Original Article

LINC00483 promotes proliferation and metastasis through the miR-19a-3p/TBK1/MAPK axis in pancreatic ductal adenocarcinoma (PDAC)

Chenming Ni[#], Kailian Zheng[#], Wenyu Liu[#], Yangliu Ou, Gang Li, Gang Jin

Department of Pancreatic Hepatobiliary Surgery, Changhai Hospital, Naval Medical University, Shanghai, China

Contributions: (I) Conception and design: C Ni, G Li, G Jin; (II) Administrative support: W Liu, G Li, G Jin; (III) Provision of study materials or patients: C Ni, K Zheng, G Li, G Jin; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Gang Jin; Gang Li. Department of Pancreatic Hepatobiliary Surgery, Changhai Hospital, Naval Medical University, Shanghai 200433, China. Email: drjingang@yeah.net; li_gang169@163.com.

Background: Long noncoding RNAs (lncRNAs) have been found to promote tumor progression. However, the role of lncRNAs in pancreatic ductal adenocarcinoma (PDAC) requires more investigation.

Methods: In this study, microarray was used to measure lncRNA levels in 3 pairs of PDAC tissues. As the highest upregulated lncRNA, LINC00483 was selected for further investigation to determine its functions in PDAC. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was used to confirm LINC00483 level in PDAC. PDAC cell lines were transfected with short hairpin RNA (shRNA) or microRNA (miRNA). 5-ethynyl-2'-deoxyuridine (EdU) assay, colony formation assay, wound healing assay, transwell assay, and xenograft mouse models were used to evaluate LINC00483 inhibition *in vitro* and *in vivo*. Luciferase reporter assay was performed to confirm binding sites of LINC00483 with miR-19a-3p, and miR-19a-3p with TANK-binding kinase 1 (TBK1). Immunohistochemistry (IHC) was performed to evaluate TBK1 and c-myc expression in PDAC tissues. Western blot was used to elucidate the LINC00483/miR-19a-3p/TBK1/mitogen-activated protein kinase (MAPK) axis.

Results: Our data showed that LINC00483 was significantly upregulated in PDAC compared to normal tissue. High level of LINC00483 was correlated with advanced clinical stage, tumor invasion and metastasis, and adverse prognosis in PDAC patients. LINC00483 suppression inhibited proliferation and invasion *in vitro* and tumor development *in vivo* via modulation of miR-19a-3p expression. Subsequently, we found that miR-19a-3p binds to TBK1 in PDAC and LINC00483 could regulate PDAC cell progression by regulating miR-19a-3p via the TBK1/MAPK pathway.

Conclusions: The results of our study suggested that the LINC00483/miR-19a-3p/TBK1/MAPK axis contributed to PDAC progression, which provides a potential therapeutic target for PDAC treatment.

Keywords: LINC00483; TANK-binding kinase 1 (TBK1); miR-19a-3p; pancreatic ductal adenocarcinoma (PDAC)

Submitted Jan 17, 2022. Accepted for publication Mar 14, 2022.

doi: 10.21037/atm-22-907

View this article at: https://dx.doi.org/10.21037/atm-22-907

Introduction

Over 80% of pancreatic cancer cases are diagnosed with pancreatic ductal adenocarcinoma (PDAC), which has become a major cause of cancer-related death worldwide (1-4). Due to late diagnosis and resistance to traditional therapeutic strategies, the 5-year survival rate of PDAC is approximately 8% (5,6). Hence, there is an urgent need to explore its pathogenesis and develop new therapeutic strategies for PDAC.

Long noncoding RNAs (lncRNAs) are RNAs with more than 200 nucleotides that are unable to be transcribed into proteins (7). Studies have shown that lncRNAs are important in regulating cellular functions, including proliferation, differentiation, metastasis, apoptosis, and drug resistance (8,9). Aberrant lncRNAs contribute to cancer development and progression. For example, lncRNA cancer susceptibility candidate 2 (CASC2) has been shown to inhibit proliferation through downregulation of the mitogen-activated protein kinase (MAPK) signaling pathway in gastric cancer (10). LncRNA metastasis-associated lung carcinoma transcript 1 (MALAT1) was increased and correlated with advanced tumor characteristics and adverse prognosis in patients with kidney cancer (11). LncRNA noncoding RNA activated by DNA damage (NORAD) boosted cellular proliferation and invasion by upregulation of SMAD interacting protein 1 (SIP1) in cervical cancer (12). Although a number of lncRNAs have been explored, their roles in PDAC remain largely unclear.

MicroRNAs have been proved to be oncogenes or tumor suppressors (13). It has been shown that the microRNA-30 (miR-30) family promotes tumor development. MiR-30-5p is a tumor suppressor through downregulation of the Wnt/β-catenin-B-cell lymphoma 9 (BCL9) pathway (14). MiR-30a/c-5p regulates DNA methyltransferase 1 (DNMT1) in cisplatin-resistant ovarian cancer (15). MiR-30c-5p targets metastasis-associated protein 1 (MTA1) and inhibits epithelial-mesenchymal transition (EMT) and invasion of gastric cancer cells (16). However, the functions of miR-19a-3p in PDAC are still unclear.

In this study, we detected lncRNA expression in 3 pairs of PDAC tissues via microarray. The functions and molecular mechanisms of LINC00483 in PDAC development were also investigated. Our results indicated that LINC00483 was significantly upregulated in PDAC and promoted PDAC progression via regulation of the miR-19a-3p/TANK-binding kinase 1 (TBK1)/MAPK axis. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-907/rc).

Methods

Patient samples

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the Research Ethics Committee of Changhai Hospital (No. CHEC2018-039). All patients were treatment-naïve and signed informed consent forms. A total of 70 pairs of PDAC and adjacent normal tissues were collected from patients who underwent pancreatectomy at Changhai Hospital.

Cell culture and transfection

Human pancreatic duct epithelial cell line H6c7 and human pancreatic cancer cell lines HPAF-II, MiaPaca-2, PANC-1, and MPANC96 were provided by American Type Culture Collection (ATCC, Rockville, MD, USA). HPAF-II cells were cultured in Eagle's Minimum Essential Medium (EMEM) containing 10% heat inactivated fetal bovine serum (HIFBS; Thermo Fisher Scientific, Waltham, MA, USA), 100 U/mL penicillin-streptomycin (Gibco, Waltham, MA, USA) and 2 mM L-glutamine (Thermo Fisher Scientific). MiaPaca-2 and PANC-1 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 10% HIFBS, antibiotics, and L-glutamine. MPANC96 and H6c7 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco) with 10% HIFBS, antibiotics, and L-glutamine. PDAC cells were transduced with short hairpin (sh)-LINC00483 lentivirus, transfected with miR-19a-3p mimics, and treated with miR-19a-3p inhibitors and negative controls (NCs) (GenePharma, Shanghai, China). Transfection with short hairpin RNA (shRNA) or microRNA (miRNA) was done with Lipofectamine 3000 reagents (Invitrogen, Carlsbad, CA, USA).

Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

RNA from tissues and cell lines were extracted with Trizol (Invitrogen). Complementary DNA (cDNA) was reverse transcribed using a PrimeScript kit (TaKaRa Bio Inc., Kusatsu, Shiga, Japan). RT-qPCR was conducted with

SYBR Green Mix (Takara, Biomedical Technology, Beijing, China) using the ABI 7500 system (Applied Biosystems, CA, USA). The PCR program was 50 ℃ for 2 minutes, 95 ℃ for 10 minutes, 38 cycles of 95 ℃ for 15 seconds, and 60 ℃ for 60 seconds. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 were used as housekeeping references. The $2^{-\Delta\Delta\text{cycle threshold (CT)}}$ method was applied to analyze relative messenger RNA (mRNA) expression.

Western blot assay

Radioimmunoprecipitation assay (RIPA) buffer (Thermo Fisher Scientific) was used to extract total protein. Protein concentration was measured with bicinchoninic acid (BCA) assay (Pierce, IL, USA). The protein sample (45 μg) was separated using 15% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) gels and then transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, St. Louis, MO, USA). Membranes were blocked with 5% non-fat milk for 1 hour in room temperature, then incubated with primary antibodies at 4 ℃ overnight, followed by secondary antibody incubation for 1 hour at room temperature and visualization with enhanced chemiluminescence (ECL) buffer (Thermo Fisher Scientific). Primary antibodies included: anti-vimentin and anti-N-Cadherin from Abcam, anti-TBK1 and anti-GAPDH from Sigma-Aldrich, anti-c-myc from Thermo Fisher Scientific, and anti-p-extracellular signal-regulated kinase (ERK), anti-ERK, anti-p65, and anti-zinc finger E-box binding homeobox 1 (ZEB1) from Cell Signaling Technology (Danvers, MA, USA).

Microarray analysis

Total RNA of 3 pairs of PDAC tissues were extracted, and microarray hybridization was performed following protocols (KangChen, Shanghai, China). Raw data were processed with Agilent Feature Extraction (version 11). Data were analyzed with GeneSpring GX v12.0 (Agilent Technologies, Santa Clara, CA, USA). $P \leq 0.05$ and a fold change (FC) ≥ 2.0 were used to differentiate downregulated and upregulated lncRNAs.

Immunohistochemistry (IHC)

IHC was done as previously described (17). IHC slides were evaluated independently by 2 pathologists. Scoring criteria of staining intensity were negative =0, weak =1, moderate =2, or strong =3. Positive area criteria were <10%=1, 10–50%=2, 50–80%=3, and >80%=4. For heterogeneous staining, intensity in each area was determined individually and the sum was calculated (3). Primary antibodies included anti-TBK1 and anti-c-myc (1:200, Cell Signaling Technology).

5-ethynyl-2'-deoxyuridine (EdU) assay

PANC-1 and MPANC96 cells with/without transfection were seeded in 12-well plates, growing overnight to reach 80% confluence. The next day, 10× EdU working solution (Millipore) was added to the cells, which were left to continue growing for another 24 hours. Formaldehyde [4% in phosphate-buffered saline (PBS)] was used to fix cells and 0.5% Triton X-100 was used to permeabilize cells, reaction buffer containing fluorescence dye was added and incubated for 30 minutes in room temperature, which were then photographed under fluorescence microscope. EdU positive rate = cells with red fluorescence (EdU-stained)/cells with blue fluorescence (Hoechst-stained).

Colony-forming assay

PANC-1 and MPANC96 cells were transfected with shRNA before plating. The cells were then seeded in 6-well plates and cultured for 2 weeks before being fixed with 30% formaldehyde and stained with 0.1% crystal violet. Colonies were then counted.

Wound healing assay

Cells were seeded in 6-well plates (1×10^6 cells/well). When confluence reached 90%, cells were starved for 24 hours in serum-free medium before a scratch was made with a pipette tip. Photos were taken at 0 and 48 hours (Olympus, Tokyo, Japan), and cell migration was calculated based on the percentage of filled wounded area.

Transwell invasion assay

Cells were seeded into the upper chamber of 24-wells plate (Corning Life Sciences, Tewksbury, MA, USA) pre-coated with Matrigel (Sigma-Aldrich, St. Louis, MO, USA), and DMEM with 10% fetal bovine serum (FBS) was placed in the lower chamber. After 24 hours, invaded cells were stained with 0.1% crystal violet for 15 minutes before counting.

Luciferase reporter assay

DNAs [LINC00483, wildtype (Wt), and mutant TBK1] were amplified and inserted into pmiR-GLO vectors (Promega Corporation, Madison, WI, USA). Cells were transfected with reporter plasmids and miR-19a-3p mimics by lipofectamine 3000 (Invitrogen) for 48 hours. Dual-luciferase reporter assay (Promega) was applied to measure luciferase.

Xenograft mouse model

To assess the effect of LINC00483 inhibition on PDAC, MPANC96 cells (5.0×10^6) stably transfected with sh-NC or sh-LINC00483 were subcutaneously injected into 4-week-old female BALB/c nude mice (around 17 g). Xenograft mouse models were kept for 7 weeks and tumor weights were recorded. Tumor volume = length × (width^2)/2. A protocol was prepared before the study without registration. Animal experiments were approved by the Committee on Ethics of Medicine, Naval Medical University, in compliance with institutional guidelines for the care and use of animals.

Statistical analysis

Data were processed by SPSS 19.0 (IBM, Armonk, NY, USA). Experimental data were presented as mean ± standard deviation (SD). Differences were analyzed using Student's *t*-test (2 groups) or one-way ANOVA (more than 2 groups). $P\leq0.05$ was considered statistically significant.

Results

Dysregulated expression profiles of lncRNAs in PDAC

There were 327 highly expressed lncRNAs and 559 low expressed lncRNAs (*Figure 1A*). LINC00483 was the highest upregulated lncRNA (log2 FC =6.35). Genetic information for LINC00483 was obtained by searching The Cancer Genome Atlas (TCGA) database (http://cancergenome.nih.gov/) and University of California Santa Cruz (UCSC) Genome Browser. The results showed that LINC00483 was significantly increased in tumor tissues, particularly in pancreatic carcinoma (*Figure 1B*). Gene ontology analysis (GO) was performed to investigate the functions of the top 10 differentially expressed (DE) lncRNAs and correlation with cancer progression, including cellular components, molecular function (MF), and biological processes (*Figure 1C-1H*). TCGA database analysis showed that highly expressed LINC00483 was correlated with adverse overall survival (OS) and disease-free survival (DFS) in PDAC patients (*Figure 1I,1J*). These results suggested that LINC00483 may be essential in PDAC progression.

High expression of LINC00483 in PDAC

To further understand the expression and clinical relevance of LINC00483 in PDAC, additional information was retrieved from TCGA database. We found that LINC00483 was significantly upregulated in PDAC compared to normal tissues, while lowly expressed in other solid tumors ($P<0.001$, *Figure 2A*). Furthermore, LINC00483 expression level in pancreatic cancer significantly increased with advanced clinical stage ($P<0.05$, *Figure 2B*).

RT-qPCR showed LINC00483 was significantly upregulated in PDAC samples compared to normal tissues (*Figure 2C*). The relationship between LINC00483 expression and PDAC clinicopathology was also analyzed. High LINC00483 expression was elevated in PDAC patients with metastasis (*Figure 2D*,*2E*, *Table 1*). LINC00483 was also overexpressed in PDAC cell lines (*Figure 2F*). These data implied that LINC00483 may be crucial in PDAC metastasis.

