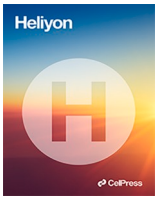
Heliyon 10 (2024) e38427

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

LINC01857 promotes cell proliferation and migration while dampening cell apoptosis in pancreatic cancer by upregulating CDC42EP3 via miR-450b-5p

Jian-Xin Zhang [1], Yan-Bin Shen [1], Dan-Dan Ma [*], Zhong-Hu Li, Zhi-Yong Zhang, Wei-Dong Jin [**]

Department of General Surgery, The Central Theater Hospital of the Chinese People's Liberation Army, Wuhan, 430070, China

ARTICLE INFO

Keywords:
Pancreatic cancer
LINC01857
miR-450b-5p
CDC42EP3
Competing endogenous RNA (ceRNA)

ABSTRACT

Background: Pancreatic cancer (PC) is a devastating human malignancy with a poor survival outcome (5-year survival less than 10 %). In recent years, the regulatory roles of long non-coding RNAs (lncRNAs) in various types of cancers have been widely reported. Based on bioinformatics analysis, LINC01857 is shown to be highly expressed in PC tissue. Nevertheless, the role of LINC01857 in PC is limitedly reported. Hence, this study aimed to explore the effects of lncRNA LINC01857 on PC cell process and the related mechanism.

Methods: RT-qPCR and fluorescence in situ hybridization (FISH) assay were conducted to measure LINC01857 expression and distribution in PANC-1 and MIA PaCa-2 cells. Colony formation and wound healing assays as well as flow cytometry analyses were employed to estimate the proliferation, migration, and apoptosis of PC cells transfected with pcDNA3.1-LINC01857 or si-LINC01857 compared with the behavior of PC cells transfected with empty pcDNA3.1 vector (control) or si-negative control (NC). Furthermore, RNA pulldown and luciferase reporter assays were utilized to demonstrate the interaction of LINC01857 and miR-450b-5p or to validate the binding of miR-450b-5p and cell division cycle 42 effector protein 3 (CDC42EP3).

Results: LINC01857 was highly expressed in PANC-1 and MIA PaCa-2 cells in contrast to its expression in pancreatic ductal epithelial cells (8.9 folds and 7.1 folds, $p < 0.001$). Silencing LINC01857 significantly reduced cell proliferation and migration while enhancing apoptosis ($p < 0.0005$). In contrast, overexpression of LINC01857 markedly ($p < 0.05$) accelerated these malignant behavior of PC cells. MiR-450b-5p was targeted and inversely regulated by LINC01857. Moreover, CDC42EP3 was verified to be targeted by miR-450b-5p, and CDC42EP3 was correlated to LINC01857 in a positive manner ($p < 0.001$). Rescue experiments manifested that silencing CDC42EP3 effectively ($p < 0.05$) reversed the promoting effect of LINC01857 on malignant behavior of PC cells.

Conclusion: LINC01857 promotes PC cell proliferation and migration while obstructing cell apoptosis by binding to miR-450b-5p and thus regulating CDC42EP3 expression. The study

* Corresponding author. the Central Theater Hospital of the Chinese People's Liberation Army, No.627 Wuluo Road, Wuchang District, Wuhan, Hubei Province, China.

** Corresponding author.

E-mail addresses: Doctor_madandan@163.com (D.-D. Ma), ptwkhero@163.com (W.-D. Jin).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e38427
Received 28 May 2024; Received in revised form 14 September 2024; Accepted 24 September 2024
Available online 25 September 2024

presents a novel and promising regulatory axis, which holds potential for the identification of biomarkers and development of therapeutic strategies for PC treatment.

1. Introduction

As one of the most lethal malignancies, pancreatic cancer (PC) occurs in the pancreas where is a gland consisted of endocrine and exocrine cells [1]. The malignancy is mainly diagnosed in men and elderly adults from 60 to 85 years old but gradually become prevalent in young patients [2]. The etiology of PC is complex and diverse, and risk factors for PC development include smoking, family heredity, type 2 diabetes, and obesity [3]. The 5-year survival rate of PC patients is below 10 % [4]. Tumor metastasis to distant organs can be quickly developed in PC, posing challenges for treatment [5]. Chemotherapy, surgical resection, targeted therapy, radiotherapy, and combination regimens were common treatment options [6]. As to the targeted therapy, the identification of abnormally expressed genes and exploration of gene functions and related mechanisms are quite necessary.

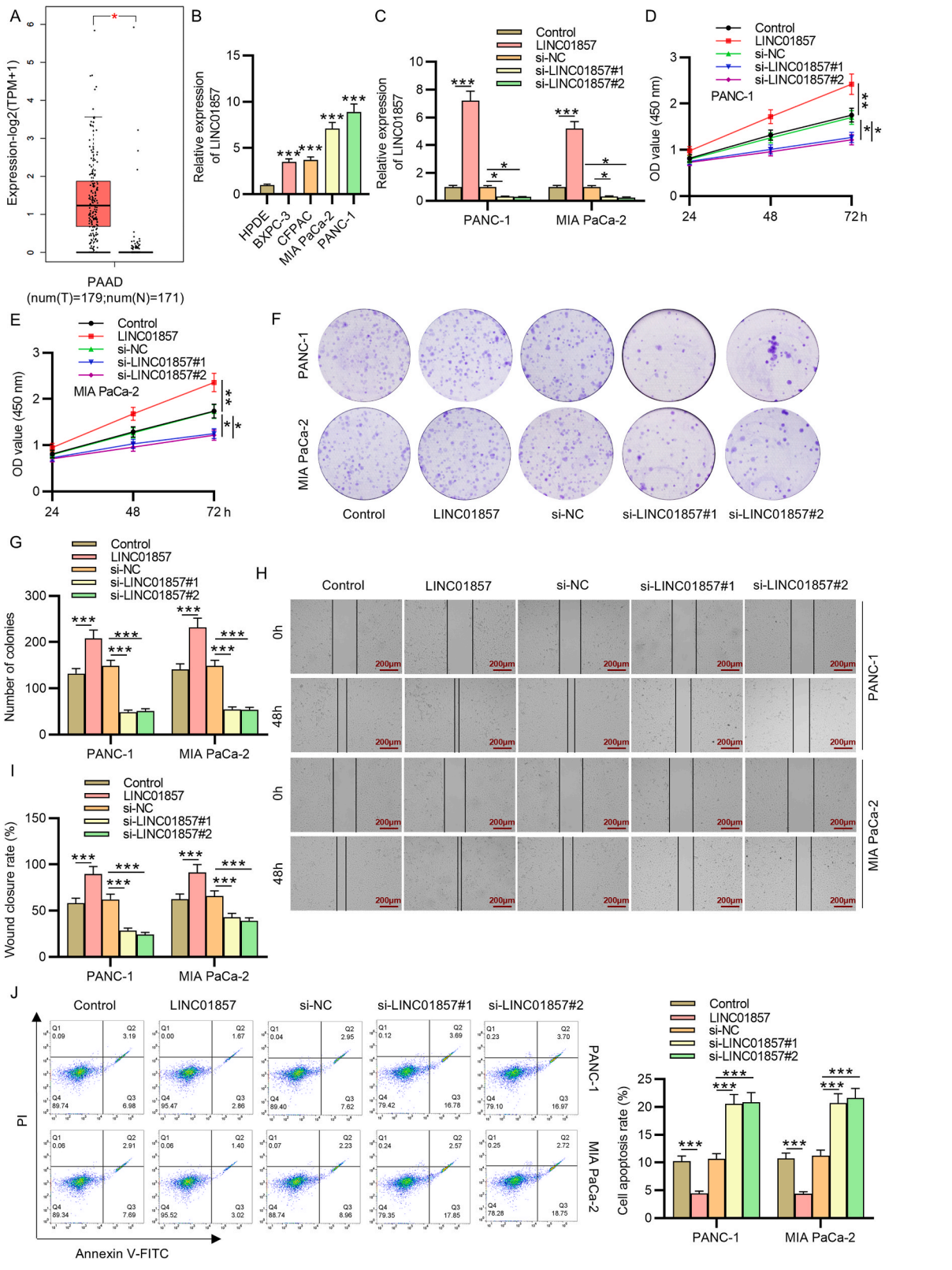
Long noncoding RNAs (lncRNAs) are composed of overall 200 nucleotides at length and lack the protein coding capability [7]. Abnormally expressed lncRNAs in human cancer are implicated with carcinogenesis by regulating proliferation, drug resistance, apoptosis and metastasis [8]. As to mechanisms, previous studies discovered that lncRNAs can act as mediators of RNA interference, scaffolds for RNA complexes, decoys for transcription factors or miRNAs, or chromatin-modifying proteins targeting specific genomic loci, and regulators of cis- or trans-transcriptional processes [9]. Among the various mechanism, the roles of lncRNAs in regulating protein-coding genes at the transcriptional or post-transcriptional level have gained increasing attention [10,11]. At the post-transcriptional level, lncRNA is known to serve as a competing endogenous RNA (ceRNA) to interact with microRNAs (miRNAs/miRs) and thus hamper the suppressive impact of miRs on the expression of specific tumorigenesis-related target genes [12,13]. Recently, many lncRNAs have been reported to promote or repress malignant behavior of PC cells by mediating ceRNA networks. For example, lncRNA forkhead box D1 antisense RNA 1 (FOXD1-AS1) promotes tumorigenesis and self-renewal of cancer stem cells in PC [14]. LncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) has recently been revealed to suppress PC cell metastasis and invasion by sponging miR-146b-5p and thus upregulating tumor necrosis factor receptor-associated factor 6 (TRAF6) [15]. LncRNA leucine zipper tumor suppressor 1 antisense RNA 1 (LZTS1-AS1) promotes PC cell proliferation and migration while repressing autophagy through increasing the expression of twist family bHLH transcription factor 1 (TWIST1) via interfering the inhibitory impact of miR-532 on TWIST1 [16].

