHD11-AS1 shRNAs and negative control were filtered into particles. Finally, all pancreatic cancer cells were infected with lentiviral particles according to the manufactures' protocol. After 48 h of incubation, stable pancreatic cancer cells were then selected by puromycin (2.5 μ g/mL, Sigma Aldrich, St. Louis, MO, USA). qRT-PCR assay was used to verify the efficiency of transfection.

For miR-1231 transfection, pancreatic cancer cells were transfected with miR-1231 agomir, miR-1231 antagomir or NC by Lipofectamine 2000 according to the previous reference.¹⁶ MiR-1231 agomir, miR-1231 antagomir and negative control RNAs were purchased from GenePharma (Shanghai, China).

CCK-8 Assay

Pancreatic cancer cells were seeded in 96-well plates (5×10^3 per well) overnight. Then, cells were treated with negative control (NC) or ABHD11-AS1 shRNA1 for 0, 24, 48 and 72 h, respectively. Ten microliters of CCK-8

reagents (GenePharma) were added to each well and further incubated for 2 h at 37°C. Finally, the absorbance of pancreatic cancer cells was measured at 450 nm using a microplate reader (Thermo Fisher Scientific).

Cell Apoptosis Analysis

Pancreatic cancer cells were trypsinized, washed with phosphate-buffered saline and resuspended in Annexin V Binding Buffer, followed by staining with 5 μ L FITC and propidium (PI) in the dark for 15 min. The data were analyzed using flow cytometer (BD, Franklin Lake, NJ, USA).

Cell Cycle Detection

Cell cycle was determined by flow cytometry using Cycle Detection Kit I (BD Biosciences, Franklin Lake, NJ, USA). Pancreatic cancer cells were seeded in a 6-well plate 1 day before cell transfection. After 24 h of transfection, the cells were lifted and fixed in pre-cold 70% ethanol at 4°C overnight. Then, cells were treated with 100 μ L PI/RNase Staining Buffer (Thermo Fisher Scientific) at room temperature in the dark for 30 min. Finally, flow cytometry (BD Biosciences) was used to detect the cell

cycle distribution, and the data were analyzed using the FlowJo software (BD Biosciences).

Dual-Luciferase Reporter Assay

The partial sequences of ABHD11-AS1 and 3'-UTR of cyclin E1 containing the putative binding sites of miR-1231 were synthesized and obtained from Sangon Biotech (Shanghai, China), then were cloned into the pmirGLO Dual-Luciferase miRNA Target Expression Vectors (Promega, Madison, WI, USA) to construct wild-type reporter vectors ABHD11-AS1 (WT/MT) and cyclin E1 (WT/MT), respectively. The ABHD11-AS1 (WT/MT) or cyclin E1 (WT/MT) were transfected into PANC-1 cells together with control, vector-control (NC) or miR-1231 agomir using Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer's instructions. The relative luciferase activity was analyzed by the Dual-Glo Luciferase Assay System (Promega).

Western Blot Detection

Total protein was isolated from cell lysates or tissues by using RIPA buffer and quantified by BCA protein assay kit (Beyotime, Shanghai, China). Proteins were separated with 10% SDS-PAGE, and then transferred onto

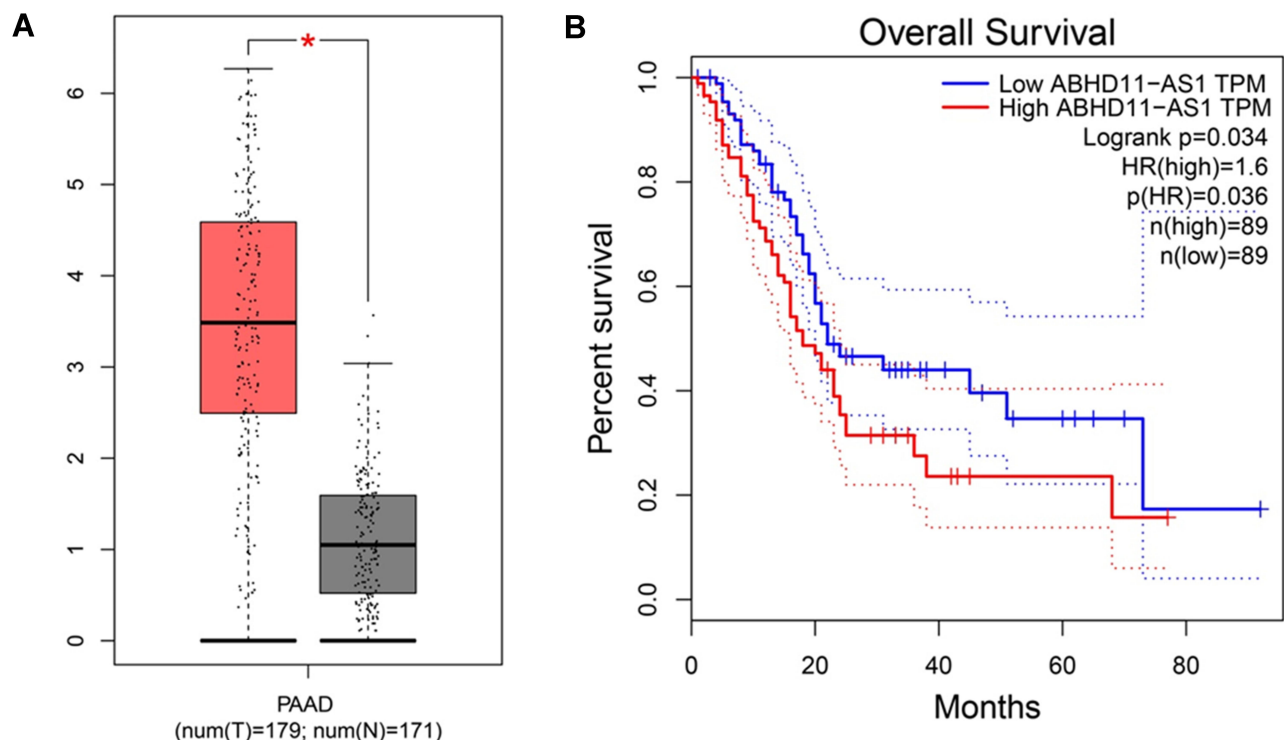


Figure 1 High expression of ABHD11-AS1 was negatively correlated with survive rate of patients with pancreatic cancer. **(A)** The expression of ABHD11-AS1 in pancreatic cancer and adjacent normal tissues was calculated based from TCGA. **(B)** Overall survival of pancreatic cancer was analyzed by TCGA. *P<0.05.

PVDF (Bio-Rad) membranes. After blocking with 3% skim milk for 1 h, the membranes were incubated with primary antibodies at 4°C overnight. Then, membranes were incubated with secondary anti-rabbit antibody (Abcam; 1:5000) at room temperature for 1 h. Membranes were scanned by using an Odyssey Imaging System and analyzed with Odyssey v2.0 software (LICOR Biosciences, Lincoln, NE, USA). The primary antibodies used in this study were as follows: anti-cyclin E1 (Abcam, Cambridge, MA, USA; 1:1000), anti-Bax (Abcam; 1:1000), anti-XIAP (Abcam; 1:1000), anti-Active caspase 3 (Abcam; 1:1000), anti-CDK2 (Abcam; 1:1000) and anti- β -actin (Abcam; 1:1000). β -Actin was used as an internal control.