To explore the underlying mechanisms of LINC00483 in PDAC development and progression, loss-of-function assays were performed. LINC00483 was significantly downregulated in MPANC96 and PANC-1 cells transfected with sh-LINC00483 (*Figure 2G*,*2H*). EdU assays showed that LINC00483 downregulation decreased proliferation of MPANC96 and PANC-1 cells (*Figure 3A-3C*). Colony-forming assays also verified the proliferation inhibition of sh-LINC00483, which showed that the number of cloned cells in MPANC96 and PANC-1 cells transfected with sh-LINC00483 was significantly reduced compared to the sh-NC group (*Figure 3D-3F*). Wound healing assays also showed sh-LINC00483 significantly repressed migration of pancreatic cancer cells (*Figure 3G-3I*).

LINC00483 is a sponge for miR-19a-3p

Next, we searched for the target of LINC00483 using miRcode, Starbase, and LncBase, which showed that miR-19a-3p was ranked as a top potential target. The secondary structure of LINC00483 and potential binding sites with miR-19a-3p are shown in *Figure 4A-4C*.

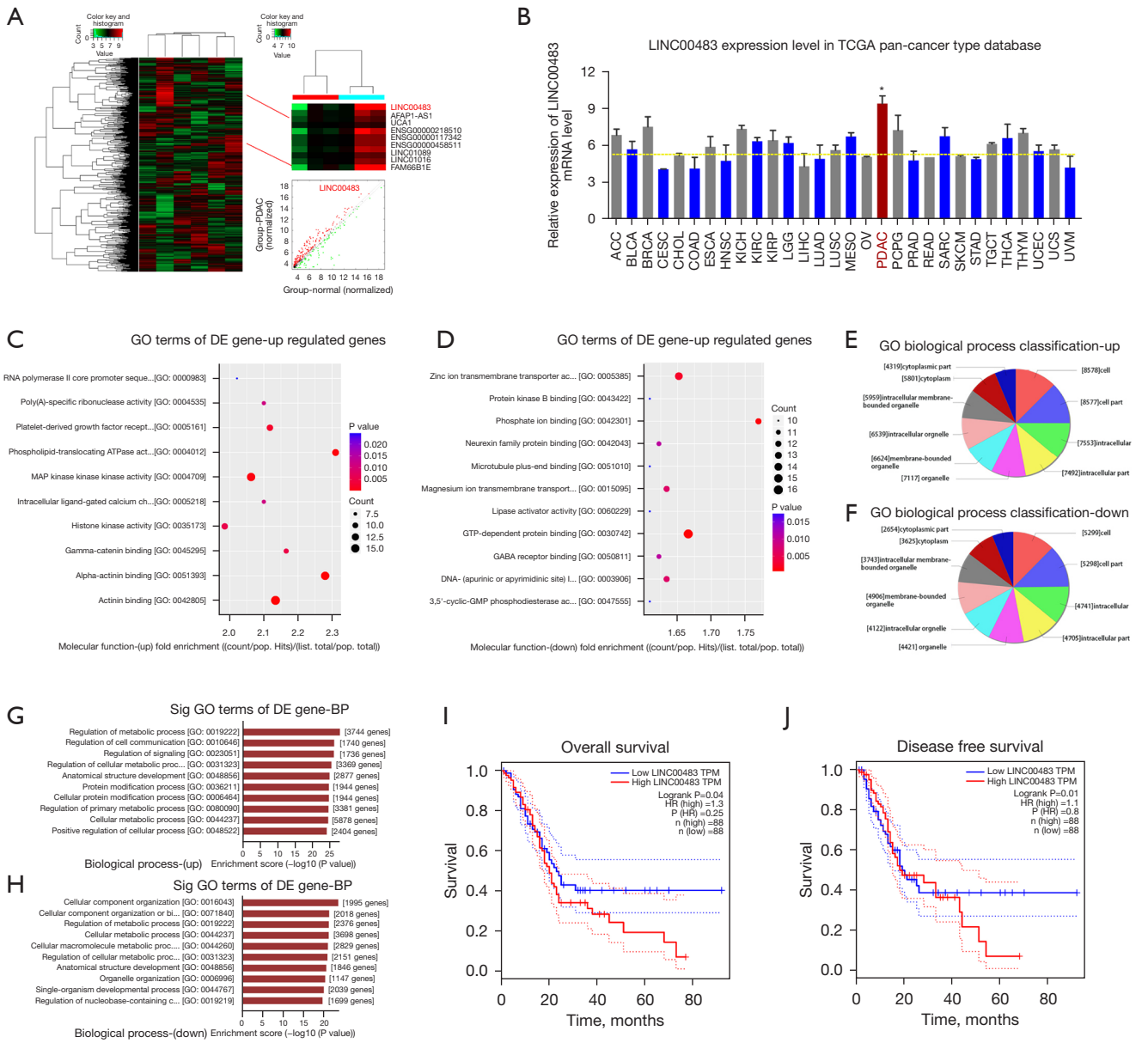

Figure 1 Expression profiles of lncRNAs and clinical implications in PDAC. (A) LncRNAs were upregulated in PDAC tissues compared to normal tissues evaluated by lncRNA expression array. (B) The genetic information for LINC00483. GO functional analysis of DE genes. The 10 most significant upregulation and downregulation of (C,D) MF, (E,F) cellular component (CC), and (G,H) BP domains. High LINC00483 level was correlated with (I) adverse OS and (J) DFS in patients with PDAC. Yellow line: mean expression of tumor classification. *P<0.05. PDAC, pancreatic ductal adenocarcinoma; mRNA, messenger RNA; TCGA, The Cancer Genome Atlas; GO, gene ontology; DE, differentially expressed; BP, biological process; TPM, transcripts per million; MF, molecular function; LncRNAs, long noncoding RNAs; OS, overall survival; DFS, disease-free survival.

Luciferase of HEK293 cells transfected with LINC00483-Wt was decreased by miR-19a-3p mimics (*Figure 4D*). Further, miR-19a-3p level was negatively associated with LINC00483 level in PDAC tissues (*Figure 4E*).

TBK1 is a target of miR-19a-3p

We then explored miR-19a-3p functions in PDAC. There was a significant decline in miR-19a-3p level in PDAC