LINC01857 has been confirmed to participate in many types of cancer, such as gastric cancer [17], breast cancer [18], glioma [19], lymphoma [20], and endometrial carcinoma [21]. Importantly, a report indicates that LINC01857 is highly expressed in PC tissues [22]. Another study validated the high expression of LINC01857 in pancreatic ductal adenocarcinoma and revealed the promoting effect of LINC01857 on epithelial-mesenchymal transition via binding with miR-19a-3p to alter the expression of secreted protein acidic and rich in cysteine-related modular calcium binding protein 2 (SMOC2) [23]. Compared with this article, our finding proposed a novel ceRNA network mediated by LINC01857 and focused on cell apoptosis in addition to cell proliferation and migration.

The miRNAs are single stranded and noncoding RNAs with 21–23 nucleotides at length, [24]. MiR-450b-5p was identified and explored in the present study. It was preliminary analyzed to be decreased in patients with ampullary adenocarcinoma, a malignancy similar to PC [25]. However, its role in PC were not reported yet. Cell division cycle 42 effector protein 3 (CDC42EP3) was chosen to be the target gene of miR450b-5p in this work. Though CDC42EP3 is a gene frequently reported in different types of cancer, there are no

Table 1
Sequences of primers used for reverse transcription-quantitative PCR.

Gene	Sequence (5'→3')
LINC01857	Forward: CTCCACTGCGCTTTGTCCAT
	Reverse: GAGGCTTTGAGGATGGGGAC
miR-2052	Forward: TGTTTTGATAACAGTAATGT
	Reverse: GAACATGTCTGCGTATCTC
miR-450b-5p	Forward: ACACTCCAGCTGGGTTTTGCAATATGTTCC
	Reverse: TGGTGTCGTGGAGTCG
miR-580-5p	Forward: TTGAGAATGATGAATCATTAG
	Reverse: GAACATGTCTGCGTATCTC
miR-4645-5p	Forward: ACAATATTTCTTGCCTGGT
	Reverse: CAGTGCGTGTCGTGGAGT
CDC42EP3	Forward: CCTGGAAACCAGGAGAAAGCAC
	Reverse: GGAGATGGCATTTTTGAGCACCG
FOXN3	Forward: TGCAAATGCACCTACTGGGTGG
	Reverse: CACCACAACGACCCTTTCCCAA
AUTS2	Forward: CCTCCTCATCACAGCAACTTCC
	Reverse: GAAGGCATTGCCACCAACTGCT
GAPDH	Forward: GTCTCCTCTGACTTCAACAGCG
	Reverse: ACCACCCTGTTGCTGTAGCCAA
U6	Forward: CTCGCTTCGGCAGCACAT
	Reverse: TTTGCGTGTCATCCTTGCG



(caption on next page)

Fig. 1. LINC01857 promotes malignant behavior of PC cells.
(A) GEPIA database was utilized to predict LINC01857 expression in PAAD and normal tissues. (B) LINC01857 expression in PC cells and HPDE cells was tested via RT-qPCR. (C) The transfection efficiency of pcDNA3.1-LINC01857 or si-LINC01857 in PANC-1 and MIA PaCa-2 cells was measured via RT-qPCR. (D–G) Cell viability and proliferation were assessed via CCK-8 and colony formation assays after transfection with pcDNA-LINC01857 or si-LINC01857. (H–I) Wound healing assays were utilized to estimate cell migration after interference of LINC01857 expression. (J) Flow cytometry was performed to evaluate PC cell apoptosis after LINC01857 overexpression or depletion. *p < 0.05, **p < 0.01, ***p < 0.001.

articles focusing on the role of CDC42EP3 in PC. The current study explored the function of two novel downstream factors of LINC01857 in PC.

In summary, the study aimed to explore the effects of LINC01857 on PC cell proliferation, migration, and apoptosis as well as a novel ceRNA network mediated by LINC01857. The study provides a promising therapeutic strategy for PC.

2. Materials and methods

2.1. Cell culture

Four PC cell lines (BXPC-3, CFPAC, MIA PaCa-2, PANC-1) and one human pancreatic ductal epithelial cell line (HPDE) were procured from ATCC (Manassas, USA). These cell lines were verified to be mycoplasma free. All the cell lines were authenticated through examination of morphology and growth characteristics and were confirmed to be free of mycoplasma. These cells were cultured in RPMI-1640 medium (Gibco, Thermo Fisher Scientific, Waltham, USA) with 10 % fetal bovine serum and 1 % penicillin/streptomycin (Gibco) at 37 °C with 5 % CO_2. The passage numbers for cell lines are 2–3.

2.2. Reverse transcription and quantitative polymerase chain reaction (RT-qPCR)

TRIzol reagent (Invitrogen, USA) was utilized for RNA extraction. PrimeScript™ II Reverse Transcriptase (Takara, Kusatsu, Japan) was utilized for cDNA synthesis through RNA reverse transcription. ABI StepOne Plus PCR system (Applied Biosystems, Foster City, USA) together with SYBR Green PCR Kit (Takara) were employed for qPCR analysis. Each PCR was performed in triplicate. Gene expression was identified via the $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH or U6. Sequences of primers used in qPCR were listed in Table 1.

2.3. Cell transfection

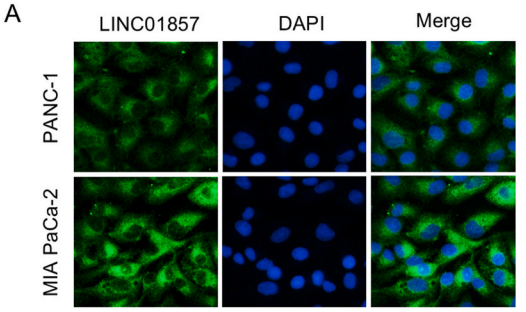
The siRNAs specifically targeting LINC01857 (si-LINC01857) or CDC42EP3 (si-CDC42EP3) were obtained from Gene Pharma (Shanghai, China) to knock down LINC01857 or CDC42EP3 expression, and the corresponding nonspecific siRNAs were utilized as negative control (si-NC). miR-450b-5p mimics and NC mimics were procured form RiboBio (Guangzhou, China) to amplify miR-450b-5p expression. LINC01857 full-length sequences were inserted into pcDNA vector (Geenseed Biotech, Guangzhou, China) for upregulation of LINC01857 in PC cells, with the empty vector served as control. For cell transfection, a total of 50 nM si-LINC01857, 50 nM si-CDC42EP3, 50 nM si-NC, 30 nM miR-450b-5p mimics, 30 nM NC mimics, 30 nM pcDNA-LINC01857 or 30 nM pcDNA-NC were subjected to cell transfection utilizing Lipofectamine 3000 (Invitrogen) for 48 h. Transfection efficiency was verified by detecting LINC01857, miR-450b-5p, or CDC42EP3 expression before and after plasmid transfection using RT-qPCR.

