Original Article

Downregulation of LINC01426 inhibits the proliferation and migration of human pancreatic cancer cells *in vivo* and *in vitro*

Zhixiang Ming[1,2], Ruoyi Zhang[3], Jianhua Xu[2], Wei Ding[4], Lei Qin[1]

[1]Department of General Surgery, The First Affiliated Hospital of Soochow University, Suzhou, China; [2]Department of General Surgery, Nantong Hospital of Traditional Chinese Medicine, Nantong, China; [3]Department of Encephalopathy, Nantong Hospital of Traditional Chinese Medicine, Nantong, China; [4]Department of Thoracic Surgery, Nantong Hospital of Traditional Chinese Medicine, Nantong, China

Contributions: (I) Conception and design: Z Ming; (II) Administrative support: None; (III) Provision of study materials or patients: R Zhang; (IV) Collection and assembly of data: W Ding, L Qin; (V) Data analysis and interpretation: J Xu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Lei Qin. Department of General Surgery, The First Affiliated Hospital of Soochow University, Suzhou, China. Email: aming6350@163.com.

Background: We performed *in vivo* and *in vitro* experiments to observe the expression of long intergenic nonprotein coding RNA 1426 (LINC01426) in pancreatic cancer tissues and cells, investigate the impact of LINC01426 on the proliferation and migration of pancreatic cancer cells, and deduce the underlying molecular mechanism.

Methods: LINC01426 expression was measured by fluorescence-based quantitative polymerase chain reaction (PCR) in pancreatic cancer tissues and cell lines. The lentiviral vector was used to create shRNA-NC, shLINC01426#2, pcDNA-NC, and pcDNA-LINC01426 pancreatic cancer cell lines. Cell Counting Kit-8 (CCK-8), clonogenic, and scratch tests were used to measure the effects of LINC01426 knockdown or overexpression on the migration and proliferation of pancreatic cancer cells. The expression levels of genes involved in migration (E-cadherin, N-cadherin, and vimentin) were examined by Western blotting after LINC01426 was either knocked down or overexpressed. The effect of LINC01426 knockdown on the proliferation of pancreatic cancer cells *in vivo* was verified in nude mice.

Results: LINC01426 was highly expressed in pancreatic cancer cells and tissues. Overexpression of LINC01426 might have promoted the proliferation, clonogenicity, and migration of pancreatic cancer cells, while knockdown of LINC01426 decreased these activities. In pancreatic cancer cells, knockdown of LINC01426 dramatically enhanced E-cadherin expression while lowering N-cadherin and vimentin expression, whereas overexpression of LINC01426 had the opposite effect. Knockdown of LINC01426 substantially decreased pancreatic cancer cell proliferation in an *in vivo* study.

Conclusions: Overexpression of LINC01426 was shown to increase the migration and proliferation of pancreatic cancer cells in both *in vivo* and *in vitro* experiments. LINC01426 could be a novel predictive biomarker and source of prospective therapeutic targets for patients with pancreatic cancer.

Keywords: Pancreatic cancer; LINC01426; proliferation; migration

Submitted Nov 01, 2022. Accepted for publication Dec 15, 2022.
doi: 10.21037/jgo-22-1167
View this article at: https://dx.doi.org/10.21037/jgo-22-1167

Introduction

Pancreatic cancer is a highly malignant tumor of the digestive system. It is clinically divided into several types, including ductal adenocarcinoma, a special type of ductal-origin cancer, acinar cell carcinoma, and small gland carcinoma, among others. The majority of cases, 85–90%, originate from the pancreatic ductal epithelium (1,2). The 5-year survival rate for pancreatic cancer is less than 1%

in both China and the United States, making it one of the deadliest tumors (3). The survival rate for those diagnosed with pancreatic cancer is dismal, and early diagnosis is uncommon. Scientists now think of tumor development as an evolutionary process that involves several genes and stages (4). Pancreatic cancer has been associated with alterations in the biological activity of a number of tumor suppressor genes, proto-oncogenes, and apoptotic genes (5). A number of recent studies have revealed that the tumor genome in pancreatic cancer is very unstable and prone to translocation, mutation, insertion or deletion, and aneuploidy. Genome-wide analysis has revealed genetic alterations in multiple core signaling pathways, including DNA replication, Kirsten rat sarcoma (*KRAS*), transforming growth factor beta (TGF-β), apoptosis, and alterations in cell cycle pathways (6,7). Consequently, our understanding of the pathogenesis of pancreatic cancer and the development of more potent and efficacious diagnostic markers for clinical prevention and treatment will benefit greatly from elucidating related molecules through research into the key molecular targets affecting the regulation of pancreatic cancer.

Increasing evidence suggests that several long noncoding RNAs (lncRNAs) are dysregulated in human malignancies. Although they lack the ability to code proteins, noncoding RNAs have attracted a great deal of research interest due to their potential role in the onset and progression of malignant illnesses through many mechanisms (8,9). LncRNAs have been shown to be potential biomarkers for the early diagnosis of pancreatic cancer (10,11), and they play an important role in the progression of this illness. Lung cancer, glioma, renal clear cell carcinoma, and other cancers all share a newly identified noncoding RNA, long intergenic nonprotein coding RNA 1426 (LINC01426), which plays a vital physiological regulatory role in their development and progression (12-14). A study has shown that LINC01426 can exert different molecular regulatory mechanisms, namely, as a spongy body to regulate microRNA (miRNA) expression or to regulate protein stability. One of the mechanisms through which LINC01426 promotes the progression of non-small cell lung cancer is the enhancement of ETS1 expression by modulating miRNA-519d-5p (15). Researchers have found that LINC01426 enhances the stability of SHH protein by binding to USP22 in tumor tissues, thereby promoting lung adenocarcinoma growth and stemness. Taken together, these findings highlight the nuanced and varied nature of LINC01426's regulatory processes. Although LINC01426's significance has been shown in many other kinds of tumors, its biological involvement in pancreatic cancer is still unclear. This study examined the expression and effects of LINC01426 in pancreatic cancer tissues and cell lines. The influence of cell biological function and the molecular mechanism of action were preliminarily discussed. We present the following article in accordance with the ARRIVE reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1167/rc).

