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Research article

Circular RNA circTMEM59 inhibits progression of pancreatic ductal adenocarcinoma by targeting miR-147b/SOCS1: An in vitro study

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ABSTRACT

Purpose: This study aimed to detect the role and mechanism of circTMEM59 in pancreatic ductal adenocarcinoma (PDAC).

Methods: 66 paired PDAC tissues and normal samples were harvested from patients diagnosed and undergoing pancreatic cancer surgery in our hospital. The expression of circTMEM59 in PDAC tissues and cell lines was detected. Based on bioinformatics information, the circTMEM59 mimics, miR-147b mimics, miR-147b inhibitor and si-suppressor of cytokine signaling 1 (SOCS1) were transfected into PDAC cells. The expression levels of circTMEM59, miR-147b and SOCS1 were detected by quantitative real-time polymerase chain reaction (qRT-PCR). RNA interaction was confirmed by dual-luciferase reporter and RNA immunoprecipitation (RIP) assays. Cell invasion and proliferation were evaluated by Transwell and Cell Counting Kit-8 (CCK-8) assays. The protein expression was detected by Western blot.

Results: CircTMEM59 was confirmed to be downregulated in PDAC tumor tissues and cells. Low expression of circTMEM59 was closely correlated with the short survival time and poor clinicopathological characteristics. By up-regulating the expression of circTMEM59 in PDAC cells, cell proliferation, invasion and epithelial-mesenchymal transition (EMT) were inhibited. More importantly, miR-147b could be sponged by circTMEM59, and knockdown of miR-147b inhibited progression of PDAC cells. Further study revealed that SOCS1 was targeted by miR-147b. SOCS1 expression was negatively related to miR-147b expression and positively related to circTMEM59 expression in PDAC tissues. Upregulated miR-147b and downregulated SOCS1 could rescue the effects of circTMEM59 on cell proliferation, EMT and invasion.

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; RNA, ribonucleic acid; CircRNA, circular ribonucleic acid; EMT, epithelial-mesenchymal transition; TMEM59, transmembrane protein 59; CRC, colorectal cancer; SOCS1, suppressor of cytokine signaling 1; NC, negative control; HPDE, human pancreatic ductal epithelial; WT, wild-type; MUT, mutant-type; qRT-PCR, quantitative real-time polymerase chain reaction; cDNA, complementary deoxyribonucleic acid; RIP, RNA immunoprecipitation; SD, standard deviation; ANOVA, analysis of variance; LSD, least significance difference; LncRNA, long non-coding ribonucleic acid; FBS, fetal bovine serum.

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Conclusion: Our data indicated that circTMEM59 inhibited cell proliferation, invasion and EMT of PDAC by regulating miR-147b/SOCS1 axis.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most lethal tumor in the world, with a five-year survival rate less than 8.5 % [1,2]. Currently, chemotherapy alone or in combination with radiotherapy is still the main treatment option for the advanced/metastatic PDAC [2]. No target therapy and immunotherapy are clearly effective for PDAC [1,3]. For this reason, better knowing the pathogenesis and molecular biology of PDAC can help develop effective strategies to fight PDAC cancer.

Circular ribonucleic acid (circRNA) is one type of the noncoding ribonucleic acids (RNAs) which were first detected in virus [4]. With the development of nucleic acid-based therapeutic tools, a number of circRNAs have been identified as functional molecules in regulating disease progression [4–6]. In tumors, circRNAs have been reported to regulate tumor proliferation, metastasis, angiogenesis, invasion, and epithelial-mesenchymal transition (EMT) [7]. Due to the high stability *in vivo*, circRNAs have been regarded as the potential therapeutic targets and clinical prognostic markers for a series of solid tumors [7].