Wound Healing Assay

Pancreatic cancer cells were plated into a 24-well Cell Culture Cluster. In addition, cells were allowed to grow to 80–90% confluence. Then, cells were underlined perpendicular to the cell culture plate with a small pipette head. After washing with PBS 3 times, serum-free medium was used for further culture, and the scratch widths at 0 and 48 h were recorded under an optical microscope. The experiment was repeated three times.

RNA Pull-Down

For the RNA pulldown assay, the Biotin RNA Labeling Mix (Roche, Basel, Switzerland) was used to transcribe and label probe-control or probe-ABHD11-AS1 from ABHD11-AS1

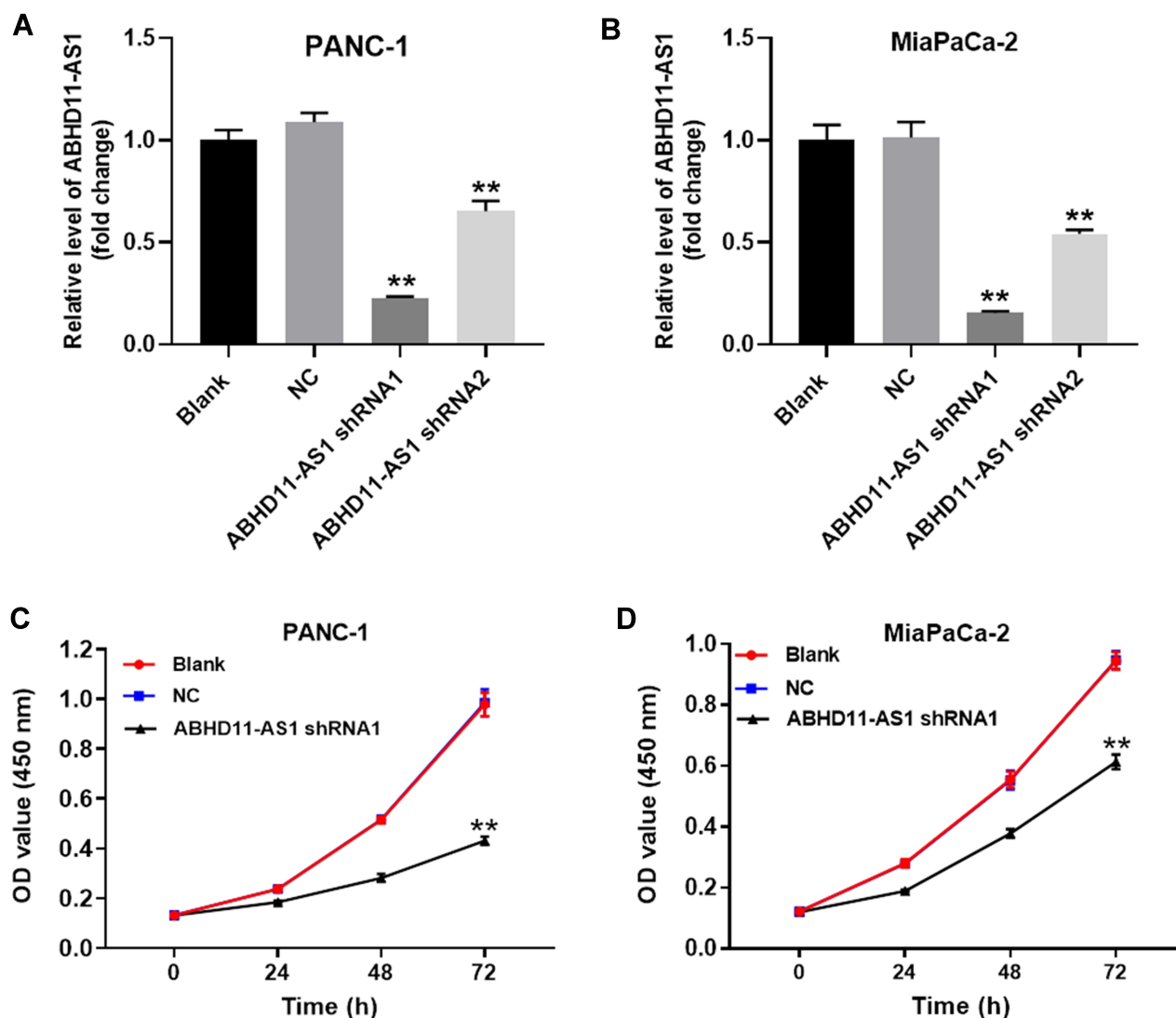


Figure 2 Knockdown of ABHD11-AS1 significantly inhibits the proliferation of pancreatic cancer cells. MiaPaCa or PANC-1 cells were transfected with ABHD11-AS1 shRNA1 or shRNA2 for 24 h. Then, (A, B) the expression of ABHD11-AS1 in pancreatic cancer cells was detected by qRT-PCR. (C, D) Cell viability of pancreatic cancer was tested by CCK-8 assay. **P<0.01 compared to control.