Highlight box

Key findings

- LINC01426 could be a novel predictive biomarker and source of prospective therapeutic targets for patients with pancreatic cancer.

What is known and what is new?

- LINC01426 has been found to play an important role in the physiological regulation of various tumors (lung cancer, glioma, renal clear cell carcinoma).
- This study preliminarily investigated the molecular mechanism of LINC01426 in pancreatic cancer.

What is the implication, and what should change now?

- LINC01426 could be used as a potential predictive biomarker to help identify novel treatment targets for pancreatic cancer.

Methods

Clinical sample collection

A total of 50 pairs of tissue specimens stored in liquid nitrogen tanks at Nantong Hospital of Traditional Chinese Medicine were obtained from fresh tumors and adjacent tissues of pancreatic ductal adenocarcinoma patients after surgical resection and pathological verification. None of the patients had received radiotherapy or chemotherapy before surgery. The clinical case data and follow-up data of these 50 patients were collected, including age, lymph node metastasis, gender, distant metastasis, degree of differentiation, survival time, tumor size, and American Joint Committee on Cancer (AJCC) clinical stage, among others.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Nantong Hospital of Traditional Chinese Medicine (No. 2022-004-16) and

informed consent was taken from all the patients.

Cell culture technology

The Shanghai Cell Bank, Chinese Academy of Sciences, provided PANC-1, MIA PaCa-2, AsPC-1, SW1990, BxPC-3, Capan-1, CEPAC-1, and HPNE pancreatic cancer cell lines. In a humidified 5% CO_2, 37 ℃ incubator, pancreatic cancer cells were grown in Dulbecco's Modified Eagle *Medium* (DMEM) with 1% penicillin-streptomycin and 10% fetal bovine serum. After seeding cells on a 6-well plate when they were in the logarithmic growth phase, we used lentivirus transfection to introduce knockdown and overexpression lentiviruses for LINC01426. shRNA-NC, shLINC01426#2, pcDNA-NC, and pcDNA-LINC01426 pancreatic cancer cell lines were created by GenePharma (Shanghai, China). Six hours after transfection, new medium was added and the cells expanded for 48 hours.

Fluorescence quantitative polymerase chain reaction (PCR) detection

The expression of LINC01426 was assessed utilizing a reverse transcription kit to produce a reaction from messenger RNA (mRNA), with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) acting as a housekeeping gene. Total RNA was extracted from surrounding tissues, cancerous tissues, and pancreatic cancer cell lines using the TRIzol method. We amplified an endogenous control using a real-time PCR instrument, and we used the $2^{-\Delta\Delta CT}$ technique to determine relative expression. The primers for LINC01426 and GAPDH sequences were used according to the previous report (12,13).

Cell Counting Kit-8 (CCK-8) detection of cell viability

Transfected cells were harvested and analyzed for their proliferation capacity using a CCK-8 kit and 4 different groups of pancreatic cancer cells: shRNA-NC, shLINC01426#2, pcDNA-NC, and pcDNA-LINC01426. Cells were sown in a 96-well plate, housed in a 5% CO_2 incubator at 37 ℃, and detected at 5-time intervals (0, 24, 48, 72, and 96 hours) after transfection. After adding 10 μL/well CCK-8 solution, a microplate reader was used to measure the absorbance.

Clonogenic assay

Single-cell suspensions were prepared from pancreatic cancer cells in the logarithmic growth phase from the shRNA-NC group, the shLINC01426#2 group, the pcDNA-NC group, and the pcDNA-LINC01426 group after transfection. Cells were plated in 6-well plates and monitored every 3 days. After 2 weeks, the medium was discarded, the cells were fixed in 4% paraformaldehyde for 15 minutes, and stained with 1% crystal violet solution. We then took pictures of the cells and analyzed the colony formation rate by determining the number of clones based on a cell mass of ≥50 cells.

Cell scratch assay

Each 6-well plate was injected with cells from either the shRNA-NC group, the shLINC01426#2 group, the pcDNA-NC group, or the pcDNA-LINC01426#2 group, all of which were pancreatic cancer cells in the logarithmic growth phase. When cell confluence reached 90% on the second day, we used a vertically-positioned 20-μL pipette tip against a ruler to make a scratch perpendicular to the horizontal line made earlier with a marker pen. The culture medium was then discarded and the cells were washed 3 times with phosphate-buffered saline (PBS) before 2 mL of 1% fetal bovine serum DMEM medium was added to the wells. The cells were then cultured in a 5% CO_2 incubator at 37 ℃. Photos were taken 24 hours after the scratches were made, and the cell scratch widths were analyzed.

Western blot detection

The logarithmic-phase pancreatic cancer cells from the shRNA-NC group, shLINC01426#2 group, pcDNA-NC group, and pcDNA-LINC01426 group were acquired. A protein concentration of 40 μg/well was utilized to electrophoretically separate protein on a sodium dodecyl sulfate-polyacrylamide gel and transfer it to a polyvinylidene fluoride (PVDF) membrane. Before incubating with the primary antibody [anti-N-cadherin (Abcam, 1:1,000), anti-vimentin (Abcam, 1:1,000), anti-E-cadherin (CST, 1:800), anti-GAPDH (Abcam, 1:800)] overnight at 4 ℃, the PVDF membrane was blocked with 5% skim milk for 2 hours at room temperature. The internal protein standard, GAPDH,

was incubated with a secondary antibody for 2 hours at room temperature after membrane washing.