Transmembrane protein 59 (TMEM59) is a membrane protein involving in many cell processes including signal transduction and cell adhesion [8]. The antitumor effects of TMEM59 have been found in glioma [9], neuroblastoma [10] and gastric cancer [11]. CircTMEM59, also named as hsa_circ_0012634, is derived from TMEM59 gene. Unlike linear TMEM59, circTMEM59 are stable and resistant to Ribonuclease R (RNase R) [12]. Currently, the detailed functions of circTMEM59 are not very clear. But according to previous studies, circTMEM59 may act as a tumor suppressor in gastrointestinal tumors. Feng et al. found that circTMEM59 was downregulated in colorectal cancer (CRC) [12]. Upregulating the expression of circTMEM59 could inhibit CRC cell growth and metastasis [12]. Similarly, Liu et al. demonstrated that circTMEM59 participated in the inhibition of CRC growth [13]. Nevertheless, the role of circTMEM59 in PDAC has not been reported previously.

This study aimed to detect the role and mechanisms of circTMEM59 in PDAC. Firstly, circTMEM59 was found to be downregulated in PDAC. Low circTMEM59 expression was closely related to shorter survival time in PDAC patients. For the underlying mechanisms, circTMEM59 was found to repress the proliferation, EMT and invasion of PDAC cells. Further investigations revealed that circTMEM59 could regulate miR-147b/suppressor of cytokine signaling 1 (SOCS1) axis to affect PDAC progression. Collectively, the present study indicated that circTMEM59 had the anti-tumor effect against PDAC via regulating miR-147b/SOCS1 axis. Our results may provide a potential treatment target for PDAC management.

2. Materials and methods

2.1. Tissue samples

66 paired PDAC tissues and normal samples were harvested from patients in the Department of Department of Hepatobiliary-pancreatic and Integrative Oncology, Fudan University Shanghai Cancer Center. All patients had not received any anti-tumor therapy before the surgery. The study was approved by the Ethics Committee of the Fudan University Shanghai Cancer Center in accordance with the Declaration of Helsinki. All enrolled patients provided informed consents.

2.2. Cell culture

A total of 4 PDAC cell lines (PANC-1, CFPAC-1, SW1990 and BXPC-3) as well as human normal pancreatic ductal epithelial (HPDE) cells were obtained from the ATCC cell bank (Shanghai, China). All the 4 cell lines were cultured in 10 % fetal bovine serum (FBS, Thermo Fisher Scientific, Waltham, USA) containing DMEM. All cells were cultured at 37 °C with 5 % CO_2.

2.3. Cell transfection

CircTMEM59 mimics, miR-147b mimics, miR-147b inhibitors, SOCS1 small interfering RNA (si-SOCS1) and corresponding negative control (NC) were constructed by Guangzhou RiboBio Co., Ltd. Using Lipofectamine 2000 (Invitrogen), the aforementioned plasmids were transfected into PANC-1 or BXPC-3 cells. The morphology of cells was observed by a microscope (Olympus Corporation, Tokyo, Japan) after transfection.

2.4. Quantitative real-time polymerase chain reaction (qRT-PCR)

1 μL total RNAs were extracted using PARIS™ kit (Thermo Fisher Scientific) according to the manufacturer's protocols, and reversely transcribed into complementary deoxyribonucleic acid (cDNA) by cDNA Synthesis Kit (TaKaRa Bio, USA). The reverse transcription conditions were 65 °C for 5 min, 42 °C for 40 min and 70 °C for 15 min qRT-PCR was conducted by an ABI StepOnePlus™ System (Applied Biosystems, USA). The primers are presented in Table 1. GAPDH and U6 were used as the internal controls. The reaction conditions are as follows: reverse transcription at 50 °C for 15 min, pre-reaction at 95 °C for 15 min, denaturation at 94 °C for

15 s, and annealing at 57 °C for 45 s, a total of 40 cycles. The data were calculated using the $2^{-\triangle\triangle Ct}$ method.