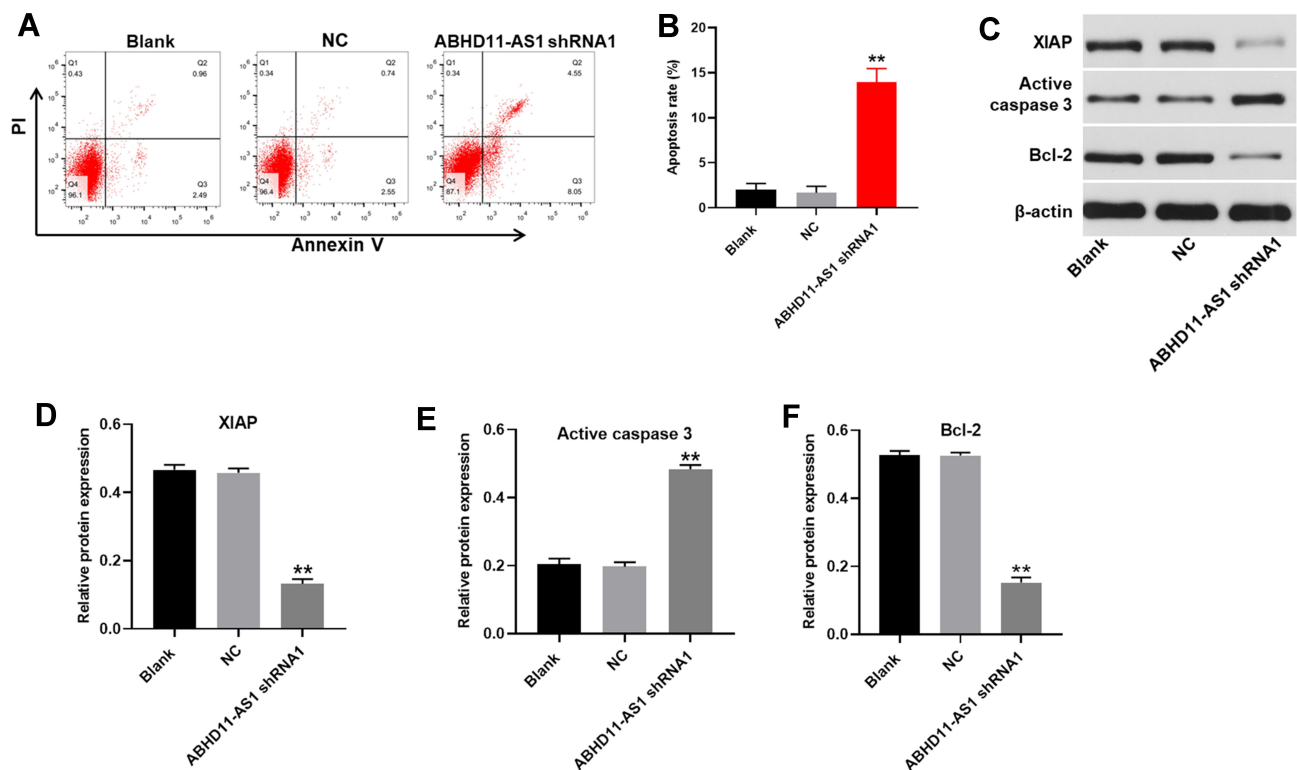


Figure 3 ABHD11-AS1 shRNA1 greatly induces the apoptosis of pancreatic cancer cells. **(A)** The apoptosis of PANC-1 cells was detected by flow cytometry. **(B)** The apoptosis rate was calculated by FACS. **(C)** The protein expressions of Active caspase 3, Bcl-2 and XIAP in PANC-1 cells were detected by western blot. The relative protein expression of **(D)** XIAP, **(E)** Active caspase 3 and **(F)** Bcl-2 were quantified by normalizing to β-actin. ** $P < 0.01$ compared to control.

shRNA lenti vector in vitro. An RNA structure buffer (Thermo Fisher Scientific) was used to induce secondary structure formation from the biotin-labeled RNAs. The biotinylated ABHD11-AS1 and negative control (bio-NC) were generated via GenePharma and coated to streptavidin-conjugated magnetic beads. Pancreatic cancer cells were lysed and then incubated with the magnetic beads for 6 h. The RNA on the beads was isolated and the enrichment level of miR-1231 was detected by PCR.

Transwell Assay

For the invasion assay, the upper chambers of the 24-well plates were pretreated with 50 μ L of Matrigel (12.5 mg/L), and the wells were pretreated with Matrigel (BD Biosciences, Franklin Lake, NJ, USA). Then, pancreatic cancer cells (1×10^6 cells/mL) in FBS-free medium were seeded into the upper chambers. The lower chambers contained RPMI 1640 medium supplemented with 1% FBS. Cells were incubated at 37°C for 24 h, and cells that had attached to the underside of the membrane were fixed and stained with a 0.1% crystal violet solution. Finally, images were captured, and the number of invading cells was counted under a microscope at 400x magnification.

In vivo Study

Twelve BALB/c nude mice (6–8 weeks old) were purchased from Vital River (Beijing, China). The mice were housed within a dedicated SPF facility (six mice per group). Pancreatic cancer cells (ABHD11-AS1 shRNA1) were injected into mice according to the previous reference.¹⁷ MiR-1231 antagomir was injected intra-tumor twice weekly. The tumor volume was measured weekly according to the formula: $\text{Length} \times \text{Width} \times \text{Width} / 2$. At the end of the experiments, mice were sacrificed and the tumors were collected and weighted. All in vivo experiments were performed in accordance with the National Institutes of Health guide for the care and use of laboratory animals, following a protocol approved by the Ethics Committees of Henan Provincial People's Hospital.

Statistical Analysis

Each study was performed with three independent experiments and all data were expressed as the mean \pm standard deviation (SD). Differences were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test (more than 2 groups, Graphpad Prism7).

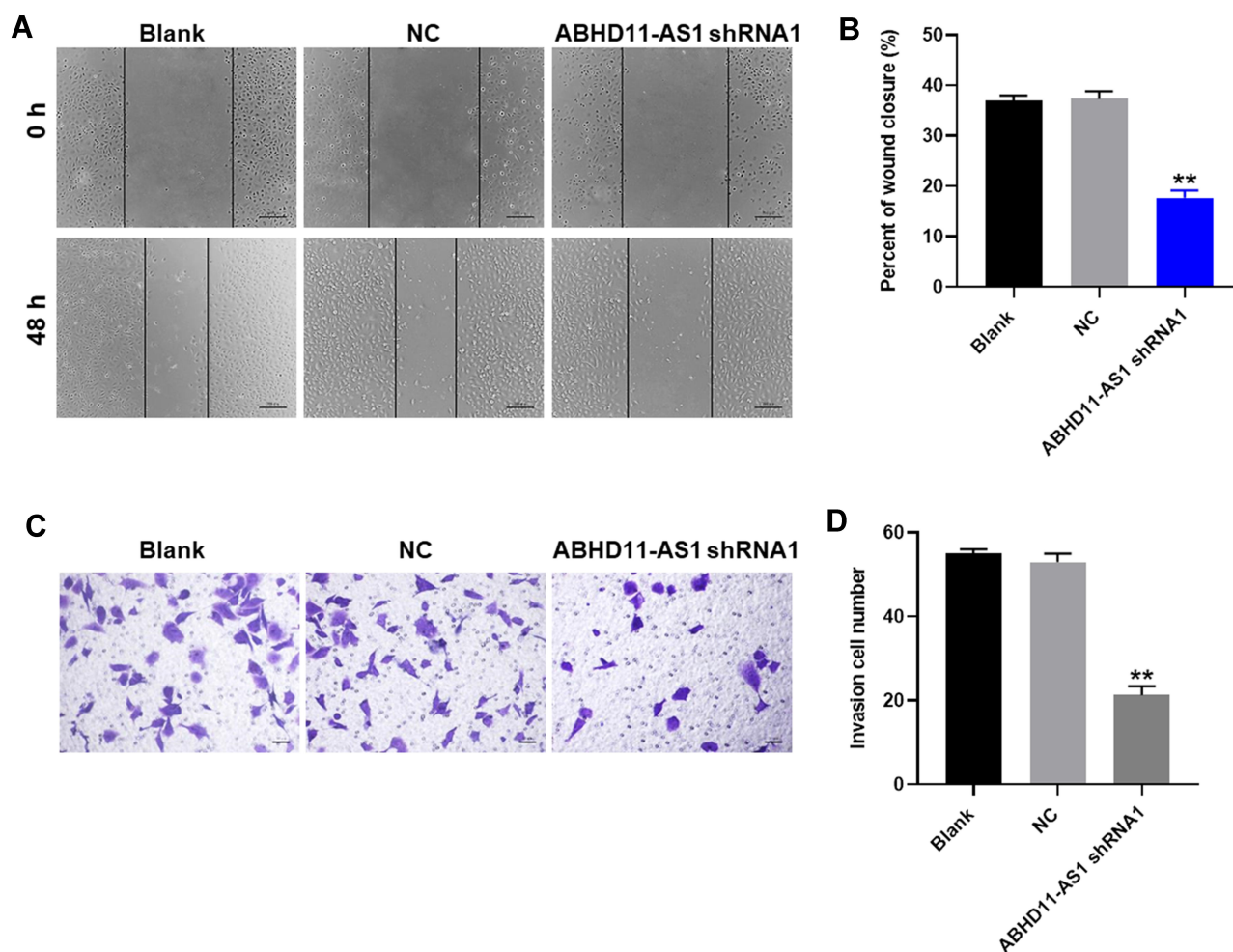


Figure 4 Downregulation of ABHD11-AS1 obviously inhibits the migration and invasion of PANC-1 cells. (A, B) Cell migration of pancreatic cancer was detected by wound healing assay. (C, D) Cell invasion of pancreatic cancer was measured by transwell assay. ** $P < 0.01$ compared to control.