2.4. Cell counting Kit-8 (CCK-8) assay

CCK-8 reagent (Beyotime, Shanghai, China) was employed for detection of cell viability. Briefly, PC cells were inoculated to 96-well plates (2×10^3 cells/well) in 100 μl of complete medium for one, two or three days of incubation. At the end of indicated days, 10 μl of CCK-8 reagent was added to each well for another 2 h of cell culture at 37 °C. The value of optical density at the wavelength of 450 nm was measured using a spectrophotometer (Molecular Devices, Gaithersburg, USA). Cell viability in the pcDNA3.1-LINC01587 group (LINC01587) was normalized to the control vector group (Control), while that in the si-LINC01857#1/2 group was compared to the si-NC group. The viability of cells in the LINC01857+si-CDC42EP3#1 group was compared to that in the LINC01857 group. Each reaction was repeated in triplicate, and the mean ± standard deviation was calculated for each value.

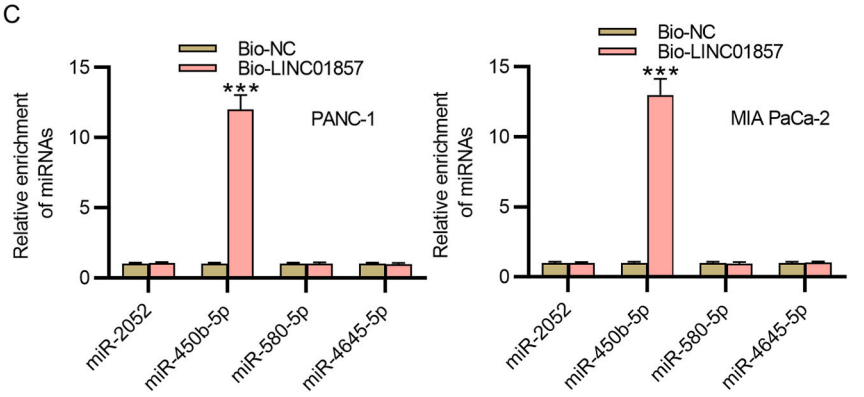
2.5. Colony formation

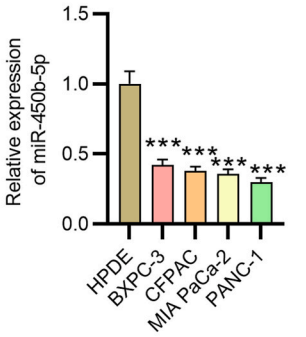
PC cells (500 cells/well) were put into culture plates (6-well) for 2 weeks of incubation. For every two or three days, the culture medium was changed with new medium. After that, colonies were first fixed with 4 % paraformaldehyde and then subjected to staining with 0.5 % crystal violet (Sigma Aldrich, St. Louis, USA). The colonies were quantified using the "ColonyArea" plugin of ImageJ software (National Institutes of Health, Bethesda, USA) after images were captured. The number of colonies containing ≥50 cells is counted and presented in a line graph.

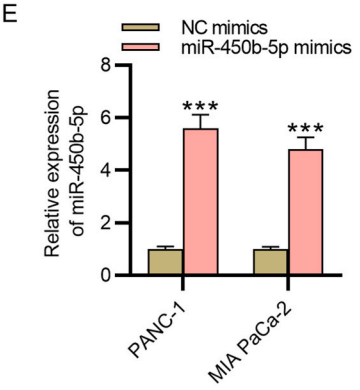


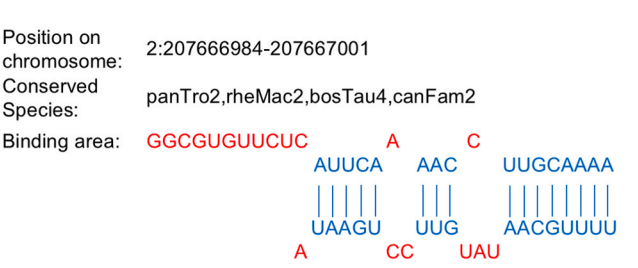
B

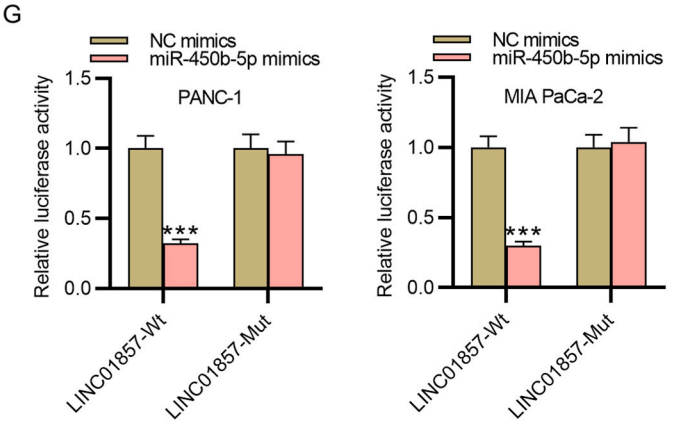
Gene	miRNA	Score	DIANA Links
AC079767.4	hsa-miR-2052	0.978	mT TB lnE mP
AC079767.4	hsa-miR-450b-5p	0.976	mT TB lnE mP
AC079767.4	hsa-miR-580-5p	0.923	mT TB lnE mP
AC079767.4	hsa-miR-4645-5p	0.846	mT TB lnE mP

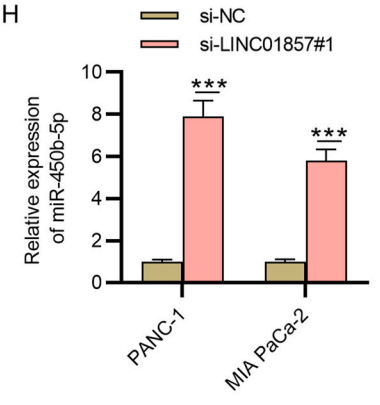












(caption on next page)

Fig. 2. LINC01857 binds with miR-450b-5p.
(A) FISH assay was utilized to determine the subcellular location of LINC01857 in PANC-1 and MIA PaCa-2 cells. (B) DIANA database was utilized to predict the candidate miRNAs for LINC01857. (C) RNA pulldown assay was employed for validating the combination of LINC01857 and candidate miRNAs (miR-2052/miR-450b-5p/miR-580-5p/miR-4645-5p). (D) MiR-450b-5p expression in PC cells was tested via RT-qPCR. (E) The transfection efficiency of miR-450b-5p mimics was measured via RT-qPCR. (F) DIANA database was applied for predicting the binding site of LINC01857 and miR-450b-5p. (G) The binding of LINC01857 and miR-450b-5p was validated through luciferase reporter assay. (H) RT-qPCR was employed for analyzing miR-4645-5p expression when LINC01857 was overexpressed or silenced. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

2.6. Wound healing assay

PC cells were put to 24-well plates (1×10^5 cells/well) for 24 h of incubation. Upon reaching 95%–100 % confluency, scratches were made on cell monolayers with a 10 μL pipette tip to make wounds. At 0 h and 48 h, images of these scratches were captured under a microscope (Nikon, Tokyo, Japan). For each wound, images were captured from five different areas. ImageJ software was used to calculate the wound closure rate according to the formula of (wound areas at 0 h– the actual wound areas at 48 h)/(wound areas at 0 h). Data are shown as the mean ± standard deviation.