2.5. Cell counting Kit-8 (CCK-8) assays

PDAC cells were seeded in 96-well plates at 1000 cells per well and incubated at 37 °C in a 5 % CO_2 incubator. The next day, the cells were washed twice with PBS, and then 100 μl DMEM was added to each well. The old solution was discarded after 0 h, 24 h, 48 and 72 h. Then, 10 μL of CCK-8 solution was added into each well and incubated for 2 h each at 37 °C. The optical density (OD) value was evaluated at 450 nm. Inhibition rate (%) = (1- $A_{experimental\ group}$ value/$A_{control\ group}$ value) × 100 %.

2.6. Transwell assay

Transwell chambers with Matrigel was used to analyze cell invasion. Cells in culture medium without FBS were added into the upper chamber of Transwell chambers, and the lower chamber was added with 10 % FBS containing medium. Then, the cells in transwell chambers were incubated for 24 h at 37 °C. After removing the cells that had not invaded, the cells on the bottom were stained with crystal violet. The invaded cells were observed by a microscope (Olympus Corporation, Tokyo, Japan).

2.7. Luciferase reporter assay

The fragments of circTMEM59 or SOCS1 3′UTR wild-type (WT) or mutant type (MUT) were cloned into pmirGLO vector (Promega, Madison, USA) using restriction enzymes Pmel and Xbal. These vectors were co-transfected into PANC-1 or BXPC-3 cells with miR-147b mimics or miR-NC. Dual-Luciferase Reporter Gene Assay Kit (Beyotime) was utilized to determine relative luciferase activity [14].

2.8. RNA immunoprecipitation (RIP) assay

RIP assay was conducted by RIP Kit (Millipore, Burlington, USA). AntiAgo2 (Millipore) was used to enrich circTMEM59 and miR-147b. The negative control was normal mouse Anti-IgG (Millipore).

2.9. Western blotting

Cells were lysed in ice-cold RIPA lysis buffer (Beyotime, Shanghai, China) to collect protein, and protein (30 μg) was electrophoresed in 10 % SDS-PAGE gel followed by transferred onto PVDF membrane (Millipore, USA). Following by blocking with 5 % skimmed milk, membranes were incubated with primary antibodies against E-cadherin [1:1000, # 96743, Cell Signaling Technology (CST), Danvers, USA], vimentin (1:1000, # 5741, CST), proliferation cell nuclear antigen (PCNA, 1:1000, # 13110, CST), SOCS1 (1:1000, # 68631, CST), and β-actin (1:1000, # 4970, CST). Subsequently, the membranes were incubated with HRP-linked secondary antibody (1:3000, 7074, CST). ECL (Millipore) solution was used to observe the protein band. Relative protein expression was quantified by BioImaging Systems.

2.10. Statistical analysis

The data were analyzed by SPSS 22.0. The measurement data were described as mean ± Standard Deviation (SD). The differences among groups were evaluated by a Student's t-test (for 2 groups) or analysis of variance (ANOVA, for more than 2 groups) followed by the least significance difference (LSD) post hoc test. The qualitative data were expressed as percentages and compared by Chi-square test. The Kaplan-Meier method was used to estimate the survival time of PDAC patients, with log-rank tests being used to assess significance. The relationship among circTMEM59, miR-147b, and SOCS1 expression levels was estimated by Pearson correlation

Table 1
Primer sequences used for qRT-PCR.

Name		Primer sequences (5′-3′)
CircTMEM59	Forward	TGATGCCAAAAATGCACCTA
	Reverse	ATTTGTAGGCTCCTGCTCCA
TMEM59	Forward	GGCAGAACTCATCAGGTCGCT
	Reverse	GCATTCTTGGCATCAGGGACA
MiR-147b	Forward	GGGGTGTGTGGAAAT
	Reverse	AACTGGTGTCGTGGAGTCGGC
SOCS1	Forward	CGCCCTCAGTGTGAAGATGG
	Reverse	GCTCGAAGAGGCAGTCGAAG
GADPH	Forward	AGAAGGCTGGGGCTCATTTG
	Reverse	AGGGGCCATCCACAGTCTTC
U6	Forward	CTCGCTTCGGCAGCACA
	Reverse	AACGCTTCACGAATTTGCGT

analysis. P < 0.05 indicated the difference was statistically significant.