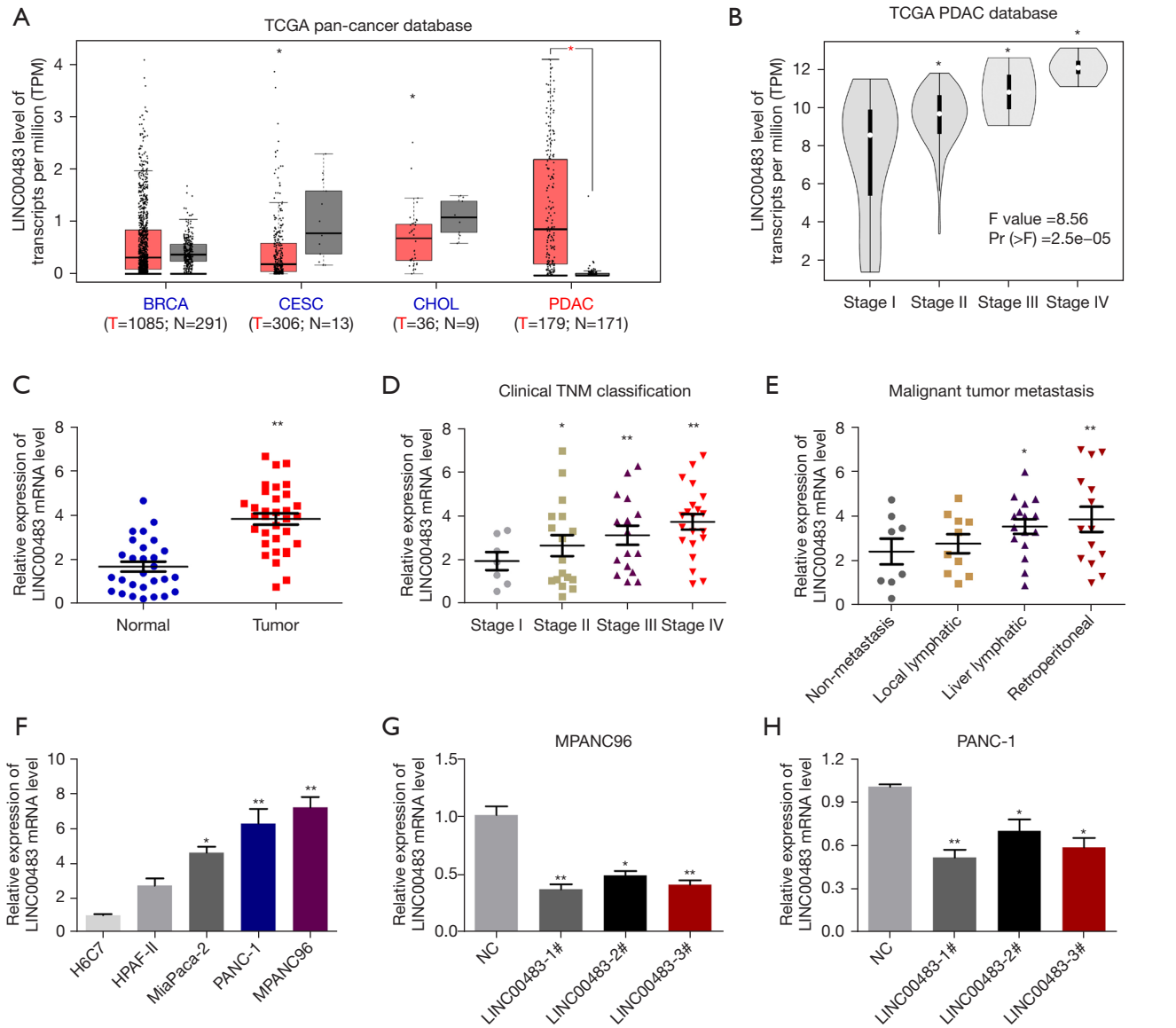

Figure 2 LINC00483 was upregulated in PDAC. (A) LINC00483 expression in BRCA, CESC, CHOL, and PDAC with corresponding normal tissues in TCGA dataset. (B) LINC00483 level in PDAC cases with distinct clinical stages in TCGA dataset. (C) LINC00483 mRNA expression in 70 PDAC cases. (D,E) High level of LINC00483 was positively related to advanced clinical stage and metastasis in PDAC patients. (F) LINC00483 expression in pancreatic cancer cell lines (HPAF-II, MiaPaca-2, PANC-1, and MPANC96) and H6C7 was measured by RT-qPCR. (G,H) Knockdown of LINC00483 by shRNA in MPANC96 and PANC-1 cells. *P<0.05; **P<0.001. TCGA, The Cancer Genome Atlas; PDAC, pancreatic ductal adenocarcinoma; BRCA, breast carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; RT-qPCR, quantitative reverse transcription polymerase chain reaction.

compared to normal tissues (*Figure 4F*). TCGA database analysis showed that low miR-19a-3p level was correlated with adverse OS and DFS in patients with pancreatic carcinoma (*Figure 4G,4H*).

We found complementary binding sites between miR-19a-3p and TBK1 (*Figures 4I,5A*). TBK1-Wt luciferase and mRNA level were remarkably decreased by miR-19a-3p mimics (*Figure 5B*), while miR-19a-3p inhibitors upregulated TBK1 mRNA level (*Figure 5C*).

We found that expression of c-myc, a common regulator

Table 1 Association of LINC00483 expression with clinicopathological parameters of pancreatic carcinoma patients

Parameters	No. of cases	LINC00483 mRNA relative expression level		P value	χ^2
		High	Low		
Age (y)				0.436	1.408
≥65	28 (0.40)	16 (0.23)	12 (0.17)		
<65	42 (0.60)	24 (0.34)	18 (0.26)		
Gender				0.582	0.763
Female	33 (0.47)	19 (0.27)	14 (0.20)		
Male	37 (0.53)	21 (0.30)	16 (0.23)		
Tumor stage				0.013*	7.836
Stage I–II	32 (0.46)	14 (0.20)	18 (0.26)		
Stage III	18 (0.26)	11 (0.16)	7 (0.10)		
Stage IV	20 (0.28)	15 (0.21)	5 (0.07)		
Lymphatic/liver metastasis[a]				0.032*	2.357
Positive	24 (0.34)	18 (0.26)	6 (0.09)		
Negative	46 (0.66)	22 (0.31)	24 (0.34)		
Tumor location				0.517	1.832
Head of pancreas	43 (0.61)	23 (0.33)	20 (0.29)		
Body of pancreas	27 (0.39)	17 (0.24)	10 (0.14)		
Vascular invasion				0.021*	5.639
Positive	49 (0.70)	32 (0.46)	17 (0.24)		
Negative	21 (0.30)	8 (0.11)	13 (0.19)		
Nerve invasion				0.037*	3.342
Positive	45 (0.64)	29 (0.41)	16 (0.23)		
Negative	25 (0.36)	11 (0.16)	14 (0.20)		

[a], lymphatic/liver metastasis: means the sum of local lymph nodes and liver metastases; *P<0.05. mRNA, messenger RNA.

in tumor progression, was positively correlated with TBK1 protein level in PDAC tissues (*Figure 5D*). IHC showed expression of TBK1 and c-myc were upregulated in PDAC and positively correlated with advanced clinical stages (*Figure 5E*), which was validated by results from TCGA database (*Figure 5F*). These data implied that TBK1 and c-myc may be important in PDAC progression.

LINC00483/miR-19a-3p/TBK1 axis promoted PDAC metastasis

We further investigated the role of LINC00483/miR-19a-3p/TBK1 in PDAC cell invasion. Transwell assay revealed that miR-19a-3p inhibitors increased invasion of PANC-1 cells. Further, the antimetastatic effect of LINC00483 suppression on PANC-1 cells was abrogated by miR-19a-3p inhibitors (*Figure 5G*). In addition, MPANC96 cell invasion was increased after transfection with pcDNA3.1_TBK1 plasmid, and the suppressive effects of LINC00483 on invasion could be rescued by TBK1 overexpression (*Figure 5H*).