2.7. Flow cytometry

In accordance with user guides, Annexin V-FITC/PI kit (Solarbio, Beijing, China) was used to detect cell apoptosis. PC cells were rinsed and then subjected to resuspension in binding buffer. Next, PC cells were dyed by 5 μl Annexin V-FITC and 5 μl PI in dark. After that, the flow cytometer (Becton Dickinson, USA) was applied to detect the percentage of apoptotic cells. Cells in the (AnnexinV-FITC)+/PI+ (Q2) area and (AnnexinV-FITC)+/PI- (Q3) area were regarded as apoptotic cells. Cell apoptosis in the pcDNA3.1-LINC01587 group (LINC01587) was normalized to the control vector group (Control), while that in the si-LINC01857#1/2 group was compared to the si-NC group. The apoptotic rate of cells in the LINC01857+si-CDC42EP3#1 group was compared to that in the LINC01857 group.

2.8. Western blot

Cell lysis was performed with radio immunoprecipitation lysis buffer (Beyotime, 50 mL, 100 μL). Proteins were extracted from PC cells and quantified by bicinchoninic acid methods. Then the protein was isolated on 12 % SDS-PAGE and then shifted to PVDF membranes. Next, the membranes were blocked using 5 % fat-free milk. Primary antibodies (Thermo Fisher Scientific, USA) of anti-CDC42EP3 (PA5-97076) and anti-GAPDH (39–8600) at the dilution of 1/500 were added to membranes for incubation overnight at 4 °C. After that, horseradish peroxidase (HRP)-conjugated secondary antibodies were supplemented for another 2 h incubation. Antibodies were validated using the Labome website (https://www.labome.com/index.html). After membrane was washed by the TBST, protein signals were detected by ECL substance (Thermo Fisher Scientific) and analyzed by the ImageJ program. GAPDH served as the loading control.

2.9. FISH

RNA FISH probe specific to LINC01857 was synthesized by RiboBio (Guangzhou, China). Streptavidin-biotin system was used to detect lncRNA. Air-dried cells were incubated with 40 nmol/L probes in a hybridization buffer (RiboBio), and the nuclei were stained by DAPI. Analysis and imaging were performed using a fluorescence microscope (Olympus). The fluorescence signal intensity was processed using ImageJ software.

2.10. RNA pulldown assay

Pierce Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific) was utilized for this assay. Transfection with biotinylated LINC01857 or biotinylated NC into PC cells for 48 h was first conducted, and then cells were incubated with lysis buffer. Next, 30 μl of Streptavidin magnetic beads (11641778001, Roche, Basel, Switzerland) was supplemented to the cell lysate after digestion with DNase I. RNA-protein mixture was treated with proteinase K, and the isolated RNA was quantified by RT-qPCR. The enrichment of miRNAs in the Bio-LINC01857 group were calculated compared with those in the bio-NC group.

2.11. Luciferase reporter gene assay

The putative binding site between LINC01857 and miR-450b-5p was predicted with the bioinformatics tool DIANA, and that between miR-450b-5p and CDC42EP3 3′-UTR was predicted via ENCORI database. LINC01857 or CDC42EP3 3′UTR segments having the binding area with miR-450b-5p were cloned to pmirGLO luciferase reporter vector (Promega, USA) for establishing the pmirGLO LINC01857-WT/Mut or CDC42EP3 3′UTR-WT/Mut. After that, the established plasmids were subjected to cotransfection with miR-450b-4p mimics/NC mimics into PC cells for 48 h using Lipofectamine 3000 (Invitrogen). Dual-Luciferase Reporter Assay System (#E1910, Promega) was applied to examine luciferase activities of fireflies (Photinus pyralis) and Renilla reniformis. The ratio of firefly luciferase activity to Renilla activity was defined as relative luciferase activity. The relative luciferase activity of LINC01857-

A

Target Detail	Target Rank	Target Score	miRNA Name	Gene Symbol	Gene Description
Details	1	100	hsa-miR-450b-5p	FOXN3	forkhead box N3
Details	2	100	hsa-miR-450b-5p	CDC42EP3	CDC42 effector protein 3
Details	3	100	hsa-miR-450b-5p	AUTS2	AUTS2, activator of transcription and developmental regulator

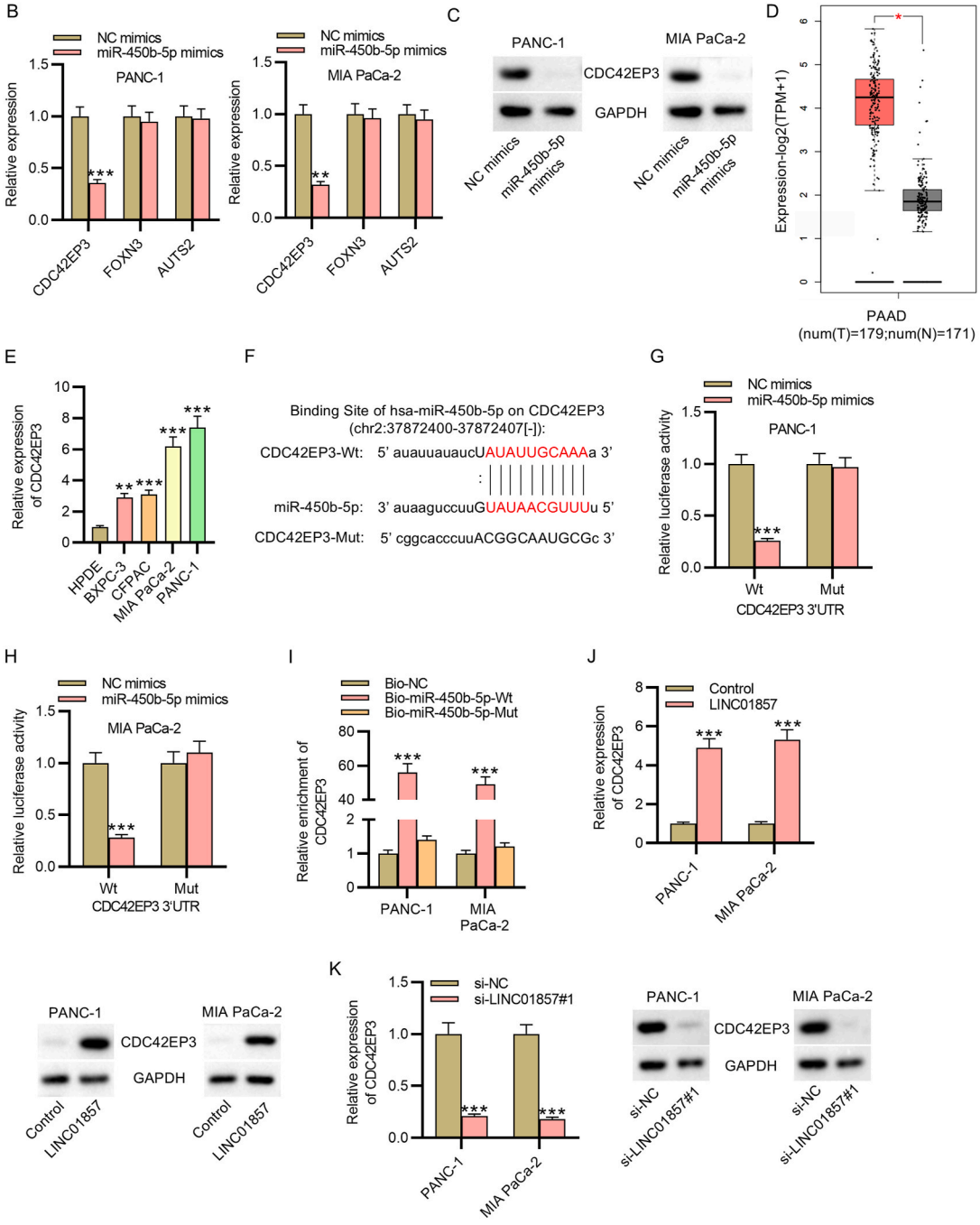


Fig. 3. CDC42EP3 is targeted by miR-450b-5p.
(A) The candidate mRNAs targeted by miR-450b-5p were predicted by miRDB database. (B) The expression of CDC42EP3, FOXN3 and AUTS2 was tested by RT-qPCR in PC cells overexpressing miR-450b-5p. (C) Western blot was utilized to test CDC42EP3 protein level in response to miR-450b-5p overexpression. Full images of blots can be found in supplementary material. (D) GEPIA database was utilized to predict CDC42EP3 expression in PAAD and normal tissues. (E) CDC42EP3 expression in PC cells and HPDE cells was tested via RT-qPCR. (F) The ENCORI database was applied for predicting the binding site of miR-450b-5p and CDC42EP3. (G–H) The binding of miR-450b-5p and CDC42EP3 was further validated through the

luciferase reporter assay. (I) RNA pulldown assays were carried out to further explore the interaction of miR-450b-5p and CDC42EP3. (J–K) Western blot was carried out for measurement of CDC42EP3 RNA and protein levels in response to LINC01857 overexpression or depletion. Full images of blots are available in supplementary material. **$p < 0.01$, ***$p < 0.001$.