3. Results

3.1. CircTMEM59 was downregulated in PDAC

In this study, the GEO database (GSE69362) was used to detect the major circRNAs in PDAC progression (Fig. 1A). Our data showed that circTMEM59 was much lower in PDAC tissues than in normal samples (p < 0.01, Fig. 1B). In order to confirm the expression of circTMEM59 in PDAC, 66 paired PDAC tissues and corresponding adjacent samples were collected. qRT-PCR analysis revealed that circTMEM59 expression was markedly decreased in PDAC tissues (p < 0.01, Fig. 1C). Using the median level of circTMEM59 as the cut-off value, the enrolled patents were assigned to high circTMEM59 expression group (n = 33) and low circTMEM59 expression group (n = 33). We found that circTMEM59 expression was related to TNM stage (p = 0.029), tumor differentiation (p = 0.003) and major vascular invasion (p = 0.046, Table 2). Furthermore, the survival analysis demonstrated that low circTMEM59 expression was markedly related to a shorter overall survival in PDAC patients (Fig. 1D). In addition, circTMEM59 expression in 4 PDAC cell lines was found to be much lower than HPDE cells (Fig. 1E). Among these cells, BXPC-3 and PANC-1 cells had the lowest circTMEM59 levels. Thus, these 2 cell lines were chosen for the further studies. Then, RNase R was added into the PANC-1 and BXPC-3 cells, and the qRT-PCR analysis showed circTMEM59 was more resistant to RNase R than linear TMEM59 (Fig. 1F), indicating circTMEM59 was more stable. Taken together, the above data suggested that circTMEM59 expression was downregulated in PDAC tissues and cell lines.

3.2. Overexpression of circTMEM59 inhibited proliferation, invasion and EMT of PDAC cells

After transfection of circTMEM59 mimics, PANC-1 or BXPC-3 cells exhibited much higher circTMEM59 expression (Fig. 2A). CCK8 assay revealed that overexpressed circTMEM59 significantly decreased cell proliferation (Fig. 2B). Transwell assay demonstrated that overexpressed circTMEM59 significantly reduced the invasion of PDAC cells (Fig. 2C). In addition, after transfection with circTMEM59, the number of round PDAC cells were increased, while the number of spindle cell were decreased (Fig. 2D), indicating circTMEM59 overexpression might inhibit EMT. Consistent with results of CCK8 assay, circTMEM59 overexpression significantly downregulated the expression of PCNA (Fig. 2E). For EMT associated proteins, overexpressed circTMEM59 significantly suppressed vimentin expression and facilitated E-cadherin expression (Fig. 2E). Therefore, our results indicated that circTMEM59 might inhibit proliferation, invasion and EMT of PDAC cells.

3.3. CircTMEM59 acted as a sponge of miR-147b to inhibit PDAC progression

Using StarBase (starbase.sysu.edu.cn), miR-147b was found to possess the potential binding sites with circTMEM59 (Fig. 3A). Then,