Nude mice subcutaneous tumor formation experiment

We obtained 12 male BALB/c nude mice aged 4–6 weeks and weighing 18–20 g each from the Laboratory Animal Center, Nantong University, and the animal experiments were done in the animal Laboratory of Nantong University. In order to study the effects of LINC01426 on the development of nude mice, 12 mice were bred and raised in two groups. Each set of cells was trypsin-digested, centrifuged, counted, resuspended at a density of 1×10^7/mL (in PBS or serum-free media), and then subcutaneously injected into the nude mice in a volume of 100 μL. A subcutaneous tumor was measured in nude mice every 5 days for volume (tumor volume = $0.5 \times L \times W^2$) using Vernier calipers. Once the tumor reached a certain size, the mice were uniformly euthanized, and the tumor that had been implanted subcutaneously was removed, sized, and photographed.

Animal experiments were performed under a project license (No. P20221209-1003) granted by the Experimental Animal Ethics Committee of Nantong University, in compliance with national guidelines for the care and use of animals. A protocol was prepared before the study without registration.

Statistical analysis

Data were analyzed using SPSS 19.0, and the mean ± SD was used to summarize the data. The statistical significance of the differences between the groups was determined using independent samples *t*-test on measurement data and one-way analysis of variance.

Results

Pancreatic tumors express LINC01426

LINC01426 expression in pancreatic cancer tissues was obtained and evaluated via Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/). Tumor tissues showed dramatically increased expression of LINC01426 (*Figure 1A*), and receiver operating characteristic (ROC) curve analysis using adjacent tissue as the control group confirmed the diagnostic utility of LINC01426 for pancreatic cancer (*Figure 1B*). Patient survival was shown to be considerably lower in the LINC01426-high group compared to the LINC01426-low group (*Figure 1C*).

We used real-time quantitative PCR to determine the expression levels of LINC01426 in 50 premalignant and malignant pancreatic tissues. Compared to nearby normal tissues, pancreatic cancer tissues revealed a dramatic rise in LINC01426 expression (*Figure 1D*). Finally, we compared LINC01426 expression with the clinicopathological features of patients with pancreatic cancer. Patients with TNM stage III–IV and tumors larger than 3 cm had significantly higher LINC01426 expression levels compared to those with smaller tumors. Expression of LINC01426 was considerably greater in moderately and well-differentiated patients compared to poorly-differentiated patients in stages I and II (*Table 1*).

Expression of LINC01426 in pancreatic cancer cell lines

We used real-time PCR to investigate LINC01426 expression in 8 different human cell lines: 1 normal pancreatic ductal epithelial cell line (HPNE), and 7 different human pancreatic cancer cell lines (AsPC-1, PANC-1, Capan-1, BxPC-3, MIA PaCa-2, SW1990, and CEPAC-1). According to the results, LINC01426 expression was found to be highest in Capan-1 and lowest in PANC-1 (*Figure 2A*), which suggested that LINC01426 played a biological function in pancreatic cancer cells. In light of these findings, we infected Capan-1 cells with LINC01426 knockdown lentiviruses (shLINC01426#1 and shLINC01426#2) and used real-time quantitative PCR to identify their presence. The findings indicated that in both shLINC01426#1 and shLINC01426, the level of LINC01426 was greatly reduced in group #2, with the knockdown impact of LINC01426 in shLINC01426 being more evident in group #2 (*Figure 2B*). Transfection of the lentiviral plasmid LINC01426 resulted in overexpression of the gene in PANC-1 cells, which was then identified by fluorescence-based quantitative PCR. The overexpression group (pcDNA-LINC01426) showed significantly increased LINC01426 expression compared to that of the control group (pcDNA-NC) (*Figure 2C*).

LINC01426 stimulates pancreatic cancer cell growth

The CCK-8 experiment showed that knocking down LINC01426 (shLINC01426#2) in Capan-1 cells dramatically decreased pancreatic cancer cell proliferation