Wt/Mut in PC cells transfected with miR-450b-5p mimics was compared to its activity in PC cells with NC mimics.

2.12. Statistical analyses

GraphPad PRISM 8 (GraphPad, La Jolla, USA) was used for statistical analysis. Data are presented as mean ± SD, $n = 3$ for all experiments. Comparison of differences among groups was evaluated using Student's *t*-test (for two groups) or one-way ANOVA (for three groups) followed by Tukey's *post hoc* analysis. The value of $p < 0.05$ was deemed as statistically significant. Specific p values were listed in Supplementary Table 1.

3. Results

3.1. LINC01857 facilitates PC cell proliferation and migration while hampering cell apoptosis

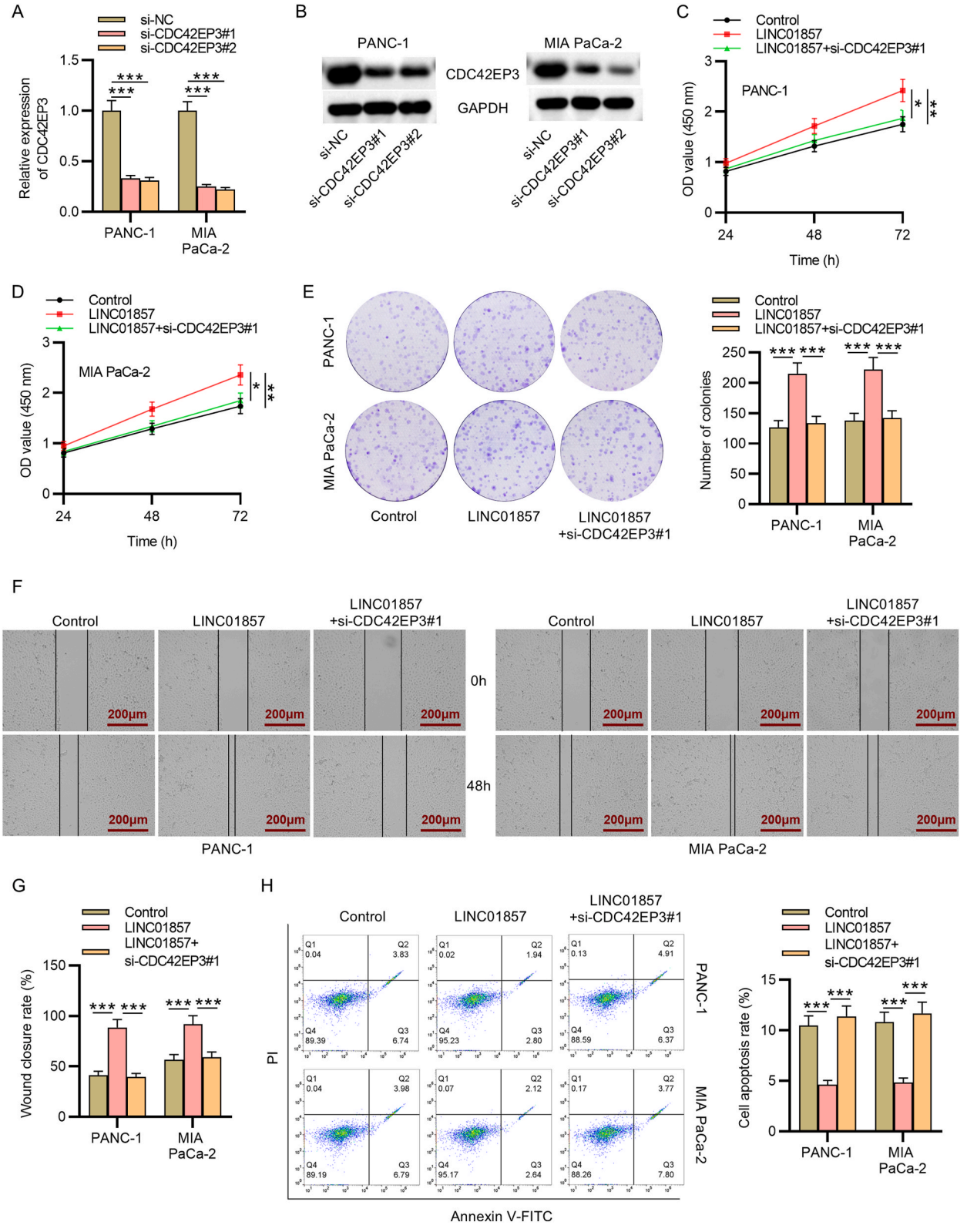
As shown by expression analysis using a bioinformatics tool GEPIA, LINC01857 is markedly upregulated in pancreatic adenocarcinoma (PAAD) tissues (Fig. 1A, $p < 0.05$). RT-qPCR was then conducted to verify LINC01857 level in PC cells compared to that in HPDE cells. It was illustrated that LINC01857 was highly expressed in four PC cell lines, including BXPC-3 ($p = 0.006$), CFPAC ($p = 0.0003$), MIA PaCa-2 ($p < 0.0001$), and PANC-1 cells ($p < 0.0001$), especially in PANC-1 and MIA PaCa-2 cells compared with its expression in HPDE cells (8.9 folds and 7.1 folds) (Fig. 1B). Therefore, the two cell lines were identified for subsequent assays. Then, functional experiments were carried out to explore the biological role of LINC01857 in PC cells. PCR revealed that LINC01857 expression was effectively amplified through transfection of pcDNA-LINC01857 ($p < 0.0001$) and its expression was successfully reduced post transfection of si-LINC01857#1 and #2 in PANC-1 and MIA PaCa-2 cells ($p < 0.05$) (Fig. 1C). CCK-8 assays indicated that the OD value was promoted by LINC01857 overexpression ($p < 0.01$) and repressed by LINC01857 depletion ($p < 0.05$), suggesting that cell viability could be facilitated by LINC01857 overexpression and inhibited by the silencing of LINC01857 (Fig. 1D and E). The quantity of colonies formed was elevated via the transfection of pcDNA3.1-LINC01857 and was declined in the presence of LINC01857 depletion (Fig. 1F–G, $p < 0.0001$). The finding indicated the promoting role of LINC01857 in cell proliferation. Moreover, wound healing assays displayed that the number of migrated cells was elevated in the pcDNA-LINC01857 group ($p < 0.0001$) and was decreased in the si-LINC01857 cell group ($p < 0.0005$), indicating that LINC01857 facilitates cell migratory capability (Fig. 1H and I). As showed by flow cytometry, cell apoptosis rate was suppressed by LINC01857 upregulation and was enhanced in the context of LINC01857 knockdown (Fig. 1J, $p \leq 0.0001$). In short, LINC01857 is abundantly expressed in PC cells and promotes PC malignant cell behavior.