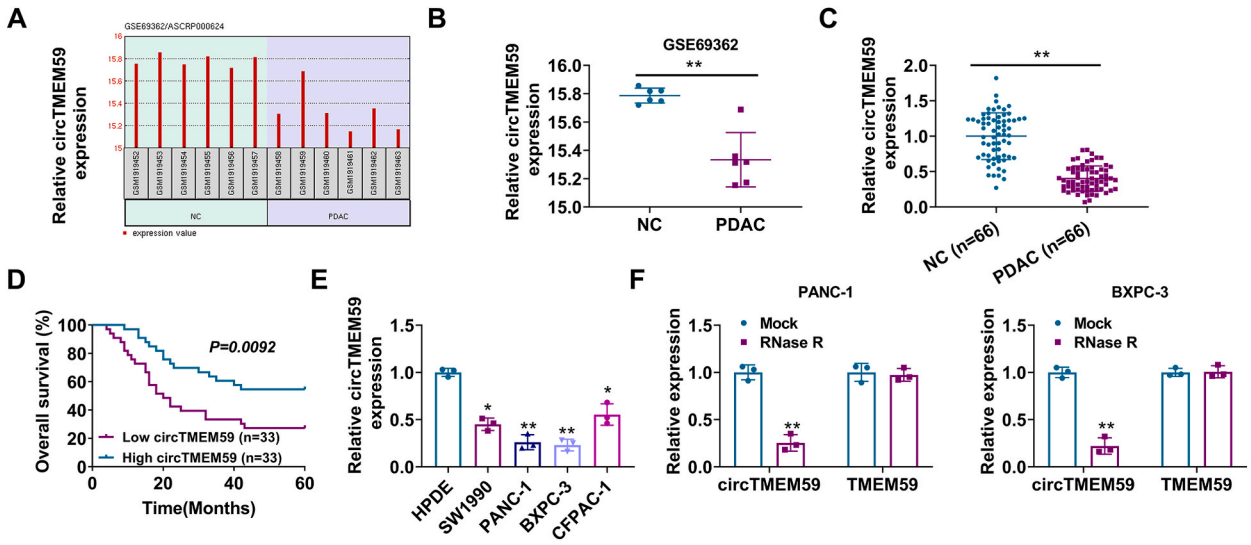

Fig. 1. CircTMEM59 was downregulated in PDAC cells and tissues. (A) The expression of circTMEM59 in PDAC tumor tissues and normal tissues was analyzed by GEO database; (B) GEO database showed that circTMEM59 was remarkably decreased in PDAC tissues; $^{**}p < 0.01$ vs. NC; (C) The circTMEM59 expression in PDAC tumor tissues and adjacent normal tissues was measured by qRT-PCR; $^{**}p < 0.01$ vs. NC; (D) Kaplan-Meier analysis of overall survival of PDAC patients according to circTMEM59 expression levels; (E) The expression levels of circTMEM59 in PDAC cell lines (SW1990, PANC-1, BXPC-3 and CFPAC-1) and human pancreatic ductal epithelial (HPDE) cells were measured by qRT-PCR; $^{*}p < 0.05$ vs. HPDE, $^{**}p < 0.01$ vs. HPDE; (F) CircTMEM59 and linear TMEM59 expression levels were detected after RNase R treatment in PDAC cells (PANC-1 and BXPC-3); $^{**}p < 0.01$ vs. mock. PDAC, pancreatic ductal adenocarcinoma.

Table 2
Correlation between circTMEM59 expression and the clinical pathological features of 66 PDAC patients.

Characteristic	All cases	circTMEM59 expression		*P*-value
		High (n = 33)	Low (n = 33)	
Age (years)				0.314
< 60	26	11	15	
≥60	40	22	18	
Gender				0.618
Male	38	20	18	
Female	28	13	15	
Major vascular invasion				0.046*
Yes	38	15	23	
No	28	18	10	
TNM stage				0.029*
I + II	29	19	10	
III + IV	37	14	23	
Tumor diferentiation				0.003**
Well + moderately	34	23	11	
poor	32	10	22	

A chi-square test was used for comparing groups between low and high circTMEM59 expression. *$P < 0.05$, **$P < 0.01$.