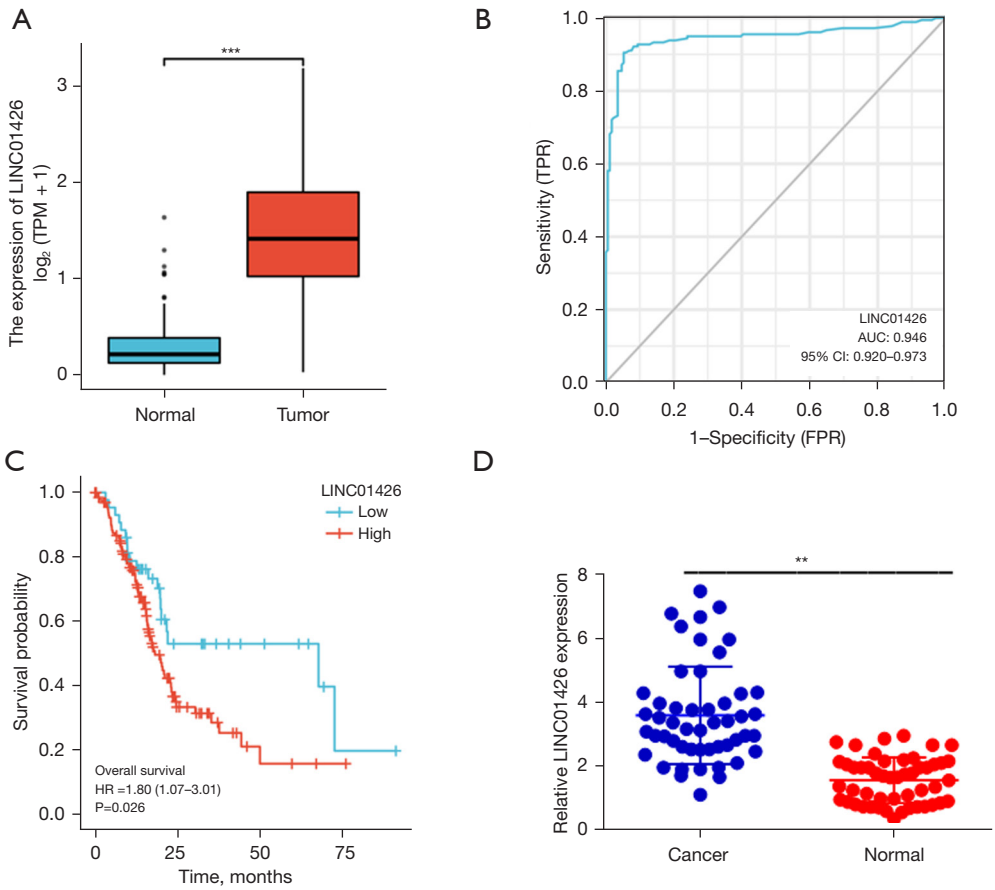

Figure 1 Expression of LINC01426 in pancreatic cancer. (A) Analysis of the GEPIA database shows that LINC01426 is substantially expressed in pancreatic cancer tissues. (B) ROC curve analysis shows that the high expression of LINC01426 in tumor tissue has diagnostic value for pancreatic cancer. (C) GEPIA database analysis of the survival time of 176 pancreatic cancer patients. (D) Fluorescence-based quantitative PCR detection analysis of LINC01426 expression in pancreatic cancer. **, P<0.01; ***, P<0.001. LINC01426, long intergenic nonprotein coding RNA 1426; TPM, transcripts per million; ROC, receiver operating characteristic curve; AUC, area under the curve; CI, confidence interval; HR, hazard ratio; GEPIA, Gene Expression Profiling Interactive Analysis; PCR, polymerase chain reaction.

compared to the shRNA-NC control group (*Figure 3A*). Concurrently, a lentiviral vector overexpressing LINC01426 transfected into PANC-1 cells increased pancreatic cancer cells' replicative potential (*Figure 3B*). Plate colony formation tests revealed that in Capan-1 cells, knockdown of LINC01426 (shLINC01426#2) greatly reduced the number of colonies produced (*Figure 3C*). In PANC-1 cells, overexpression of LINC01426 (pcDNA-LINC01426) significantly increased the number of cell clones (*Figure 3D*). These findings suggested that LINC01426 may have promoted pancreatic cancer development.

LINC01426 encourages metastasis of pancreatic cancer cells

An early cell scratch migration assay revealed LINC01426's effect on pancreatic cancer cell migration by observing the relative ratio of the blank area after scratching, which reflected the cells' capacity to migrate. The results exhibited that in Capan-1 cells, knockdown of LINC01426 (shLINC01426#2) significantly reduced pancreatic cancer cell migration compared to the control group (shRNA-NC) (*Figure 4A*). However, overexpression of LINC01426

Table 1 Analysis of associations between patient clinicopathological features and LINC01426 expression

Pathological features	n	LINC01426 expression (mean ± standard deviation)	*t*	P
Gender			0.1799	0.8580
Female	22	3.59±1.51		
Male	28	3.67±1.60		
Age			0.4005	0.6906
≤60 years	19	3.48±1.46		
>60 years	31	3.66±1.59		
Tumor size			2.046	0.0462
≤3 cm	21	3.26±1.15		
>3 cm	29	4.08±1.84		
Degree of differentiation			2.550	0.0140
Medium/high differentiation	27	4.13±1.52		
Poor differentiation	23	3.05±1.46		
Lymph node metastasis			1.910	0.0622
No	21	3.15±1.33		
Yes	29	3.92±1.46		
Tumor position			1.212	0.2315
Head of pancreas	20	3.19±1.41		
Pancreas tail	30	3.72±1.58		
TNM staging			2.482	0.0166
I–II	24	2.95±1.17		
III–IV	26	3.84±1.35		

LINC01426, long intergenic nonprotein coding RNA 1426; TNM, tumor/lymph nodes/metastasis.

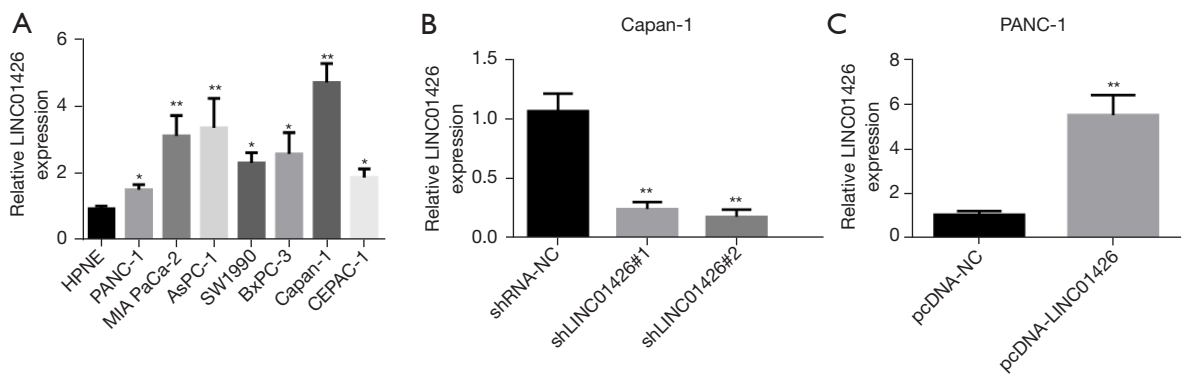

Figure 2 LINC01426 expression in pancreatic cancer cell lines. (A) Detection of lncRNA LINC01426 expression by real-time quantitative PCR. (B) Transfection and knockdown of the LINC01426 lentiviral vector in Capan-1. (C) Transfection of PANC-1 cells with the LINC01426 overexpressing lentiviral vector. *, P<0.05; **, P<0.01. LINC01426, long intergenic nonprotein coding RNA 1426; PCR, polymerase chain reaction; NC, negative control.