The comparison between two groups was analyzed by unpaired Student's *t*-test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

High Expression of ABHD11-AS1 is Negatively Correlated with Survival Rate of Patients with Pancreatic Cancer

To explore the gene expression data of pancreatic cancer and adjacent normal tissue, TCGA data were used. As indicated in Figure 1A, the expression of ABHD11-AS1 in pancreatic cancer tissues was significantly upregulated, compared with adjacent normal tissues. In addition, high expression of ABHD11-AS1 was negatively correlated with the survival rate of patients with pancreatic cancer (Figure 1B).

Knockdown of ABHD11-AS1 Significantly Inhibits the Proliferation of Pancreatic Cancer Cells

Next, to detect the efficiency of cell transfection, qRT-PCR was used. As indicated in Figure 2A and B, the expression of ABHD11-AS1 in pancreatic cancer cells was significantly downregulated in the presence of ABHD11-AS1 shRNAs. Since pancreatic cancer cells were more sensitive to ABHD11-AS1 shRNA1, ABHD11-AS1 shRNA1 was selected of use in the following experiments. Moreover, the viability of pancreatic cancer cells was significantly decreased by ABHD11-AS1 shRNA1 (Figure 2C and D). In addition, PANC-1 cells were more susceptible to ABHD11-AS1 shRNA1 compared with MiaPaCa-2. Therefore, PANC-1 cells were selected for use in subsequent investigations. Taken together, knockdown of ABHD11-AS1 significantly inhibited the proliferation of pancreatic cancer cells.

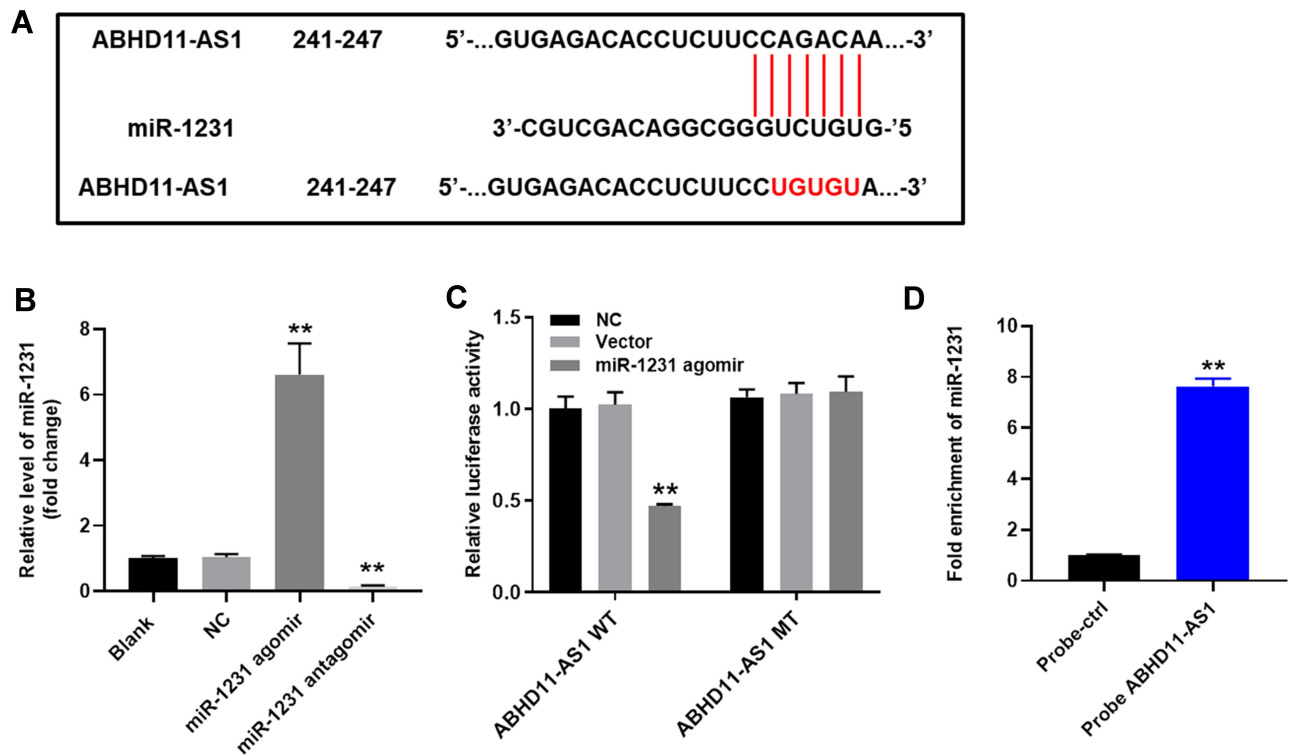


Figure 5 ABHD11-AS1 mediates the tumorigenesis of pancreatic cancer via sponging miR-1231. **(A)** Gene structure of ABHD11-AS1 at the position of 241-247 indicates the predicted target site of miR-1231 in its 3'UTR. **(B)** PANC-1 cells were transfected with miR-1231 agomir/antagomir for 24 h. Then, the expression of miR-1231 in PANC-1 cells was detected by qRT-PCR. **(C)** The luciferase activity was measured after co-transfecting with WT/MT ABHD11-AS1 3'-UTR plasmid and miR-1231 agomir in PANC-1 cells using the dual luciferase reporter assay. The results were normalized to Renilla luciferase. **(D)** RNA pull-down was performed to confirm the correlation between ABHD11-AS1 and miR-1231. ** $P < 0.01$ compared to control.

ABHD11-AS1 shRNA1 Greatly Induces the Apoptosis of Pancreatic Cancer Cells

In order to test the cell apoptosis, flow cytometry was used. As demonstrated in Figure 3A and B, ABHD11-AS1 shRNA1 notably induced the apoptosis of PANC-1 cells. Meanwhile, as revealed in 3C-3F, the expressions of XIAP and Bcl-2 in PANC-1 cells were notably decreased in the presence of ABHD11-AS1 shRNA1. In contrast, knockdown of ABHD11-AS1 obviously upregulated the expression of active caspase 3 in pancreatic cancer cells (Figure 3C-F). Altogether, ABHD11-AS1 shRNA1 greatly induced the apoptosis of pancreatic cancer cells.

Downregulation of ABHD11-AS1 Obviously Inhibits the Migration and Invasion of PANC-1 Cells

Next, wound healing assay was performed to detect the effect of ABHD11-AS1 knockdown on cell migration. The data indicated that knockdown of ABHD11-AS1 notably

inhibited the migration of pancreatic cancer cells (Figure 4A and B). Consistently, the invasion ability of PANC-1 cells was significantly inhibited in the presence of ABHD11-AS1 shRNA1 (Figure 4C and D). In summary, downregulation of ABHD11-AS1 obviously inhibited the migration and invasion of PANC-1 cells.