3.2. LINC01857 binds with miR-450b-5p

As showed by FISH, LINC01857 majorly localized in cytoplasm of PANC-1 and MIA PaCa-2 cells, implying that LINC01857 may exert its function post-transcriptionally (Fig. 2A). Hence, LINC01857 has the potential to function as a ceRNA to interact with miRNAs in PC. DIANA database was utilized to predict the possible miRNAs for LINC01857 [26]. According to the score ranking, the top four miRNAs were selected (Fig. 2B). RNA pulldown assay illustrated that only miR-450b-5p was prominently enriched in response to biotinylated LINC01857 (Bio-LINC01857) probe compared with its enrichment in the Bio-NC group (PANC-1: 12 folds; MIA PaCa-2: 13 folds, $p < 0.0001$), suggesting that LINC01857 could interact with miR-450b-5p (Fig. 2C). Furthermore, RT-qPCR showed the low levels of miR-450b-5p in PC cells (Fig. 2D, $p < 0.0001$). RT-qPCR results also showed the successful transfection of miR-450b-5p mimics, because miR-450b-5p level was markedly increased in PANC-1 and MIA PaCa-2 cells overexpressing miR-450b-5p in contrast to its expression in NC mimics group (5.6 folds and 4.8 folds) (Fig. 2E, $p < 0.0001$). The binding area of LINC01857 and miR-450b-5p was obtained in the DIANA database (Fig. 2F). Then, it was found that miR-450b-5p upregulation declined the luciferase activity of LINC01857-WT in PANC-1 cells (68 % decrease, $p < 0.0001$) and MIA PaCa-2 cells (70 % decrease, $p < 0.0001$), whereas LINC01857-Mut activity was not significantly changed between miR-450b-5p mimics group and NC mimics group (PANC-1: 1 vs 0.96, $p = 0.8135$; MIA PaCa-2: 1 vs 1.04, $p = 0.8027$) (Fig. 2G). These data confirmed that LINC01857 could combine with miR-450b-5p. Additionally, miR-450b-5p expression was elevated via LINC01857 depletion (7.9 and 5.8 folds) (Fig. 2H, $p < 0.0001$). Overall, LINC01857 binds to miR-450b-5p and inversely modulates its expression in PC cells.

3.3. CDC42EP3 is a target of miR-450b-5p

Downstream mRNAs that can be regulated by miR-450b-5p were further explored. In miRDB (http://mirdb.org/) databases with the searching criterion of Target Score = 100, three potential mRNAs were identified (Fig. 3A). RT-qPCR results illustrated that CDC42EP3 expression was decreased by miR-450b-5p overexpression (64 % decrease, $p < 0.0001$), while the expression of the other two mRNAs was almost unchanged (Fig. 3B–$p > 0.8$). Western blot further illustrated that CDC42EP3 level was repressed following miR-450b-5p upregulation (Fig. 3C). GEPIA database displays that CDC42EP3 is highly expressed in PAAD samples (Fig. 3D). In our



(caption on next page)

Fig. 4. LINC01857 promotes malignant cell behavior in PC by upregulating CDC42EP3.
(A–B) RT-qPCR and Western blot were conducted for estimating the transfection efficiency of si-CDC42EP3. Full images of blots can be found in supplementary material. (C–E) Cell viability and proliferation were assessed using CCK-8 and colony formation assays in the control group, the pcDNA3.1-LINC01857 group, and the pcDNA3.1-LINC01857+si-CDC42EP3#1 group. (F–G) Wound healing assay was performed for estimating cell migration in the above three groups. (H) Flow cytometry was utilized for measuring cell apoptosis in the above three groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

experiments, CDC42EP3 was discovered to be abundantly expressed in four PC cells (Fig. 3E, $p = 0.0013$, $p = 0.0006$, $p < 0.0001$, $p < 0.0001$). The binding area of CDC42EP3 and miR-450b-5p was predicted from ENCORI (https://rnasysu.com/encori/) database (Fig. 3F). CDC42EP3-Wt luciferase activity was reduced by miR-450b-5p overexpression ($p < 0.0001$), while the CDC42EP3-Mut activity was not significantly altered by miR-450b-5p upregulation in PANC-1 and MIA PaCa-2 cells (Fig. 3G–H, $p = 0.8873$, $p = 0.4031$). RNA pulldown assays further validated the binding of miR-450b-5p to CDC42EP3. As shown by Fig. 3I, CDC42EP3 abundantly existed in the Bio-miR-450b-5p-Wt groups compared with its level in the Bio-NC group (56 and 49 folds, $p < 0.0001$). Additionally, there was no significant changes between Bio-NC and Bio-miR-450b-5p-Mut groups (Fig. 3I, $p = 0.9771$, $p = 0.9942$). The mRNA and protein levels of CDC42EP3 were elevated in response to LINC01857 upregulation and were reduced in the context of LINC01857 knockdown (Fig. 3J–K, $p < 0.0001$). In summary, CDC42EP3 is targeted and negatively modulated by miR-450b-5p in PC cells. Additionally, CDC42EP3 expression is positively correlated with LINC01857 expression.

3.4. LINC01857 influences cell malignant behavior in PC by upregulating CDC42EP3

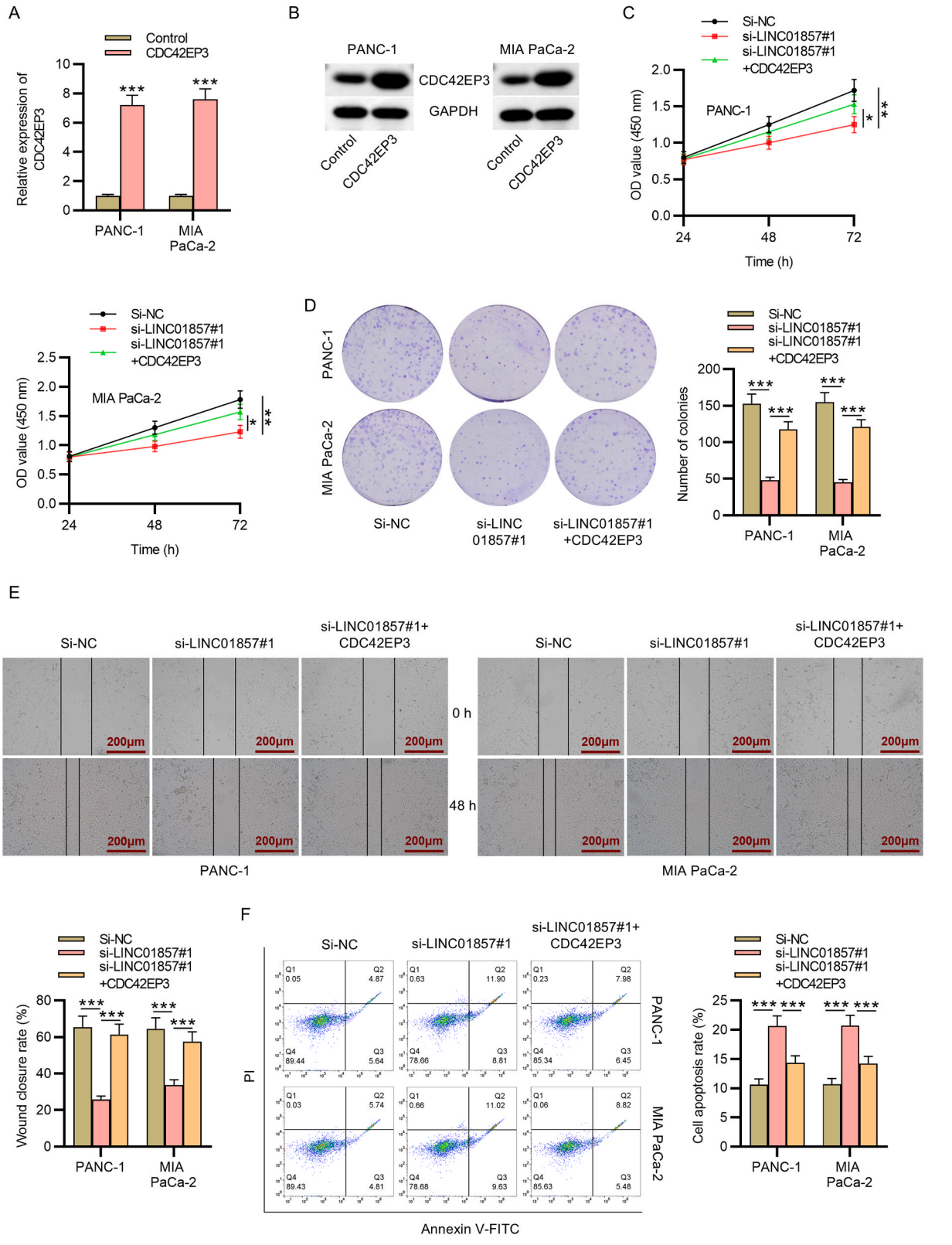
At last, rescue assays were conducted to verify whether LINC01857 promotes malignant PC cell behavior by upregulating CDC42EP3. First, CDC42EP3 was silenced in PC cells via the transfection of si-CDC42EP3 plasmids. It was manifested that the levels of CDC42EP3 were inhibited by the si-CDC42EP3 transfection ($p < 0.0001$, Fig. 4A and B). Cell viability promoted by LINC01857 amplification could be reversed via CDC42EP3 downregulation (Fig. 4C and D, *$p < 0.05$, **$p < 0.01$). Further, colony formation assays indicated that CDC42EP3 silencing counteracted the promoting impact of LINC01857 overexpression on cell proliferation (Fig. 4E, $p < 0.0001$). Wound healing assays manifested that the promotive role of LINC01857 in cell migration was offset by knockdown of CDC42EP3 (Fig. 4F–G, $p \leq 0.0008$). According to flow cytometry, cell apoptosis was inhibited by LINC01857 upregulation, while co-transfection of si-CDC42EP3 counteracted the inhibitory effect (Fig. 4H, $p < 0.0001$). In short, LINC01857 facilitates PC cell process via upregulating CDC42EP3 expression. More experiments were performed to investigate whether overexpressed CDC42EP3 can rescue the inhibitory effect of LINC01857 knockdown on malignant behavior of PC cells. As shown by Fig. 5A and B, CDC42EP3 mRNA and protein expression levels were successfully amplified post transfection of pcDNA3.1-CDC42EP3 vectors ($p < 0.0001$). Cells in the si-LINC01857 group had relatively low viability and proliferative capability compared with the si-NC group, and the trend was countervailed by CDC42EP3 upregulation (Fig. 5C–D, $p < 0.0001$). In addition, the reduction of PC cell migratory ability mediated by LINC01857 depletion was improved by overexpressed CDC42EP3 (Fig. 5E–$p \leq 0.0001$). On the contrary, the silencing of LINC01857 contributed to a high apoptotic rate of PC cells ($p < 0.0001$), and the alteration was suppressed by CDC42EP3 overexpression in PANC-1 and MIA PaCa-2 cells ($p = 0.0002$, $p = 0.0001$) (Fig. 5F). The above findings demonstrated that LINC01857 promotes PC cell process by upregulating CDC42EP3.