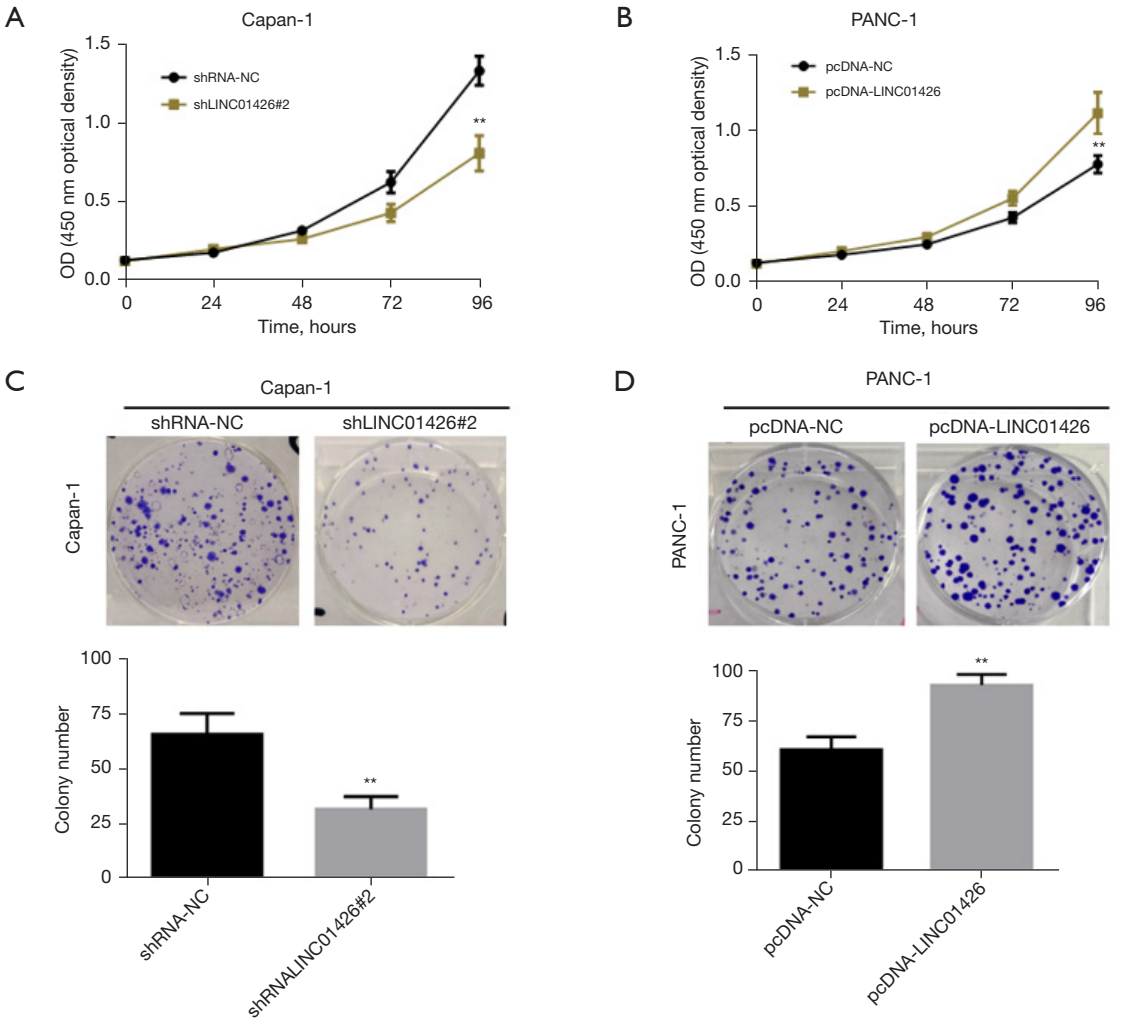

Figure 3 *In vitro* tests confirm the impact of LINC01426 expression on pancreatic cancer cell growth. (A,B) Plate clone creation assay to determine how LINC01426 knockdown or overexpression affects pancreatic cancer cells' capacity to produce clones. (C,D) CCK-8 assay to assess the impact of LINC01426 knockdown or overexpression on the proliferation of pancreatic cancer cells (crystal violet staining). **, P<0.01. LINC01426, long intergenic nonprotein coding RNA 1426; OD, optical density; NC, negative control; CCK-8, Cell Counting Kit-8.

(pcDNA-LINC01426) after transfection of LINC01426 lentiviral vector significantly promoted pancreatic cancer cell migration compared to the control group (pcDNA-NC) (*Figure 4B*).

To investigate LINC01426's effect on pancreatic cancer cell migration, Western blotting was used to measure vimentin, E-cadherin, and N-cadherin levels. E-cadherin protein expression was considerably lower in pancreatic cancer cells overexpressing LINC01426 (pcDNA-LINC01426) compared to the control group (pcDNA-NC) (*Figure 5*). E-cadherin was overexpressed in pancreatic cells with knocked down LINC01426, while vimentin and N-cadherin expression decreased.

LINC01426 knockdown prevents tumor development in nude mice with subcutaneously implanted tumors

After subcutaneous injection of transfected Capan-1 cells from the control group (shRNA-NC) and the knockdown LINC01426 group (shLINC01426#2) into nude mice, tumor volume was measured daily from day 10 to day 35, after which tumor tissue was removed by a surgical procedure and photographed. Compared to the control group (shRNA-NC), the volume of tumor-bearing tissue