ABHD11-AS1 Sponges with miR-1231

For the purpose of exploring the mechanism by which ABHD11-AS1 mediates the progression of pancreatic cancer, starbase (<http://starbase.sysu.edu.cn/>) and miRDB (<http://www.mirdb.org/>) were used. As indicated in Figure 5A, ABHD11-AS1 had a putative miR-1231 targeting site. Meanwhile, among the miRNAs sponged by ABHD11-AS1, miR-1231 was closely correlated with the tumorigenesis of pancreatic cancer.¹⁸ Thus, miR-1231 was selected for further investigations. Next, miR-1231 agomir and miR-1231 antagomir were stably transfected into PANC-1 cells (Figure 5B). We found co-transfection of the wild-type ABHD11-AS1 vector (WT-ABHD11-AS1) with miR-1231 agomir

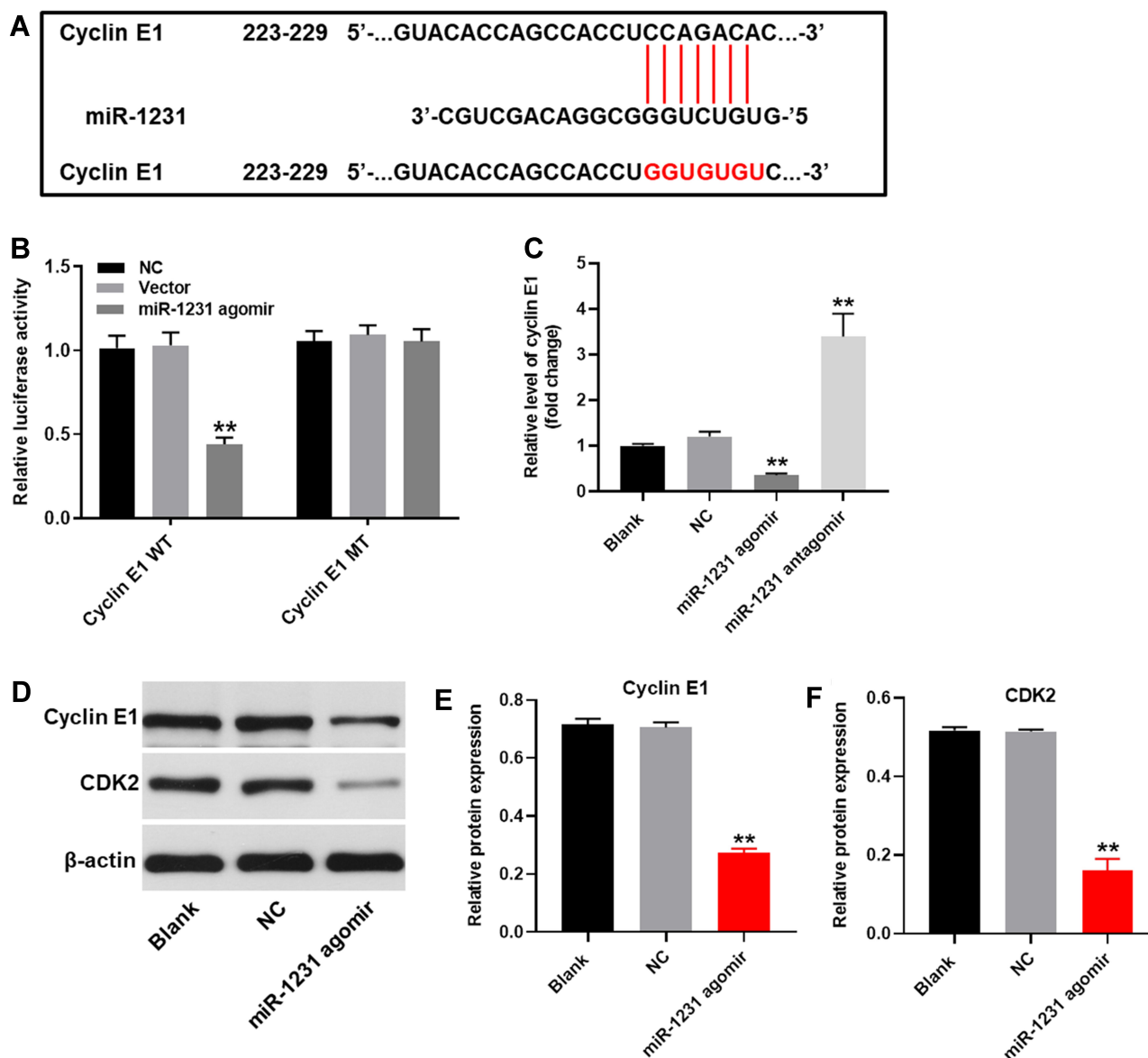


Figure 6 MiR-1231 directly targets Cyclin E1. (A) Gene structure of Cyclin E1 at the position of 223-229 indicates the predicted target site of miR-1231 in its 3'UTR. (B) The luciferase activity was measured after co-transfecting with WT/MT Cyclin E1 3'UTR plasmid and miR-1231 agomir in PANC-1 cells using the dual luciferase reporter assay. The results were normalized to Renilla luciferase. (C) The expression of Cyclin E1 in PANC-1 cells was detected by qRT-PCR. (D) The protein expressions of Cyclin E1 and CDK2 in PANC-1 cells were detected by western blot. (E, F) The relative protein expressions were quantified by normalizing to β-actin. **P<0.01 compared to control.

significantly reduced luciferase activities compared with mutant ABHD11-AS1 vector (MT-ABHD11-AS1) (Figure 5C). Furthermore, pull-down study confirmed ABHD11-AS1 bound to miR-1231 (Figure 5D). All these data revealed that ABHD11-AS1 regulated the tumorigenesis of pancreatic cancer via sponging miR-1231.

MiR-1231 Directly Targets cyclin E1

To find the target of miR-1231, TargetScan (http://www.targetscan.org/vert_71/) and dual-luciferase report

assay were performed. As revealed in Figure 6A and B, cyclin E1 was found to be the direct target of miR-1231. In addition, the expression of cyclin E1 in pancreatic cancer cells was significantly downregulated by miR-1231 agomir (Figure 6C). In contrast, miR-1231 antagonist notably increased the expression of cyclin E1 (Figure 6C). Furthermore, the protein expressions of cyclin E1 and CDK2 in PANC-1 cells were notably decreased by miR-1231 agomir (Figure 6D-F). All these results showed that miR-1231 directly targeted cyclin E1.