4. Discussion

PC is regarded as a fatal malignancy because the disease can quickly invade surrounding tissue and organs with no early symptoms [2]. Therefore, understanding the molecular mechanism of PC progression is vital to develop new treatments. Increasing evidence has confirmed that lncRNAs exert crucial functions in cancer tumorigenesis [27]. In PC, many lncRNAs are identified to be upregulated or downregulated in human cancers and participates in regulating PC progression, such as BCAB-AS1 [28], LINC01268 [29], and GATA6-AS1 [30]. In this study, LINC01857 was verified to be upregulated in PC cells compared with its expression in pancreatic ductal epithelial cells. The finding is consistent with the conclusion of a previous article authored by Giulietti et al., which identified the upregulation of LINC01857 in PC tissues [22]. This work further investigated the biological role of LINC01857 in PC. The silencing of LINC01857 repressed cell proliferative and migratory capabilities while promoting cell apoptosis in PC. LINC01857 overexpression had the opposite effect, indicating the oncogenic role of LINC01857 in PC. Results of functional experiments regarding the effect of LINC01857 on cell proliferation and migration were in line with those reported in a recent study [23]. Moreover, the carcinogenic role of LINC01857 has also been mentioned in other types of cancer such as diffuse large B-cell lymphoma and gastric cancer [17,20].

Recently, the ceRNA regulatory network of lncRNA/miRNA/mRNA has been confirmed in various cancers [27]. The regulatory axis involves the interaction of lncRNAs with miRNAs, leading to indirect regulation of mRNA levels at a post-transcriptional stage [31]. The ceRNA network represents a promising regulatory mechanism for RNA interactions, and it has been experimentally validated to govern the progression and development of cancer [13,32]. The prerequisite for ceRNA mechanism is the cytoplastic localization of lncRNA [33]. Consistent with available literature, LINC01857, in this study, was discovered to predominantly exist in cytoplasm of PC cells. The ceRNA function of LINC01857 verified in this study is in accordance with previous articles centered on its role in glioma [19] and lymphoma [20].

Moreover, miR-450b-5p was verified to be the downstream miRNA inversely regulated by LINC01857. Like its role in the present work, miR-450b-5p was frequently reported to participate in ceRNA networks mediated by lncRNAs. For example, it was reported to



(caption on next page)

Fig. 5. LINC01857 depletion inhibits PC cell process via downregulation of CDC42EP3.
(A–B) RT-qPCR and western blotting were performed to measure the overexpression efficiency of CDC42EP3 in PANC-1 and MIA PaCa-2 cells. Full images of uncropped blots are available in Supplementary material. (C–D) CCK-8 and colony formation assays were performed to examine PC cell viability and proliferation in the si-NC, si-LINC01857#1, and si-LINC01857#1 + CDC42EP3 groups. (E–F) Wound healing assays and flow cytometry analyses were conducted to assess PC cell migration and apoptosis in the si-NC, si-LINC01857#1, and si-LINC01857#1 + CDC42EP3 groups. *p < 0.05, **p < 0.01, ***p < 0.001.

interact with LINC00441 and target RAB10 in cervical cancer [34]. miR-450b-5p acts as a downstream factor of LINC00641 in colorectal cancer and constitutes the LINC00641/miR-450b-5p/GOLPH3 axis [35]. In addition, the low expression of miR-450b-5p in PC cells in the current work is in line with the results of miRNA profiling in PC authored by Calatayud et al., which also confirmed the prognostic role of miR-450b-5p for patients with PC [25].

CDC42EP3 is a member of Cdc42 effector protein family and exerts a crucial role in assorted cellular process, including cell polarization and neural progression [36]. In the present study, CDC42EP3 was targeted and negatively modulated by miR-450b-5p in PC cells. High CDC42EP3 expression levels in PC cells are in accordance with bioinformatics analysis using GEPIA (data source: The Cancer Genome Atlas) [37]. Moreover, rescue experiments manifested that CDC42EP3 depletion offset the promotive impact of LINC01857 upregulation on malignant cellular behavior, while overexpressed CDC42EP3 reversed the suppressive impact of LINC01857 knockdown on PC cell process. The current findings verified the oncogenic role of CDC42EP3 in regulating PC cells, and LINC01857 facilitates PC cell proliferation and metastasis while repressing apoptosis by upregulating CDC42EP3. The expression and function of CDC42EP3 were first reported in PC. Its carcinogenic role in PC is not aberrant from previous reports on CDC42EP3 in other types of cancer. For example, CDC42EP3 facilitates colorectal cancer progression by modulating cell proliferation and migration [38]. CDC42EP3 is a critical modulator participating in the development of gastric cancer [39].

Overall, this study demonstrates that overexpression of LINC01857 facilitates cell proliferation and migration via interacting with miR-450b-5p to elevate CDC42EP3 expression. These discoveries may provide new therapeutic targets for precision medicine in PC. The limitations of this study lie in the absence of *in vivo* experiments for further verification of the regulatory axis mediated by LINC01857 in PC and the lack of exploration regarding the upstream transcription factor of LINC01857 and the signaling pathways mediated by CDC42EP3. Animal experiments and in-depth exploration of molecules involved in the axis can be the direction for future work.

Ethics approval

Not applicable.

Funding

This work is supported by the National Natural Science Foundation of China (No.81902501).

Data availability statement

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Jian-Xin Zhang: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yan-Bin Shen:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dan-Dan Ma:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Zhong-Hu Li:** Supervision, Software, Resources, Project administration, Methodology. **Zhi-Yong Zhang:** Validation, Supervision, Software, Resources. **Wei-Dong Jin:** Supervision, Software.