We explored *in vivo* effects of LINC00483 in xenograft mouse models. LINC00483 repression significantly suppressed tumor development and growth (*Figure 6A-6C*). The MAPK signaling pathway and epithelial-mesenchymal transition (EMT) are crucial in metastasis. In our study, LINC00483 repression significantly downregulated EMT regulators vimentin and N-cadherin in addition to the

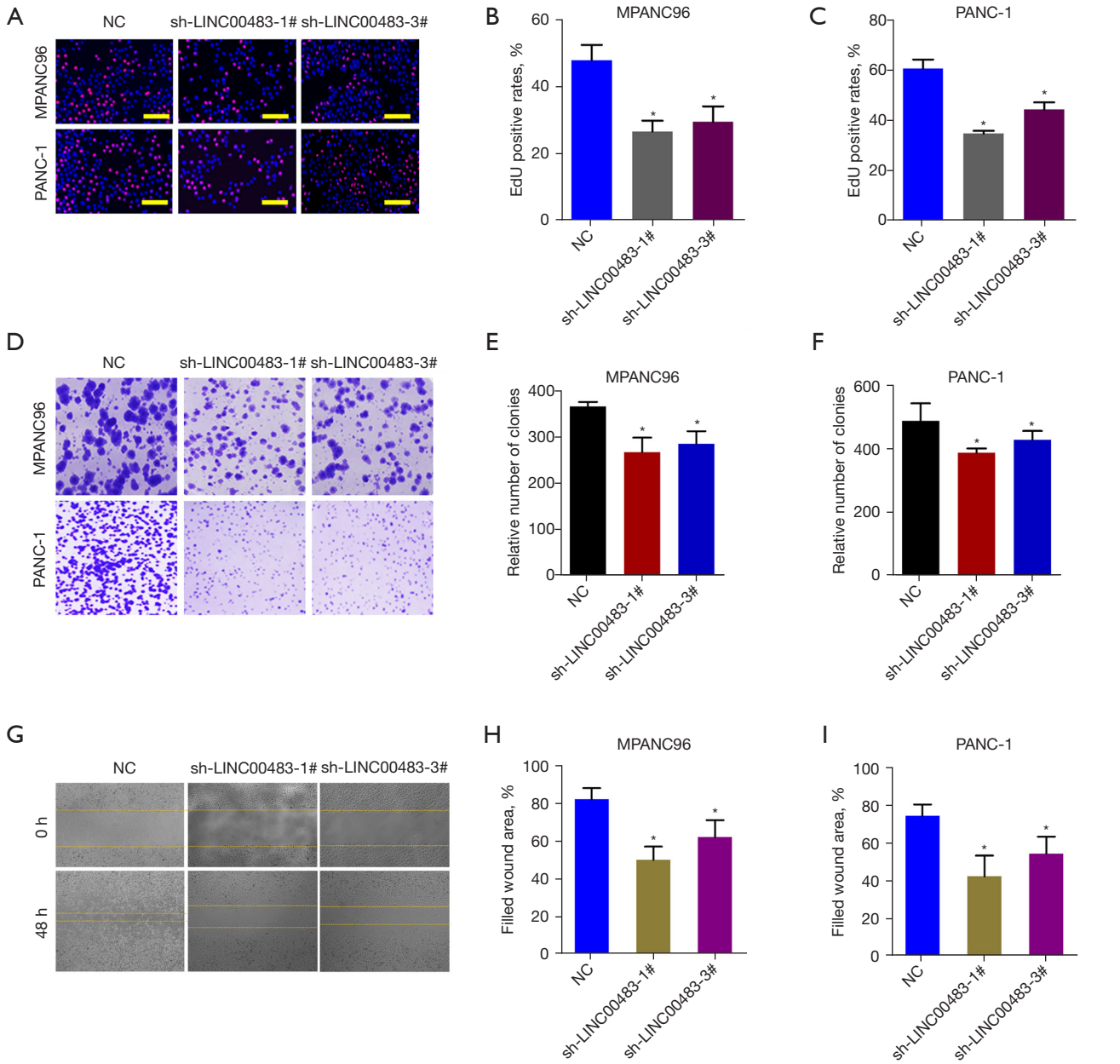

Figure 3 LINC00483 promotes PDAC the development of prometastatic phenotype *in vitro*. (A-C) EdU assays were used to explore PDAC cells viability. EdU-stained cells were red and Hoechst-stained cells were blue under fluorescence microscope. Scale bars: 100 μm (yellow). (D-F) Colony-forming assays verified the proliferation inhibition rate of LINC00483; the number of cloned cells stained with 0.1% crystal violet in MPANC96 and PANC-1 cells transfected with sh-LINC00483 was reduced when compared to the control group significantly. Magnification, ×100. (G-I) Knockdown of LINC00483 in MPANC96 and PANC-1 cells significantly inhibited cell migration. 1# and 3# sh-plasmids with high efficiency were used. *P<0.05. NC, negative control; PDAC, pancreatic ductal adenocarcinoma; EdU, 5-ethynyl-2'-deoxyuridin.

MAPK pathway, including c-myc, p-ERK1/2, ZEB1, and p65. LINC00483's inhibitory effects on EMT and the MAPK pathway were rescued by miR-19a-3p inhibitors (*Figure 6D*).

Overall, our results showed that LINC00483 boosted proliferation and metastasis of PDAC through regulation of the miR-19a-3p/TBK1/MAPK axis (*Figure 6E*).