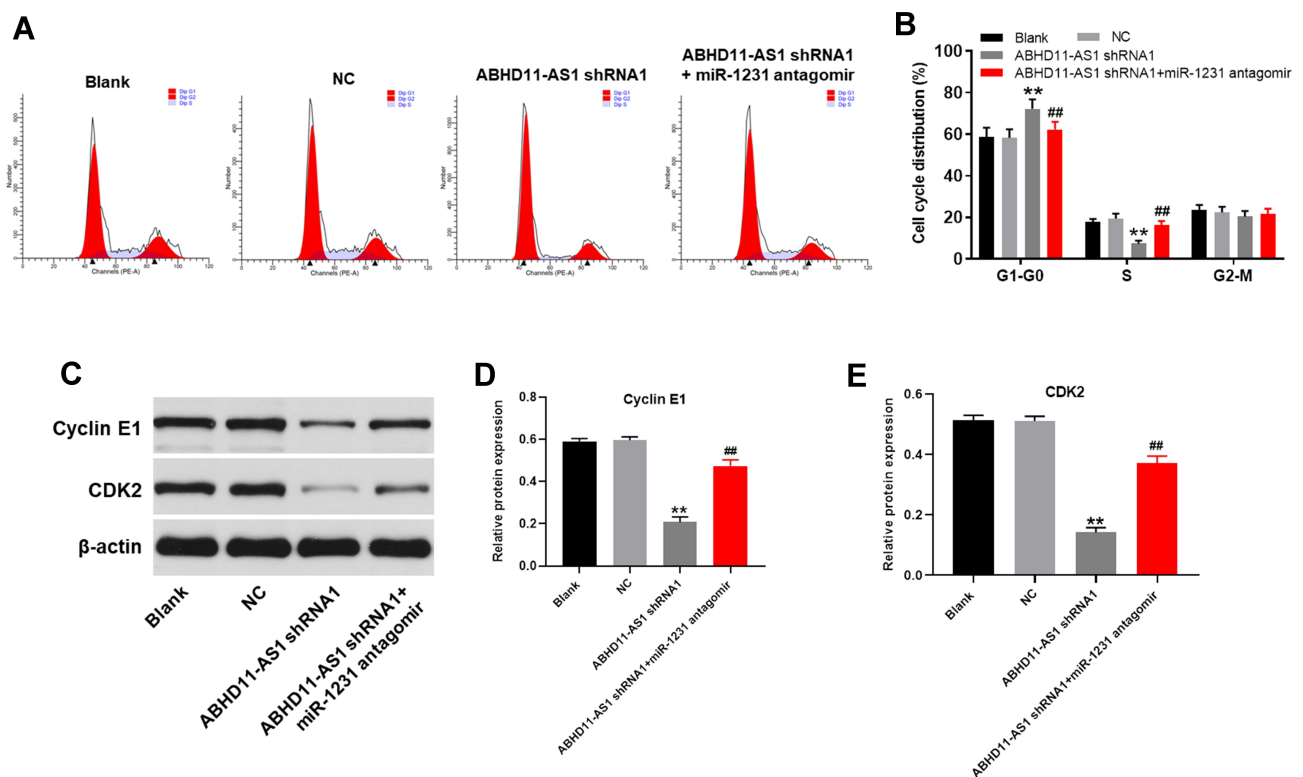


Figure 7 ABHD11-AS1 shRNA1-induced G1 arrest in pancreatic cancer cells is partially reversed by miR-1231 antagonist. **(A, B)** The cell cycle distribution in G0/G1, S, and G2 phase after propidium iodide staining of pancreatic cancer cells were determined by FACS. **(C)** The protein expressions of Cyclin E1 and CDK2 in PANC-1 cells were detected by western blot. **(D, E)** The relative protein expressions were quantified by normalizing to β -actin. ** $P < 0.01$ compared to control. ### $P < 0.01$ compared to ABHD11-AS1 shRNA1.

ABHD11-AS1 shRNA1-Induced G1 Arrest in Pancreatic Cancer Cells is Partially Reversed by miR-1231 Antagonist

In order to detect the effect of ABHD11-AS1 knockdown cell cycle, flow cytometry was used. As shown in Figure 7A and B, knockdown of ABHD11-AS1 significantly induced G1 arrest in pancreatic cancer cells, while ABHD11-AS1 knockdown-induced G1 arrest was partially reversed by miR-1231 antagonist. In addition, protein expressions of cyclin E1 and CDK2 were significantly downregulated by ABHD11-AS1 shRNA, while this phenomenon was partially reversed by miR-1231 antagonist (Figure 7C–E). Taken together, ABHD11-AS1 shRNA1-induced G1 arrest in pancreatic cancer cells is partially reversed by miR-1231 antagonist.

Knockdown of ABHD11-AS1 Significantly Inhibits the Tumor Growth of Pancreatic Cancer in vivo

Finally, xenograft mice model was established to investigate the role of ABHD11-AS1 in pancreatic cancer

in vivo. As showed in Figure 8A and B, ABHD11-AS1 shRNA1 significantly decreased tumor growth in vivo, while the anti-tumor effect of ABHD11-AS1 shRNA1 was partially abrogated by miR-1231 antagonist. Consistently, tumor weight in mice was greatly reduced in ABHD11-AS1 knockdown group compared with the control group (Figure 8C). However, ABHD11-AS1 shRNA1-induced decrease in tumor weight was notably reversed in the presence of miR-1231 antagonist (Figure 8C). Furthermore, the protein expressions of cyclin E1 and CDK2 in tumor tissues of mice were significantly down-regulated by ABHD11-AS1 shRNA1, while this phenomenon was significantly reversed by miR-1231 antagonist (Figure 8D–F). In summary, knockdown of ABHD11-AS1 significantly inhibited the tumor growth of pancreatic cancer in vivo.

Discussion

It has been confirmed that lncRNAs played a key role in the progression of pancreatic cancer.^{6,7} In the current study, we confirmed that downregulation of ABHD11-AS1 significantly inhibited the growth of pancreatic cancer cells. Wen J et al found that the expression of ABHD11-