Declaration of competing interest

None.

Acknowledgements

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38427.

References

[1] S. Lanfredini, A. Thapa, E. O'Neill, RAS in pancreatic cancer, Biochem. Soc. Trans. 47 (4) (2019) 961–972.
[2] Z. Zhao, W. Liu, Pancreatic cancer: a review of risk factors, diagnosis, and treatment, Technol. Cancer Res. Treat. 19 (2020) 1533033820962117.
[3] J.D. Mizrahi, et al., Pancreatic cancer, Lancet 395 (10242) (2020) 2008–2020.
[4] L. Del Nero, et al., Familial pancreatic cancer, Clin Res Hepatol Gastroenterol 47 (3) (2023) 102079.
[5] Z. Guo, et al., Molecular profile of metastasis, cell plasticity and EMT in pancreatic cancer: a pre-clinical connection to aggressiveness and drug resistance, Cancer Metastasis Rev. (2023).
[6] H. Duan, L. Li, S. He, Advances and prospects in the treatment of pancreatic cancer, Int. J. Nanomed. 18 (2023) 3973–3988.
[7] T. Ali, P. Grote, Beyond the RNA-dependent function of LncRNA genes, Elife 9 (2020).
[8] E. Bakinowska, et al., Non-coding RNA as biomarkers and their role in the pathogenesis of gastric cancer-A narrative review, Int. J. Mol. Sci. 25 (10) (2024).
[9] J. Xu, et al., LncRNA MIR17HG suppresses breast cancer proliferation and migration as ceRNA to target FAM135A by sponging miR-454-3p, Mol. Biotechnol. 65 (12) (2023) 2071–2085.
[10] S. Adnane, A. Marino, E. Leucci, LncRNAs in human cancers: signal from noise, Trends Cell Biol. 32 (7) (2022) 565–573.
[11] Q. Dong, et al., LncRNA SNHG4 promotes prostate cancer cell survival and resistance to enzalutamide through a let-7a/RREB1 positive feedback loop and a ceRNA network, J. Exp. Clin. Cancer Res. 42 (1) (2023) 209.
[12] R. Li, H. Xu, X. Gao, The ceRNA network regulates epithelial-mesenchymal transition in colorectal cancer, Heliyon 9 (3) (2023) e14143.
[13] G. Xiong, et al., Long noncoding competing endogenous RNA networks in pancreatic cancer, Front. Oncol. 11 (2021) 765216.
[14] L. Ouyang, et al., LncRNA FOXD1-AS1 regulates pancreatic cancer stem cell properties and 5-FU resistance by regulating the miR-570-3p/SPP1 axis as a ceRNA, Cancer Cell Int. 24 (1) (2024) 4.
[15] F. Wei, et al., LncRNA-NEAT1 inhibits the occurrence and development of pancreatic cancer through spongy miR-146b-5p/traf6, Biotechnol. Genet. Eng. Rev. (2023) 1–19.
[16] H. Wu, et al., LncRNA LZTS1-AS1 induces proliferation, metastasis and inhibits autophagy of pancreatic cancer cells through the miR-532/TWIST1 signaling pathway, Cancer Cell Int. 23 (1) (2023) 130.
[17] S. Chen, et al., LINC01857 promotes the proliferation, migration, and invasion of gastric cancer cells via regulating miR-4731-5p/HOXC6, Can. J. Physiol. Pharmacol. 100 (7) (2022) 689–701.
[18] W. Qian, et al., LncRNA LINC01857 reduces metastasis and angiogenesis in breast cancer cells via regulating miR-2052/CENPQ axis, Open Med. 17 (1) (2022) 1357–1367.
[19] G. Hu, et al., LncRNA LINC01857 promotes growth, migration, and invasion of glioma by modulating miR-1281/TRIM65 axis, J. Cell. Physiol. 234 (12) (2019) 22009–22016.
[20] Q. Li, et al., LncRNA LINC01857 promotes cell growth and diminishes apoptosis via PI3K/mTOR pathway and EMT process by regulating miR-141-3p/MAP4K4 axis in diffuse large B-cell lymphoma, Cancer Gene Ther. 28 (9) (2021) 1046–1057.
[21] R. Cao, J. Zhao, J. Zhang, LINC01857 exacerbates the malignant behaviors of endometrial carcinoma cells by sponging miR-19b-3p and recruiting ELAVL1 to upregulate MYCN, Gynecol. Obstet. Invest. 88 (1) (2023) 16–29.
[22] M. Giulietti, et al., LncRNA co-expression network analysis reveals novel biomarkers for pancreatic cancer, Carcinogenesis 39 (8) (2018) 1016–1025.
[23] Y. Lu, et al., LncRNA LINC01857 drives pancreatic adenocarcinoma progression via modulating miR-19a-3p/SMOC2, Clinics 77 (2022) 100047.
[24] M. Correia de Sousa, et al., Deciphering miRNAs' action through miRNA editing, Int. J. Mol. Sci. 20 (24) (2019).
[25] D. Calatayud, et al., Tissue MicroRNA profiles as diagnostic and prognostic biomarkers in patients with resectable pancreatic ductal adenocarcinoma and periampullary cancers, Biomark. Res. 5 (2017) 8.
[26] M.D. Paraskevopoulou, et al., DIANA-LncBase: experimentally verified and computationally predicted microRNA targets on long non-coding RNAs, Nucleic Acids Res. 41 (Database issue) (2013) D239–D245.
[27] J. Xu, et al., The role of lncRNA-mediated ceRNA regulatory networks in pancreatic cancer, Cell Death Dis. 8 (1) (2022) 287.
[28] G. Wu, et al., LncRNA BCAN-AS1 stabilizes c-Myc via N(6)-methyladenosine-mediated binding with SNIP1 to promote pancreatic cancer, Cell Death Differ. 30 (10) (2023) 2213–2230.
[29] S. Liu, et al., Exosomal lncRNA LINC01268 promotes pancreatic cancer progression via the miR-217-KIF2A-PI3K/AKT axis, Genes Dis 10 (5) (2023) 1799–1801.
[30] Y. Zhou, et al., GATA6-AS1 suppresses epithelial-mesenchymal transition of pancreatic cancer under hypoxia through regulating SNAI1 mRNA stability, J. Transl. Med. 21 (1) (2023) 882.
[31] K. Li, T. Yao, Z. Wang, lncRNA-mediated ceRNA network in bladder cancer, Noncoding RNA Res 8 (2) (2023) 135–145.
[32] W.J. Wang, et al., A competing endogenous RNA network reveals novel potential lncRNA, miRNA, and mRNA biomarkers in the prognosis of human colon adenocarcinoma, J. Surg. Res. 235 (2019) 22–33.
[33] C.L. Smillie, T. Sirey, C.P. Ponting, Complexities of post-transcriptional regulation and the modeling of ceRNA crosstalk, Crit. Rev. Biochem. Mol. Biol. 53 (3) (2018) 231–245.
[34] H. Han, Q. Shao, X. Liu, LINC00441 promotes cervical cancer progression by modulating miR-450b-5p/RAB10 axis, Cancer Cell Int. 20 (2020) 368.
[35] Z. Hong, et al., Long intergenic noncoding RNA 00641 promotes growth and invasion of colorectal cancer through regulating miR-450b-5p/GOLPH3 Axis, J Oncol 2022 (2022) 8259135.
[36] H. Farhan, V.W. Hsu, Cdc42 and cellular polarity: emerging roles at the golgi, Trends Cell Biol. 26 (4) (2016) 241–248.
[37] Z. Tang, et al., GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses, Nucleic Acids Res. 45 (W1) (2017) W98–w102.
[38] Q. Feng, et al., CDC42EP3 promotes colorectal cancer through regulating cell proliferation, cell apoptosis and cell migration, Cancer Cell Int. 21 (1) (2021) 169.
[39] W. Chen, et al., CDC42EP3 is a key promoter involved in the development and progression of gastric cancer, Carcinogenesis 42 (9) (2021) 1179–1188.