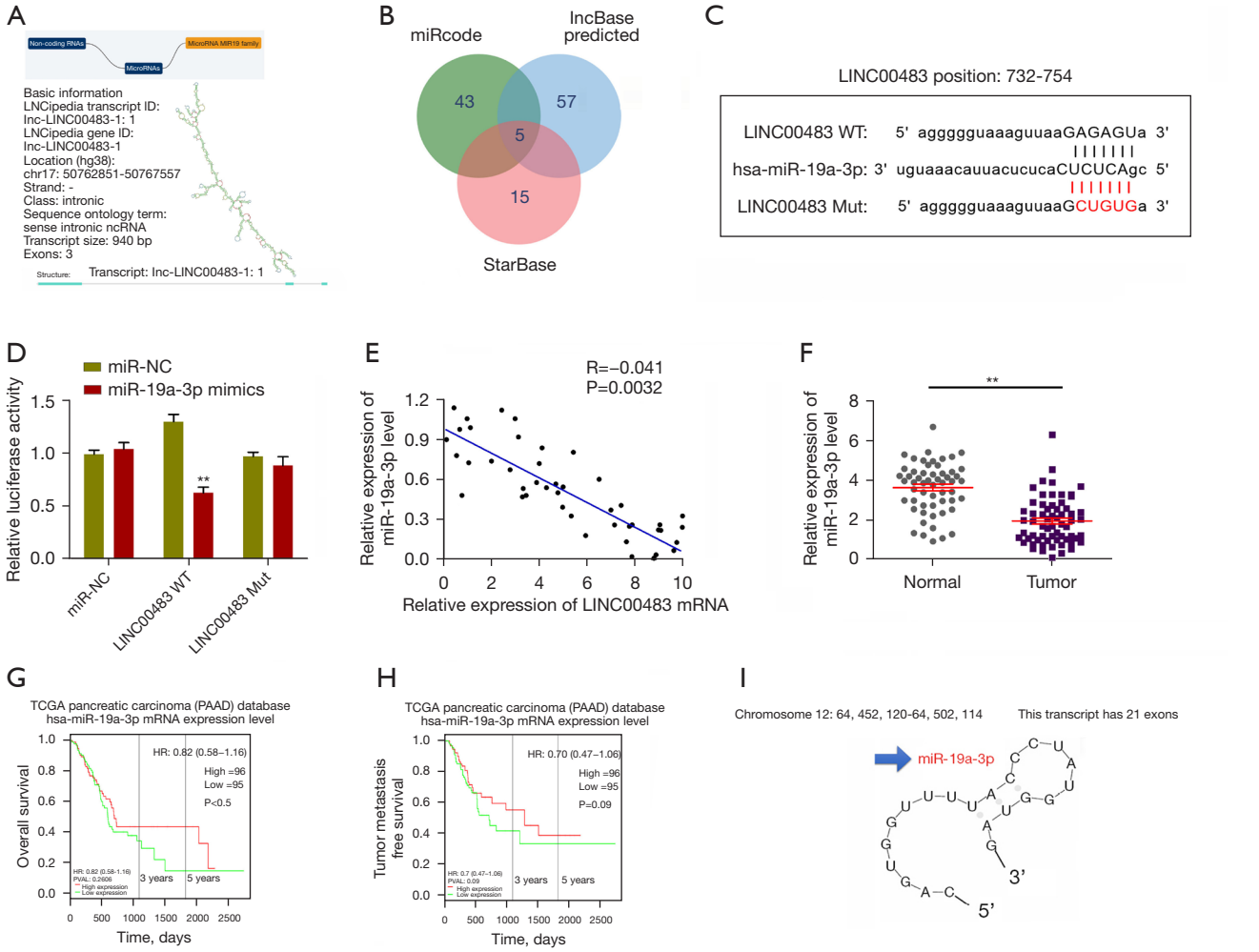

Figure 4 LINC00483 is a sponge for miR-19a-3p. (A) The location information and predicted secondary structure of LINC00483. (B) Potential targets of LINC00483 presented with Venn diagrams from 3 databases. (C) Prediction of complementary binding sites of LINC00483 and miR-19a-3p. (D) MiR-19a-3p mimics decreased LINC00483-Wt luciferase. (E) MiR-19a-3p level was negatively correlated with LINC00483 level in PDAC tissues. (F) MiR-19a-3p level in 70 pairs of PDAC tissues measured by RT-qPCR. (G,H) Low miR-19a-3p level was associated with adverse OS and DFS in patients with PDAC. (I) MiR-19a-3p is located on chromosome 6 upstream of TBK1 promoter. **P<0.001. NC, negative control; TCGA, The Cancer Genome Atlas; PAAD, pancreatic adenocarcinoma; mRNA, *messenger RNA*; PDAC, pancreatic ductal adenocarcinoma; RT-qPCR, quantitative reverse transcription polymerase chain reaction; OS, overall survival; DFS, disease-free survival.

Discussion

PDAC is one of the most malignant diseases world-wide (2), and the prognosis for patients is poor (5). Thus, it is important to identify genes that are critical in PDAC pathogenesis. LncRNAs have recently been found to be potential cancer therapeutic targets (18,19). For example, Wang *et al.* found lncRNA BRAF-activated nonprotein coding RNA (BANCR) could promote proliferation and metastasis in PDAC by regulating EMT (20). LncRNA HOXA transcript at the distal tip (HOTTIP) has been shown to modulate pancreatic cancer stem cell characteristics via HOXA9 (21). Wang *et al.* suggested that lncRNA AB209630 inhibited gemcitabine-resistant PDAC cell proliferation through the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway (22).

We revealed that LINC00483 was significantly elevated in PDAC tissues and cell lines. LINC00483 overexpression