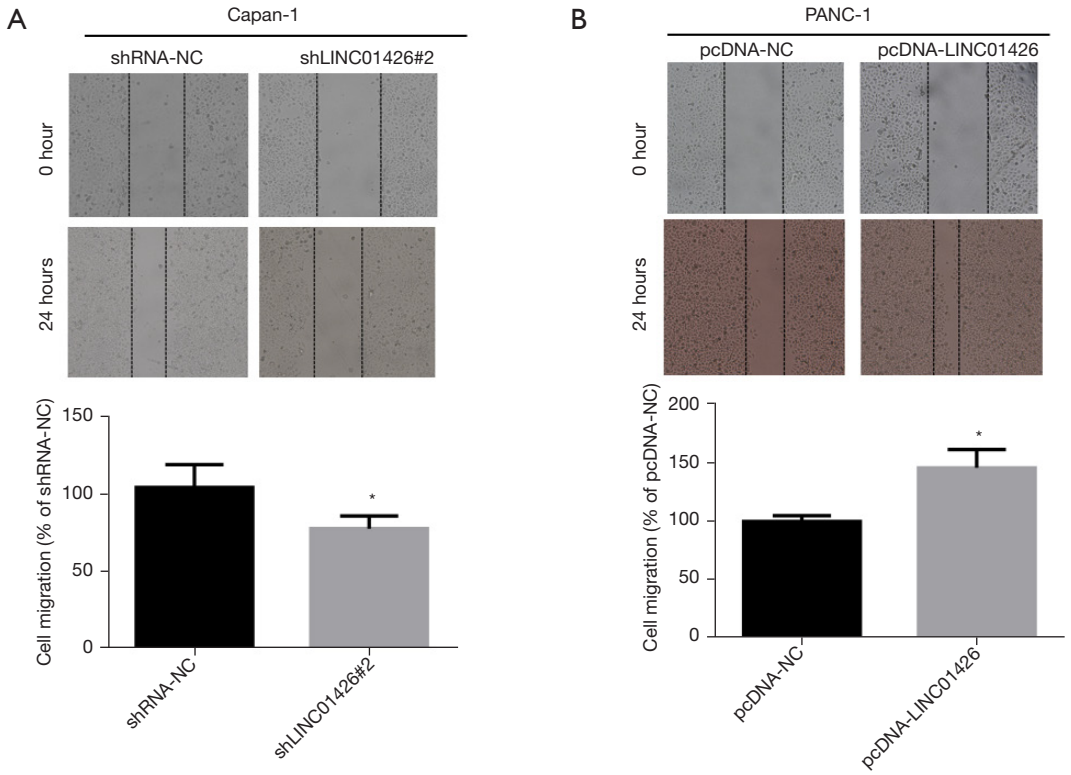

Figure 4 LINC01426 affects the ability of pancreatic cancer cells to migrate. The cell scratch test shows how LINC01426's knockdown (A) or overexpression (B) affects the capacity of pancreatic cancer cells to migrate (microscope, ×100). *, P<0.05. LINC01426, long intergenic nonprotein coding RNA 1426; NC, negative control.

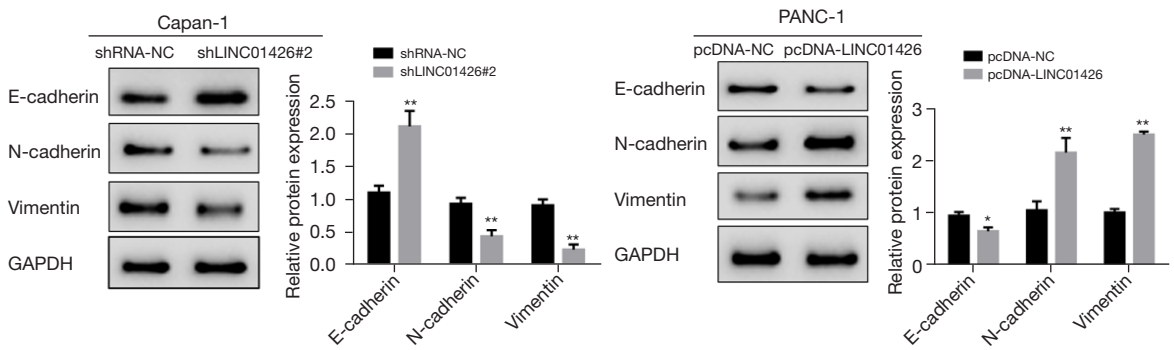

Figure 5 LINC01426 affects the expression of proteins involved in migration, including vimentin, E-cadherin, and N-cadherin, in pancreatic cancer cells. *, P<0.05; **, P<0.01. LINC01426, long intergenic nonprotein coding RNA 1426; NC, negative control; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

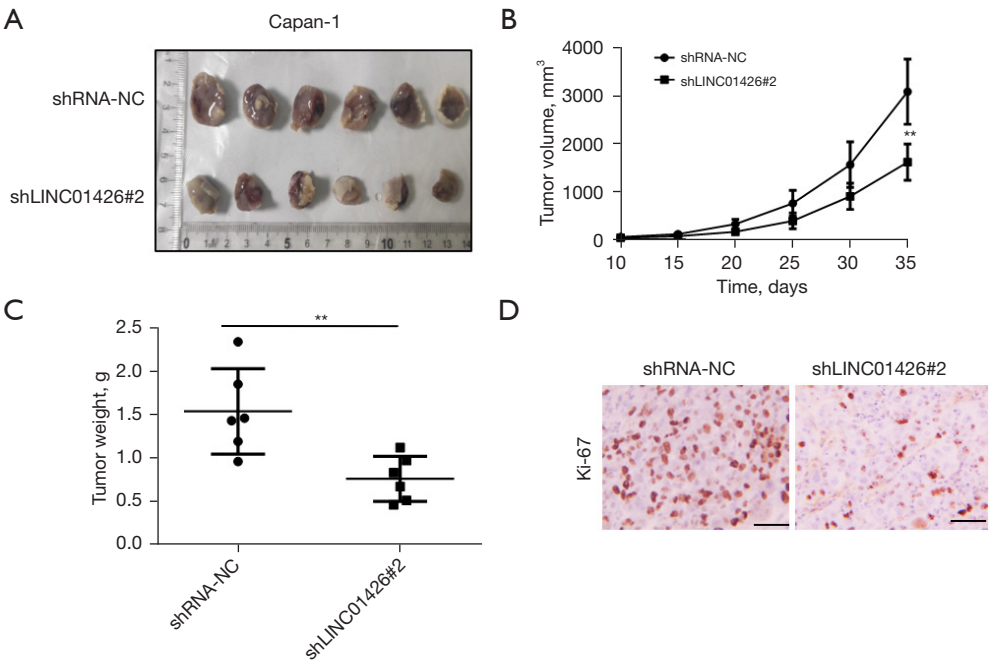

Figure 6 In nude mice, LINC01426 may slow the formation of subcutaneous tumors. (A) After transfection, Capan-1 cells, including control group (shRNA-NC) and knockdown LINC01426 (shLINC01426#2) cells, were subcutaneously injected into mice, and formation of subcutaneous tumors was compared on the 35th day. (B) The subcutaneous tumor volume was calculated every 5 days starting on the 10th day of inoculation, and a formed volume curve was drawn. (C) Tumor mass. (D) Ki-67 protein expression in tissues, scale bar: 50 μm (immunohistochemical staining). **, P<0.01. NC, negative control; LINC01426, long intergenic nonprotein coding RNA 1426.