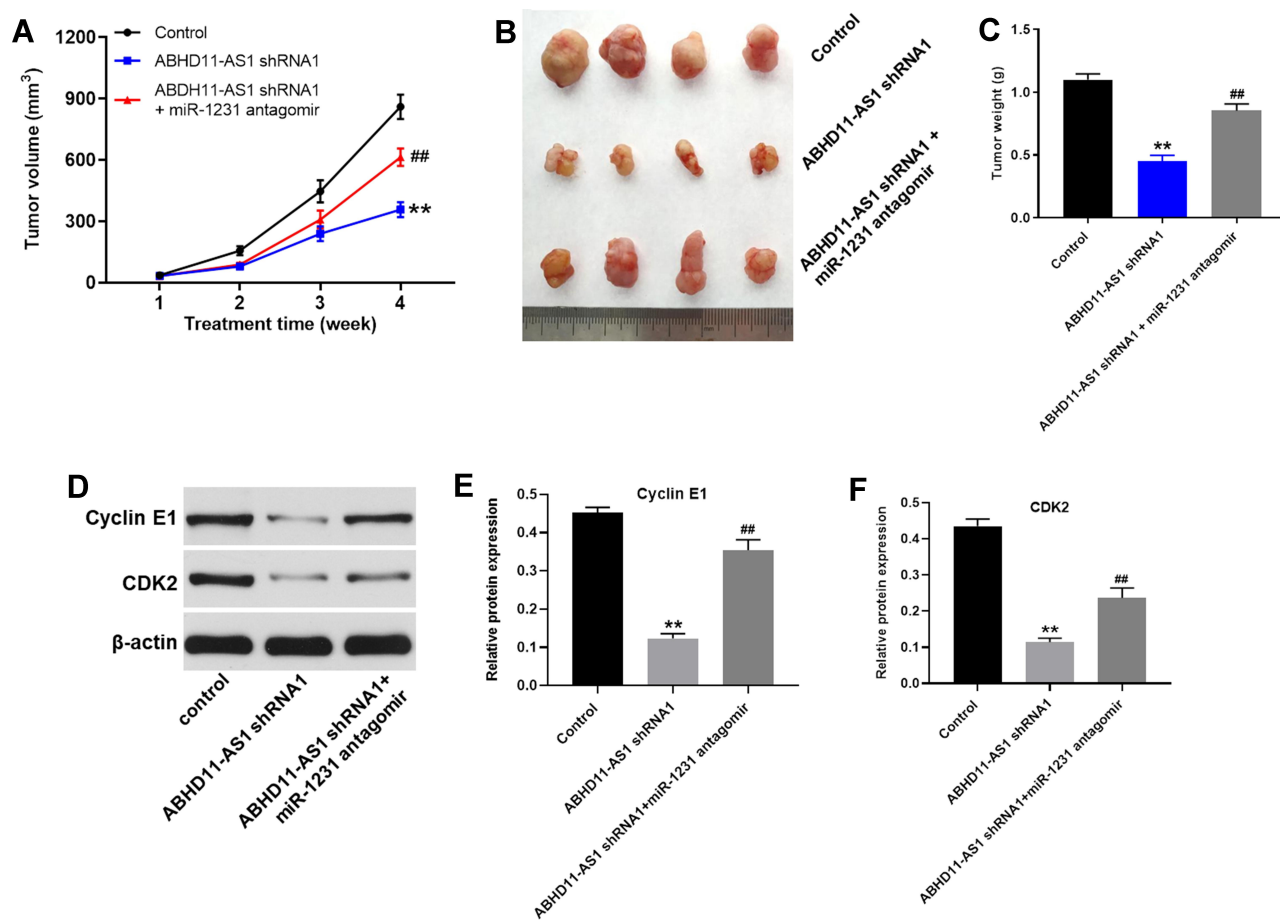


Figure 8 Knockdown of ABHD11-AS1 significantly inhibits the growth of pancreatic cancer in vivo. PANC-1 cells were subcutaneously injected into nude mice to establish xenograft mice model. ABHD11-AS1 shRNA1 or ABHD11-AS1 shRNA1 plus miR-1231 antagonist was directly injected into the tumors twice a week. (A) Tumor volumes of mice were measured weekly. (B) At the end of the study, tumor tissues of mice were collected and pictured. (C) Tumor weights in each group of mice were calculated. (D) The protein expressions of CDK2 and Cyclin E1 in tumor tissues of mice were detected by western blot. (E, F) The relative protein expressions were quantified by normalizing to β -actin. ** $P < 0.01$ compared to control. ## $P < 0.01$ compared to ABHD11-AS1 shRNA1.

AS1 was notably upregulated in papillary thyroid carcinoma (PTC).¹⁹ Our study was consistent with this research, suggesting that ABHD11-AS1 could act as a promoter during tumorigenesis. On the other hand, some studies have shown that ABHD11-AS1 participated in some malignancies. For instance, ABHD11-AS1 could promote the proliferation, migration and invasion of colorectal cancer cells via regulating the miR-1254-WNT11 pathway.²⁰ Meanwhile, Qiao Xi et al found ABHD11-AS1 could promote the growth of pancreatic cancer cells via PI3K/Akt signaling.²¹ The present study further explored the function of ABHD11-AS1, confirming that ABHD11-AS1 could play a key role in the development of pancreatic cancer.

The present study also found that knockdown of ABHD11-AS1 could induce the apoptosis of pancreatic cancer cells. Moreover, ABHD11-AS1 silencing

significantly upregulated the expression of Active caspase3 and reduced the levels of Bcl-2 and XIAP. Active caspase 3, XIAP and Bcl-2 were key regulators of cell apoptosis.^{22–24} Active caspase 3 has been verified to be the pro-apoptosis protein during the cell apoptosis,²⁵ while upregulation of Bcl-2 and XIAP could inhibit the apoptosis of cancer cells.²⁶ The present outcome was consistent with these data, indicating that ABHD11-AS1 silencing induced the apoptosis of pancreatic cancer cells through upregulating Active caspase3 and downregulating Bcl-2 and XIAP.

Next, the mechanism by which ABHD11-AS1 knockdown suppressed the progression of pancreatic cancer was explored. We found miR-1231 was the downstream miRNA of ABHD11-AS1. MiRNAs are considered to be differentially expressed in multiple diseases.^{27,28} Shang et al revealed that exosomal miRNA-1231 derived from bone marrow

mesenchymal stem cells could inhibit the activity of pancreatic cancer.¹⁸ Our research was consistent to this research, suggesting that miR-1231 could play inhibitory role in pancreatic cancer. Otherwise, a recent report found that ABHD11-AS1 mediated the progression of papillary thyroid cancer via sponging miR-199a-5p.²⁹ This difference may be due to different tumor types.

It has been confirmed that miRNAs could exert their biological function mainly by binding their target mRNAs.^{30,31} In the current study, we found cyclin E1 was a direct target of miR-1231, which has been regarded as a key regulator in cell cycle.³² In addition, CDK2 was found to be downregulated in pancreatic cancer cells in the presence of ABHD11-AS1 shRNA1. CDK2 has been confirmed to be cell cycle regulators in many cancers.^{33,34} The present study was consistent with these backgrounds, revealing that ABHD11-AS1 knockdown notably induced G1 arrest in pancreatic cancer cells through inactivating CDK2 and cyclin E1. Meanwhile, our finding indicated that ABHD11-AS1 regulated the tumorigenesis of pancreatic cancer via sponging miR-1231, which led to the dysfunction of miR-1231 according to competitive endogenous RNA network.^{35,36} Thus, it can be concluded that ABHD11-AS1 indirectly targets cyclin E1.

Frankly speaking, there are some limitations to this research. First, this research focused only on cell cycle-related proteins so far. In addition, only one miRNA was found to be sponged by ABHD11-AS1. Moreover, this research did not study the effect of ABHD11-AS1 shRNA on PI3K/Akt signaling. Therefore, more investigations are needed in the future.

In conclusion, knockdown of ABHD11-AS1 significantly suppressed the tumorigenesis of pancreatic cancer via mediation of miR-1231/cyclin E1 axis. Therefore, ABHD11-AS1 may serve as a new target for the treatment of pancreatic cancer.

Ethical Approval

All in vivo experiments were performed in accordance with the National Institutes of Health guide for the care and use of laboratory animals, following a protocol approved by the Ethics Committees of Henan Provincial People's Hospital.

Disclosure

The authors declared no competing interests in this study.