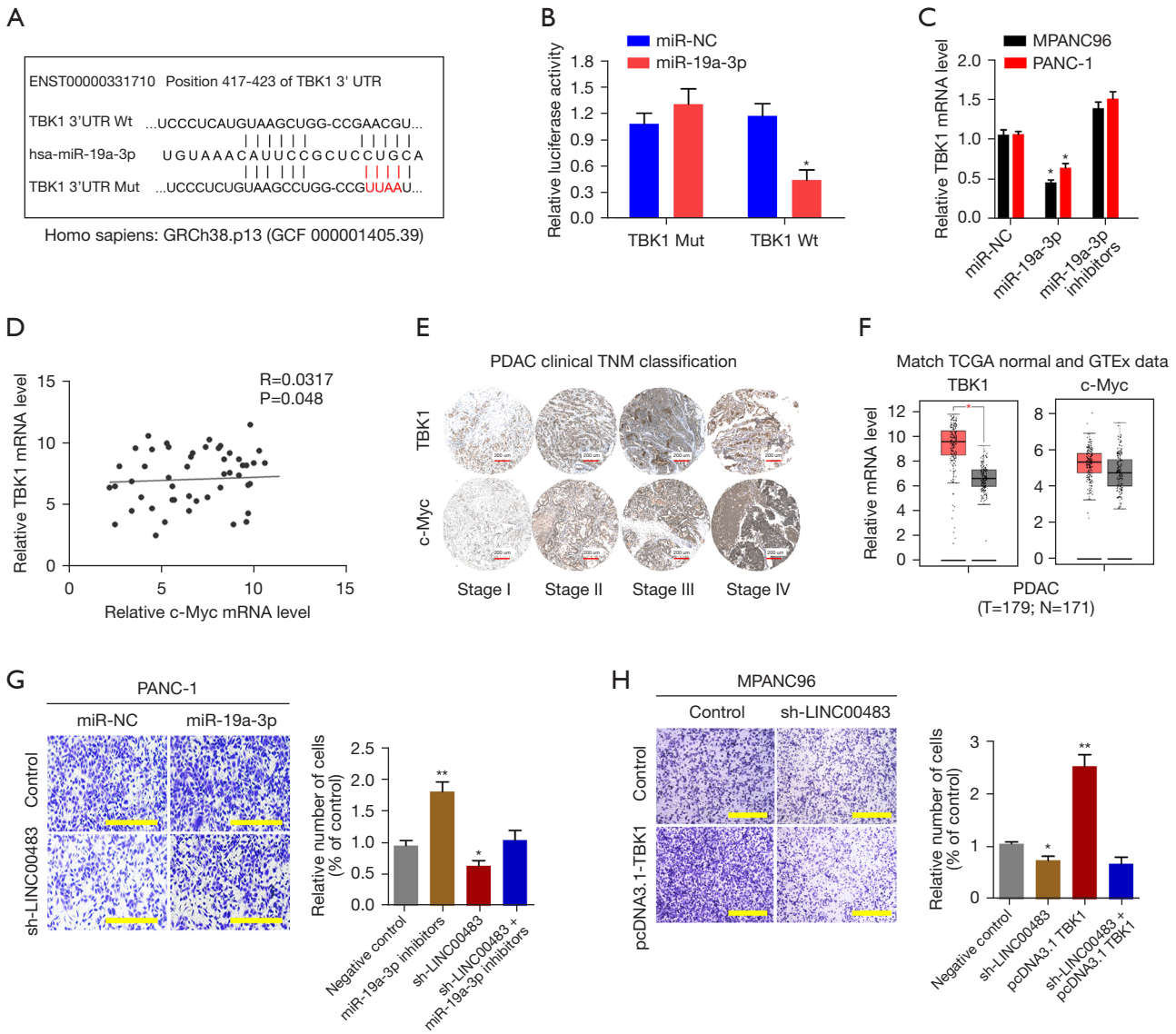

Figure 5 MiR-19a-3p binds to TBK1 and is correlated with adverse prognosis in PDAC. (A) Prediction of binding sites between miR-19a-3p and TBK1. (B) MiR-19a-3p mimics reduced TBK1-Wt luciferase. (C) MmiR-19a-3p mimics downregulated TBK1 level in PDAC cells. Conversely, miR-19a-3p inhibitors upregulated TBK1. (D) Expression level of c-myc was positively correlated with TBK1 level in PDAC. (E) IHC assays showed expression level of c-myc and TBK1 were upregulated and correlated with advanced stage in PDAC patients. (F) GEPIA revealed that c-myc and TBK1 expression was increased in PDAC tissues. Red box is from PDAC tissue, grey box is from normal tissue. (G) The antimetastatic effect of LINC00483 repression on PANC-1 cells was abrogated by miR-19a-3p inhibitors. (H) LINC00483 suppression on MPANC96 cells invasion was rescued by TBK1 overexpression. Transwell assays in (G) and (H) were stained with 0.1% crystal violet. Magnification, ×100. Scale bars: 100 μm (yellow). *P<0.05; **P<0.001. NC, negative control; TBK1, TANK-binding kinase 1; mRNA, *messenger RNA*; PDAC, pancreatic ductal adenocarcinoma; TCGA, The Cancer Genome Atlas; GEPIA, Gene Expression Profiling Interactive Analysis.

was positively correlated with metastasis and adverse prognosis in PDAC patients. LINC00483 inhibition could repress proliferation and invasion of PDAC cells and inhibit tumor development in xenograft mouse models. These data proved that LINC00483 is essential in PDAC tumorigenesis.

Emerging evidence has demonstrated that lncRNAs could act as miRNA sponges to mediate gene expression (23).

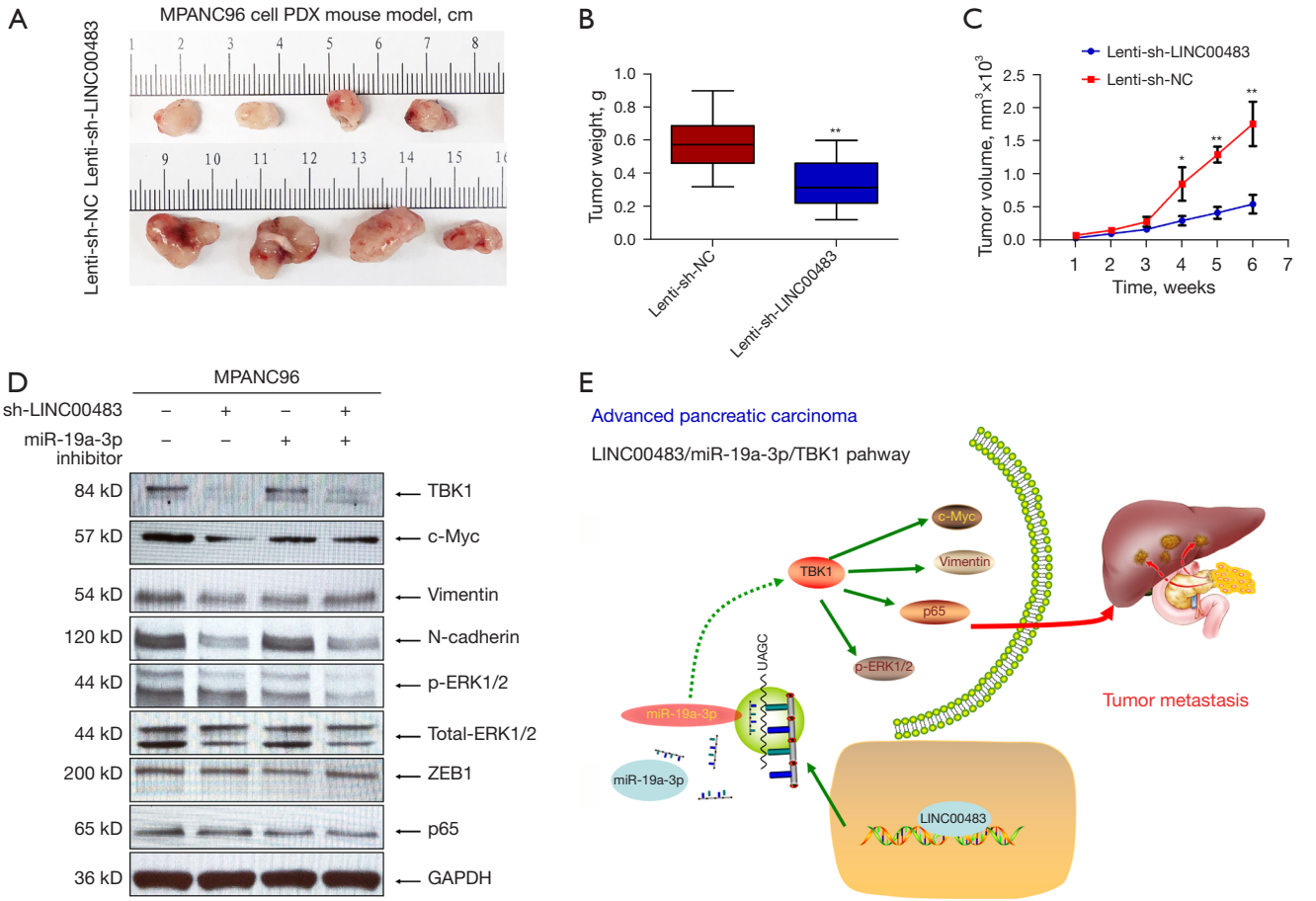

Figure 6 LINC00483 repression decreased PDAC development in xenograft mouse model, and the LINC00483/miR-19a-3p/TBK1 axis boosted PDAC metastasis. (A) Representative tumor images, (B) relative weight, and (C) growth curves in xenograft mouse models. (D) The effects of sh-LINC00483 and/or miR-19a-3p inhibitors on the MAPK pathway and EMT processes as determined by Western blot. (E) Schematic diagram of LINC00483 in PDAC metastasis. *P<0.05; **P<0.001. NC, negative control; TBK1, TANK-binding kinase 1; PDAC, pancreatic ductal adenocarcinoma; MAPK, mitogen-activated protein kinase; EMT, epithelial-mesenchymal transition.

For example, lncRNA FLVCR1 antisense RNA 1 (FLVCR1-AS1) was shown to promote lung cancer cell proliferation and invasion by sponging miR-573 (24). LncRNA ABHD11 antisense RNA 1 (ABHD11-AS1) has been found to assist colorectal cancer progression by regulating the miR-1254-Wnt Family Member 11 (WNT11) signaling pathway (25). Another study showed that lncRNA SBF2 antisense RNA 1 (SBF2-AS1) facilitated cervical cancer progression via the miR-361-5p/forkhead box M1 (FOXM1) axis (26). In our study, bioinformatics analysis identified miR-19a-3p as the target of LINC00483, which was confirmed by dual-luciferase reporter assay. Transwell assay proved that LINC00483 facilitated PDAC cell invasion by miR-19a-3p downregulation. Taken together, our results suggested that LINC00483 acted as a sponge for miR-19a-3p in PDAC.