generated in the knockdown LINC01426 (shLINC01426#2) group was much lower (*Figure 6A*). On days 30 and 35 after inoculation, the knockdown LINC01426 group had significantly fewer tumors than the control group (shRNA-NC) (*Figure 6B*). Finally, the weight of the peeled tumor tissue demonstrated that the shLINC01426#2 group had considerably less tumor-bearing tissue than the shRNA-NC group (*Figure 6C*). After tumor tissue was peeled and stained with immunohistochemistry, Ki-67 protein expression was high in shRNA-NC and low in shLINC01426#2 (*Figure 6D*). Based on these findings, knocking down LINC01426 in nude mice appeared to slow the development of subcutaneous tumors.

Discussion

Rapid advances in molecular biology have allowed researchers to uncover several gene molecules and signaling pathways connected to pancreatic cancer, providing crucial insight into the disease's etiology and pathogenesis (4). However, pancreatic cancer detection and therapy have not improved substantially. Therefore, continuing in-depth research into the pathogenesis of pancreatic cancer and pursuing targeted therapeutic strategies for key molecules is of utmost clinical significance and scientific benefit. This study confirmed that pancreatic cancer tissues and cell lines express LINC01426 at significant levels. LINC01426 knockdown decreased cancer proliferation, clonal growth, and migration, while overexpression increased them. LINC01426 knockdown increased E-cadherin expression in pancreatic cancer cells, whereas overexpression decreased it. LINC01426 knockdown dramatically decreased pancreatic cancer cell growth *in vivo*.

Several clinical and basic investigations have demonstrated that lncRNAs are implicated in molecular mechanisms (16-18). Some lncRNAs regulate the incidence and development of pancreatic cancer. LncRNAs affect proliferation, apoptosis, invasion, migration, treatment resistance, and angiogenesis of resistance (10,19,20). LncRNA plasmacytoma variant translocation 1 (PVT1) stimulates the Wnt/β-catenin pathway, promoting resistance to gemcitabine in pancreatic cancer (21). PVT1

regulates miR-619-5p/Pygo2/ATG14. PMSB8-AS1 regulates Signal transducers and activators of transcription (STATs) expression and sponges miR-382-3p to promote pancreatic cancer (22). Understanding how these lncRNAs affect cellular biology is essential to understanding pancreatic cancer's pathogenic mechanism. We first found that pancreatic cancer tissues and cell lines expressed LINC01426 at high levels. When LINC01426 was knocked down, pancreatic cancer cell proliferation and clonogenicity were considerably suppressed, but when LINC01426 was overexpressed, they were significantly stimulated.

Cancer cell invasion and tumor metastasis are intricate processes. The main pathological changes involve malignant tumor cells breaking through the basement membrane and infiltrating the surrounding tissue, and tumor cells from the primary site traveling via lymphatic and blood circulation to distant organs (23). Most pancreatic cancer patients develop distant metastases to the liver, lungs, or other organs (24,25). The results of both national and international research have found that several lncRNAs impact epithelial-mesenchymal transition and other pathways, which affect pancreatic cancer invasion and metastasis. AGAP2-AS1 decreases ANGPTL4 and ANKRD1 to inhibit pancreatic cancer development and migration (26). The lncRNA DANCR regulates miRNA-33b, which promotes pancreatic cancer growth (27). These results imply that lncRNAs control pancreatic cancer's development and may be a molecular target for targeted therapy. A scratch experiment showed that reduced LINC01426 expression slowed pancreatic cancer cell migration, whereas enhanced expression accelerated it. Overexpression of LINC01426 had the opposite impact of knockdown on vimentin, E-cadherin, and N-cadherin expression in pancreatic cancer cells, as shown by analyses of the underlying molecular pathways. Knockdown of LINC01426 dramatically decreased pancreatic cancer cell growth identified via *in vivo* tumorigenic testing using nude mice. In addition, it was well known that lncRNAs serve as competing endogenous RNAs (ceRNAs) through competing with mRNAs to bind with miRNAs. So in the following study, we will further explore the mechanism of LINC01426 in pancreatic cancer.

Conclusions

In conclusion, LINC01426 was substantially expressed in pancreatic cancer tissues and cell lines. LINC01426 knockdown decreased cancer proliferation, clonal growth, and migration. When LINC01426 was overexpressed, the opposite was observed. Further, LINC01426 knockdown markedly reduced pancreatic cancer cell proliferation in an *in vivo* experiment. Our results suggest that LINC01426 could be used as a potential predictive biomarker to help identify novel treatment targets for pancreatic cancer.

Acknowledgments

Funding: This study was supported by the Research project of Nantong Municipal Health Commission (No. MS2022035).

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1167/rc

Data Sharing Statement: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1167/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1167/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Nantong Hospital of Traditional Chinese Medicine (No. 2022-004-16) and informed consent was taken from all the patients. Animal experiments were performed under a project license (No. P20221209-1003) granted by the Experimental Animal Ethics Committee of Nantong University, in compliance with national guidelines for the care and use of animals.