References

- Meng X, Ma J, Wang B, Wu X, Liu Z. Long non-coding RNA OIP5-AS1 promotes pancreatic cancer cell growth through sponging miR-342-3p via AKT/ERK signaling pathway. *J Physiol Biochem*. 2020.
- Yang Q, Wang J, Zhong P, et al. The clinical prognostic value of lncRNA FAM83H-AS1 in cancer patients: a meta-analysis. *Cancer Cell Int*. 2020;20:72.
- Choi HCW, Lam KO, Pang HHM, et al. Global comparison of cancer outcomes: standardization and correlation with healthcare expenditures. *BMC Public Health*. 2019;19(1):1065. doi:10.1186/s12889-019-7384-y
- Bender E. Will a test to detect early pancreatic cancer ever be possible? *Nature*. 2020;579(7800):S12–S13. doi:10.1038/d41586-020-00846-3
- Pausch TM, Aue E, Wirsik NM, et al. Metastasis-associated fibroblasts promote angiogenesis in metastasized pancreatic cancer via the CXCL8 and the CCL2 axes. *Sci Rep*. 2020;10(1):5420. doi:10.1038/s41598-020-62416-x
- Liu CS, Zhou Q, Zhang YD, Fu Y. Long noncoding RNA SOX2OT maintains the stemness of pancreatic cancer cells by regulating DEK via interacting with miR-200a/141. *Eur Rev Med Pharmacol Sci*. 2020;24(5):2368–2379.
- Dacheng W, Songhe L, Weidong J, et al. LncRNA SNHG3 promotes the growth and metastasis of colorectal cancer by regulating miR-539/RUNX2 axis. *Biomed Pharmacother*. 2020;125:110039. doi:10.1016/j.biopha.2020.110039
- Guo JQ, Yang ZJ, Wang S, et al. LncRNA SNHG16 functions as an oncogene by sponging miR-200a-3p in pancreatic cancer. *Eur Rev Med Pharmacol Sci*. 2020;24(4):1718–1724.
- Jin J, Xu H, Li W, et al. LINC00346 acts as a competing endogenous RNA regulating development of hepatocellular carcinoma via modulating CDK1/CCNB1 axis. *Front Bioeng Biotechnol*. 2020;8:54. doi:10.3389/fbioe.2020.00054
- Jin KT, Lu ZB, Lv JQ, Zhang JG. The role of long non-coding RNAs in mediating chemoresistance by modulating autophagy in cancer. *RNA Biol*. 2020;1–14. doi:10.12659/MSM.920725
- Gao J, Dai C, Yu X, Yin XB, Zhou F. Long noncoding RNA LINC00324 exerts protumorigenic effects on liver cancer stem cells by upregulating fas ligand via PU box binding protein. *FASEB J*. 2020;34(4):5800–5817. doi:10.1096/fj.201902705RR
- Wong CH, Li CH, He Q, et al. Ectopic HOTTIP expression induces noncanonical transactivation pathways to promote growth and invasiveness in pancreatic ductal adenocarcinoma. *Cancer Lett*. 2020;477:1–9. doi:10.1016/j.canlet.2020.02.038
- Bi S, Wang Y, Feng H, Li Q. Long noncoding RNA LINC00657 enhances the malignancy of pancreatic ductal adenocarcinoma by acting as a competing endogenous RNA on microRNA-433 to increase PAK4 expression. *Cell Cycle*. 2020;19(7):801–816. doi:10.1080/15384101.2020.1731645
- Liu Y, Feng W, Liu W, et al. Circulating lncRNA ABHD11-AS1 serves as a biomarker for early pancreatic cancer diagnosis. *J Cancer*. 2019;10(16):3746–3756. doi:10.7150/jca.32052
- Tang Z, Li C, Kang B, et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res*. 2017;45(W1):W98–W102. doi:10.1093/nar/gkx247
- Wang P, Liu Q, Zhao H, et al. miR-216a-targeting theranostic nanoparticles promote proliferation of insulin-secreting cells in type 1 diabetes animal model. *Sci Rep*. 2020;10(1):5302. doi:10.1038/s41598-020-62269-4
- Pichinuk E, Chalikh M, Benhar I, et al. In vivo anti-MUC1(+) tumor activity and sequences of high-affinity anti-MUC1-SEA antibodies. *Cancer Immunol Immunother*. 2020;69(7):1337–1352. doi:10.1007/s00262-020-02547-2

18. Shang S, Wang J, Chen S, et al. Exosomal miRNA-1231 derived from bone marrow mesenchymal stem cells inhibits the activity of pancreatic cancer. *Cancer Med*. 2019;8(18):7728–7740. doi:10.1002/cam4.2633
19. Wen J, Wang H, Dong T, et al. STAT3-induced upregulation of lncRNA ABHD11-AS1 promotes tumour progression in papillary thyroid carcinoma by regulating miR-1301-3p/STAT3 axis and PI3K/AKT signalling pathway. *Cell Prolif*. 2019;52(2):e12569. doi:10.1111/cpr.12569
20. He D, Yue Z, Liu L, et al. Long noncoding RNA ABHD11-AS1 promote cells proliferation and invasion of colorectal cancer via regulating the miR-1254-WNT11 pathway. *J Cell Physiol*. 2019;234(7):12070–12079. doi:10.1002/jcp.27877
21. Qiao X, Lv SX, Qiao Y, et al. Long noncoding RNA ABHD11-AS1 predicts the prognosis of pancreatic cancer patients and serves as a promoter by activating the PI3K-AKT pathway. *Eur Rev Med Pharmacol Sci*. 2018;22(24):8630–8639.
22. Huang YF, Lu L, Shen HL, Lu XX. LncRNA SNHG4 promotes osteosarcoma proliferation and migration by sponging miR-377-3p. *Mol Genet Genomic Med*. 2020;e1349.
23. Elisia I, Hay M, Cho B, et al. Low carbohydrate diets containing soy protein and fish oil slow the growth of established NNK-induced lung tumors. *Carcinogenesis*. 2020;41(8):1083–1093. doi:10.1093/carcin/bgaa028
24. Lohmann B, Rhun EL, Silginer M, Epskamp M, Weller M. Interferon-beta sensitizes human glioblastoma cells to the cyclin-dependent kinase inhibitor, TG02. *Oncol Lett*. 2020;19(4):2649–2656.
25. Jablonska O, Juchno D, Leska A, Kowalewska K, Boron A. Variable occurrence of apoptosis in the testes of diploid and sterile allotetraploid Cobitis (Teleostei, Cobitidae) males during the reproductive cycle. *J Exp Biol*. 2020;223(9):jeb212050. doi:10.1242/jeb.212050
26. Liu XF, Wang K, Du HC. LncRNA SNHG6 regulating Hedgehog signaling pathway and affecting the biological function of gallbladder carcinoma cells through targeting miR-26b-5p. *Eur Rev Med Pharmacol Sci*. 2020;24(14):7598–7611.
27. Wang J, Yao S, Diao Y, et al. miR-15b enhances the proliferation and migration of lung adenocarcinoma by targeting BCL2. *Thorac Cancer*. 2020.
28. Chen G, Chen Z, Zhao H. MicroRNA-155-3p promotes glioma progression and temozolomide resistance by targeting Six1. *J Cell Mol Med*. 2020.
29. Zhuang X, Tong H, Ding Y, et al. Long noncoding RNA ABHD11-AS1 functions as a competing endogenous RNA to regulate papillary thyroid cancer progression by miR-199a-5p/SLC1A5 axis. *Cell Death Dis*. 2019;10(8):620. doi:10.1038/s41419-019-1850-4
30. Li M, Ding X, Zhang Y, et al. Antisense oligonucleotides targeting lncRNA AC104041.1 induces antitumor activity through Wnt2B/beta-catenin pathway in head and neck squamous cell carcinomas. *Cell Death Dis*. 2020;11(8):672. doi:10.1038/s41419-020-02820-3
31. Chen F, Ni X, Chen L, Wang X, Xu J. miR-939-3p promotes epithelial-mesenchymal transition and may be used as a prognostic marker in hepatocellular carcinoma. *Oncol Lett*. 2020;19(4):2727–2732.
32. Zeng Y, Zhang Z, Wang W, et al. Underlying mechanisms of apoptosis in HepG2 cells induced by polyphyllin I through Fas death and mitochondrial pathways. *Toxicol Mech Methods*. 2020;1–10.
33. Luo JY, Yan SB, Chen G, et al. RNA-sequencing, connectivity mapping, and molecular docking to investigate ligand-protein binding for potential drug candidates for the treatment of wilms tumor. *Med Sci Monit*. 2020;26:e920725.
34. Zheng HK, Dong MH, Liu GH, et al. Dysregulation of the urothelial cancer associated 1 long noncoding RNA promotes proliferation of vascular smooth muscle cells by modulating expression of P27KIP1/CDK2. *Genet Test Mol Biomarkers*. 2020;24(4):204–211. doi:10.1089/gtmb.2019.0241
35. Hu D, Zhang B, Yu M, Shi W, Zhang L. Identification of prognostic biomarkers and drug target prediction for colon cancer according to a competitive endogenous RNA network. *Mol Med Rep*. 2020;22(2):620–632. doi:10.3892/mmr.2020.11171
36. Wang QY, Peng L, Chen Y, et al. Characterization of super-enhancer-associated functional lncRNAs acting as ceRNAs in ESCC. *Mol Oncol*. 2020;14(9):2203–2230. doi:10.1002/1878-0261.12726

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