Previous studies have shown TBK1 to be a key player in cancer proliferation, apoptosis, EMT, and metastasis (27-30). In our study, we used bioinformatics analysis and functional experiments to prove that TBK1 was a direct target of miR-19a-3p in PDAC. The antimetastatic effect of LINC00483 suppression of PDAC cell invasion could be abrogated by cotransfection with miR-19a-3p inhibitors. LINC00483 inhibition significantly downregulated EMT and MAPK pathway-related proteins (vimentin, N-cadherin, c-myc, p-ERK1/2, ZEB1, and p65), which were rescued by cotransfection with miR-19a-3p inhibitors. Overall, our results showed that LINC00483 fostered proliferation and

invasion in PDAC through miR-19a-3p-regulated TBK1/MAPK pathway.

To summarize, our study identified LINC00483 to be important in PDAC and characterized its interaction with miR-19a-3p and TBK1. The LINC00483/miR-19a-3p/TBK1/MAPK axis might be a potential novel treatment target for PDAC.

Acknowledgments

Funding: This work was supported by National Natural Science Foundation of China (Grant No. 81871992).

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-907/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-907/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-907/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. Animal experiments were approved by the Committee on Ethics of Medicine, Naval Medical University, in compliance with institutional guidelines for the care and use of animals. Research involving patients was approved by the Research Ethics Committee of Changhai Hospital (No. CHEC2018-039) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All patients signed informed consent forms.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424. Erratum in: CA Cancer J Clin 2020;70:313.
2. Hezel AF, Kimmelman AC, Stanger BZ, et al. Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev 2006;20:1218-49.
3. Ryan DP, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. N Engl J Med 2014;371:2140-1.
4. Capasso M, Franceschi M, Rodriguez-Castro KI, et al. Epidemiology and risk factors of pancreatic cancer. Acta Biomed 2018;89:141-6.
5. Principe DR, Underwood PW, Korc M, et al. The Current Treatment Paradigm for Pancreatic Ductal Adenocarcinoma and Barriers to Therapeutic Efficacy. Front Oncol 2021;11:688377.
6. Pereira SP, Oldfield L, Ney A, et al. Early detection of pancreatic cancer. Lancet Gastroenterol Hepatol 2020;5:698-710.
7. Schmitt AM, Chang HY. Long Noncoding RNAs in Cancer Pathways. Cancer Cell 2016;29:452-63.
8. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell 2009;136:629-41.
9. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet 2009;10:155-9.
10. Li P, Xue WJ, Feng Y, et al. Long non-coding RNA CASC2 suppresses the proliferation of gastric cancer cells by regulating the MAPK signaling pathway. Am J Transl Res 2016;8:3522-9.
11. Zhang HM, Yang FQ, Chen SJ, et al. Upregulation of long non-coding RNA MALAT1 correlates with tumor progression and poor prognosis in clear cell renal cell carcinoma. Tumour Biol 2015;36:2947-55.
12. Huo H, Tian J, Wang R, et al. Long non-coding RNA NORAD upregulate SIP1 expression to promote cell proliferation and invasion in cervical cancer. Biomed Pharmacother 2018;106:1454-60.
13. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6:857-66.
14. Zhao JJ, Lin J, Zhu D, et al. miR-30-5p functions as a tumor suppressor and novel therapeutic tool by targeting the oncogenic Wnt/β-catenin/BCL9 pathway. Cancer Res

2014;74:1801-13.
15. Han X, Zhen S, Ye Z, et al. A Feedback Loop Between miR-30a/c-5p and DNMT1 Mediates Cisplatin Resistance in Ovarian Cancer Cells. Cell Physiol Biochem 2017;41:973-86.
16. Cao JM, Li GZ, Han M, et al. MiR-30c-5p suppresses migration, invasion and epithelial to mesenchymal transition of gastric cancer via targeting MTA1. Biomed Pharmacother 2017;93:554-60.
17. Li Z, Zhao X, Zhou Y, et al. The long non-coding RNA HOTTIP promotes progression and gemcitabine resistance by regulating HOXA13 in pancreatic cancer. J Transl Med 2015;13:84.
18. Li Y, Al Hallak MN, Philip PA, et al. Non-Coding RNAs in Pancreatic Cancer Diagnostics and Therapy: Focus on lncRNAs, circRNAs, and piRNAs. Cancers (Basel) 2021;13:4161.
19. Ye S, Yang L, Zhao X, et al. Bioinformatics method to predict two regulation mechanism: TF-miRNA-mRNA and lncRNA-miRNA-mRNA in pancreatic cancer. Cell Biochem Biophys 2014;70:1849-58.
20. Wang W, Zhai J M, Fan Y G, et al. Long non-coding RNA BANCR promotes pancreatic ductal adenocarcinoma cell growth and metastasis via affecting epithelial-mesenchymal transition. Int J Clin Exp Pathol 2016;9:9297-303.
21. Fu Z, Chen C, Zhou Q, et al. LncRNA HOTTIP modulates cancer stem cell properties in human pancreatic cancer by regulating HOXA9. Cancer Lett 2017;410:68-81.
22. Wang L, Wang F, Na L, et al. LncRNA AB209630 inhibits gemcitabine resistance cell proliferation by regulating PI3K/AKT signaling in pancreatic ductal adenocarcinoma. Cancer Biomark 2018;22:169-74.
23. Paraskevopoulou MD, Hatzigeorgiou AG. Analyzing miRNA-lncRNA interactions/Long Non-Coding RNAs. Humana Press, New York, NY, 2016:271-86.
24. Gao X, Zhao S, Yang X, et al. Long non-coding RNA FLVCR1-AS1 contributes to the proliferation and invasion of lung cancer by sponging miR-573 to upregulate the expression of E2F transcription factor 3. Biochem Biophys Res Commun 2018;505:931-8.
25. 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;234:12070-9.
26. Gao F, Feng J, Yao H, et al. LncRNA SBF2-AS1 promotes the progression of cervical cancer by regulating miR-361-5p/FOXM1 axis. Artif Cells Nanomed Biotechnol 2019;47:776-82.
27. Kharbanda A, Rajabi H, Jin C, et al. MUC1-C confers EMT and KRAS independence in mutant KRAS lung cancer cells. Oncotarget 2014;5:8893-905.
28. Nath S, Mukherjee P. MUC1: a multifaceted oncoprotein with a key role in cancer progression. Trends Mol Med 2014;20:332-42.
29. Ponnusamy K, Tzioni MM, Begum M, et al. The innate sensor ZBP1-IRF3 axis regulates cell proliferation in multiple myeloma. Haematologica 2022;107:721-32.
30. Tréhoux S, Lahdaoui F, Delpu Y, et al. Micro-RNAs miR-29a and miR-330-5p function as tumor suppressors by targeting the MUC1 mucin in pancreatic cancer cells. Biochim Biophys Acta 2015;1853:2392-403.

Cite this article as: Ni C, Zheng K, Liu W, Ou Y, Li G, Jin G. LINC00483 promotes proliferation and metastasis through the miR-19a-3p/TBK1/MAPK axis in pancreatic ductal adenocarcinoma (PDAC). Ann Transl Med 2022;10(6):317. doi: 10.21037/atm-22-907