References

1. Wood LD, Canto MI, Jaffee EM, et al. Pancreatic Cancer: Pathogenesis, Screening, Diagnosis, and Treatment. Gastroenterology 2022;163:386-402.e1.
2. Cai J, Chen H, Lu M, et al. Advances in the epidemiology of pancreatic cancer: Trends, risk factors, screening, and prognosis. Cancer Lett 2021;520:1-11.
3. Xia C, Dong X, Li H, et al. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. Chin Med J (Engl) 2022;135:584-90.
4. Lee JJ, Bernard V, Semaan A, et al. Elucidation of Tumor-Stromal Heterogeneity and the Ligand-Receptor Interactome by Single-Cell Transcriptomics in Real-world Pancreatic Cancer Biopsies. Clin Cancer Res 2021;27:5912-21.
5. Chen X, Zeh HJ, Kang R, et al. Cell death in pancreatic cancer: from pathogenesis to therapy. Nat Rev Gastroenterol Hepatol 2021;18:804-23.
6. Connor AA, Gallinger S. Pancreatic cancer evolution and heterogeneity: integrating omics and clinical data. Nat Rev Cancer 2022;22:131-42.
7. Yakar DÖ, Bozkırlı BO, Ceyhan GO. Genetic landscape of pancreatic cancer: a narrative review. Chin Clin Oncol 2022;11:5.
8. Huang W, Li H, Yu Q, et al. LncRNA-mediated DNA methylation: an emerging mechanism in cancer and beyond. J Exp Clin Cancer Res 2022;41:100.
9. Yan H, Bu P. Non-coding RNA in cancer. Essays Biochem 2021;65:625-39.
10. Xu J, Xu J, Liu X, et al. The role of lncRNA-mediated ceRNA regulatory networks in pancreatic cancer. Cell Death Discov 2022;8:287.
11. Ghafouri-Fard S, Fathi M, Zhai T, et al. LncRNAs: Novel Biomarkers for Pancreatic Cancer. Biomolecules 2021;11:1665.
12. Jiang Y, Zhang H, Li W, et al. LINC01426 contributes to clear cell renal cell carcinoma progression by modulating CTBP1/miR-423-5p/FOXM1 axis via interacting with IGF2BP1. J Cell Physiol 2021;236:427-39.
13. Liu X, Yin Z, Xu L, et al. Upregulation of LINC01426 promotes the progression and stemness in lung adenocarcinoma by enhancing the level of SHH protein to activate the hedgehog pathway. Cell Death Dis 2021;12:173.
14. Wang SJ, Wang H, Zhao CD, et al. Long noncoding RNA LINC01426 promotes glioma progression through PI3K/AKT signaling pathway and serves as a prognostic biomarker. Eur Rev Med Pharmacol Sci 2018;22:6358-68.
15. Dai J, Wang B, Zhao Y, et al. Long Noncoding RNA LINC01426 Sequesters microRNA-519d-5p to Promote Non-Small Cell Lung Cancer Progression by Increasing ETS1 Expression. Cancer Manag Res 2020;12:12697-708.
16. Xu F, Huang M, Chen Q, et al. LncRNA HIF1A-AS1 Promotes Gemcitabine Resistance of Pancreatic Cancer by Enhancing Glycolysis through Modulating the AKT/YB1/HIF1α Pathway. Cancer Res 2021;81:5678-91.
17. Li J, Zhang J, Tao S, et al. Prognostication of Pancreatic Cancer Using The Cancer Genome Atlas Based Ferroptosis-Related Long Non-Coding RNAs. Front Genet 2022;13:838021.
18. Zhang X, Zheng S, Hu C, et al. Cancer-associated fibroblast-induced lncRNA UPK1A-AS1 confers platinum resistance in pancreatic cancer via efficient double-strand break repair. Oncogene 2022;41:2372-89.
19. Takahashi K, Taniue K, Ono Y, et al. Long Non-Coding RNAs in Epithelial-Mesenchymal Transition of Pancreatic Cancer. Front Mol Biosci 2021;8:717890.
20. Li J, Hou S, Ye Z, et al. Long Non-Coding RNAs in Pancreatic Cancer: Biologic Functions, Mechanisms, and Clinical Significance. Cancers (Basel) 2022;14:2115.
21. Zhou C, Yi C, Yi Y, et al. LncRNA PVT1 promotes gemcitabine resistance of pancreatic cancer via activating Wnt/β-catenin and autophagy pathway through modulating the miR-619-5p/Pygo2 and miR-619-5p/ATG14 axes. Mol Cancer 2020;19:118.
22. Zhang H, Zhu C, He Z, et al. LncRNA PSMB8-AS1 contributes to pancreatic cancer progression via modulating miR-382-3p/STAT1/PD-L1 axis. J Exp Clin Cancer Res 2020;39:179.
23. Babaei G, Aziz SG, Jaghi NZZ. EMT, cancer stem cells and autophagy; The three main axes of metastasis. Biomed Pharmacother 2021;133:110909.
24. Ren B, Cui M, Yang G, et al. Tumor microenvironment participates in metastasis of pancreatic cancer. Mol Cancer 2018;17:108.
25. Wang SS, Xu J, Ji KY, et al. Epigenetic Alterations in Pancreatic Cancer Metastasis. Biomolecules 2021;11:1082.
26. Hui B, Ji H, Xu Y, et al. RREB1-induced upregulation of the lncRNA AGAP2-AS1 regulates the proliferation and

migration of pancreatic cancer partly through suppressing ANKRD1 and ANGPTL4. Cell Death Dis 2019;10:207.
27. Luo Y, Wang Q, Teng L, et al. LncRNA DANCR promotes proliferation and metastasis in pancreatic cancer by regulating miRNA-33b. FEBS Open Bio 2020;10:18-27.

(English Language Editor: A. Muijlwijk)

Cite this article as: Ming Z, Zhang R, Xu J, Ding W, Qin L. Downregulation of LINC01426 inhibits the proliferation and migration of human pancreatic cancer cells *in vivo* and *in vitro*. J Gastrointest Oncol 2022;13(6):3278-3289. doi: 10.21037/jgo-22-1167