miR-147b mimics and NC were transfected into PDAC cells, and the miR-147b levels were detected by qRT-PCR. As expected, miR-147b expression was significantly increased followed by miR-147b mimics transfection ($p < 0.001$, Fig. 3B). Then, circTMEM59-wt and circTMEM59-mut were cloned into a dual-luciferase system. The results revealed that PDAC cells co-transfected with circTMEM59-wt and miR-147b mimics strongly decreased the activity of luciferase, while those co-transfected with circTMEM59-mut and miR-147b mimics did not affect the activity (Fig. 3C). The RIP assay revealed that both circTMEM59 and miR-147b were enriched in Ago2 immunoprecipitates compared to IgG-pellet, indicating circTMEM59 and miR-147b were both in the same RNA-induced silencing complex (Fig. 3D–E). Furthermore, comparing to the adjacent normal samples, the expression of miR-147b in PDAC tissues was much higher ($p < 0.001$, Fig. 3F). In 66 PDAC patients, the level of miR-147b was negatively correlated with circTMEM59 expression ($p < 0.001$, Fig. 3G). Thus, the above data revealed that circTMEM59 might act as a molecular sponge of miR-147b.

3.4. Knockdown of miR-147b inhibited proliferation, invasion and EMT of PDAC cells

After transfection of miR-147b inhibitors, PDAC cells exhibited much lower miR-147b expression (Fig. 4A). CCK8 assay revealed that miR-147b inhibitors significantly reduced cell proliferation (Fig. 4B). Transwell assay confirmed that miR-147b inhibitors could reduce the invasion of PDAC cells significantly (Fig. 4C). Furthermore, after transfection of miR-147b inhibitors, the round PDAC cells were increased, while spindle cells were decreased (Fig. 4D). The western blotting analysis revealed that the levels of PCNA and vimentin were markedly reduced after transfection of miR-147b inhibitors (Fig. 4E). However, miR-147b inhibitors could significantly upregulate the expression of E-cadherin (Fig. 4E). The above results confirmed that knockdown of miR-147b could inhibit proliferation, invasion and EMT of PDAC cells.

3.5. SOCS1 was a target of miR-147b

To further detect the downstream targets of circTMEM59/miR-147b in PDAC, TargetScan (targetscan.org/vert_71) was used. Interestingly, a common binding site between miR-147b and SOCS1 was observed (Fig. 5A). The luciferase reporter assay further confirmed that co-transfected with SOCS1-wt and miR-147b mimics strongly decreased the luciferase activity (Fig. 5B). Furthermore, it should be noticed that the levels of SOCS1 protein were markedly downregulated in PDAC cells transfected with miR-147b mimics as comparing to the control ($p < 0.01$, Fig. 5C). The above findings suggested that SOCS1 might be a target of miR-147b. In order to confirm our hypothesis, circTMEM59 mimics and circTMEM59 mimics + miR-147b mimics were transfected into PDAC cells, respectively. CircTMEM59 mimics treatment increased SOCS1 levels, while miR-147b mimics reversed the increase of SOCS1 resulting from circTMEM59 mimics (Fig. 5D). In addition, SOCS1 levels were also found to be decreased in PDAC tissues ($p < 0.001$, Fig. 5E). Correlation analysis revealed that the SOCS1 expression was negatively related to miR-147b expression (Fig. 5F) and positively related to circTMEM59 expression (Fig. 5G).

3.6. CircTMEM59 inhibited PDAC progression by regulating miR-147b/SOCS1 axis

A series of studies were conducted to confirm the effect of circTMEM59, miR-147b and SOCS1 on PDAC progression. Firstly, si-SOCS1 was found to decrease the expression levels of SOCS1 (Fig. 6A). Then, circTMEM59 mimics, circTMEM59 mimics + miR-147b mimics and circTMEM59 mimics + si-SOCS1 were transfected into PDAC cells. As shown in Fig. 6B, circTMEM59 mimics significantly reduced cell proliferation. However, miR-147b mimics and si-SOCS1 rescued the functions of circTMEM59 mimics on cell proliferation ($p < 0.05$, Fig. 6B). Similar results were obtained in the Transwell assay. Overexpression of circTMEM59 decreased the invasion cell number, while miR-147b mimics and si-SOCS1 rescued the effects (Fig. 6C). Western blot data confirmed that the

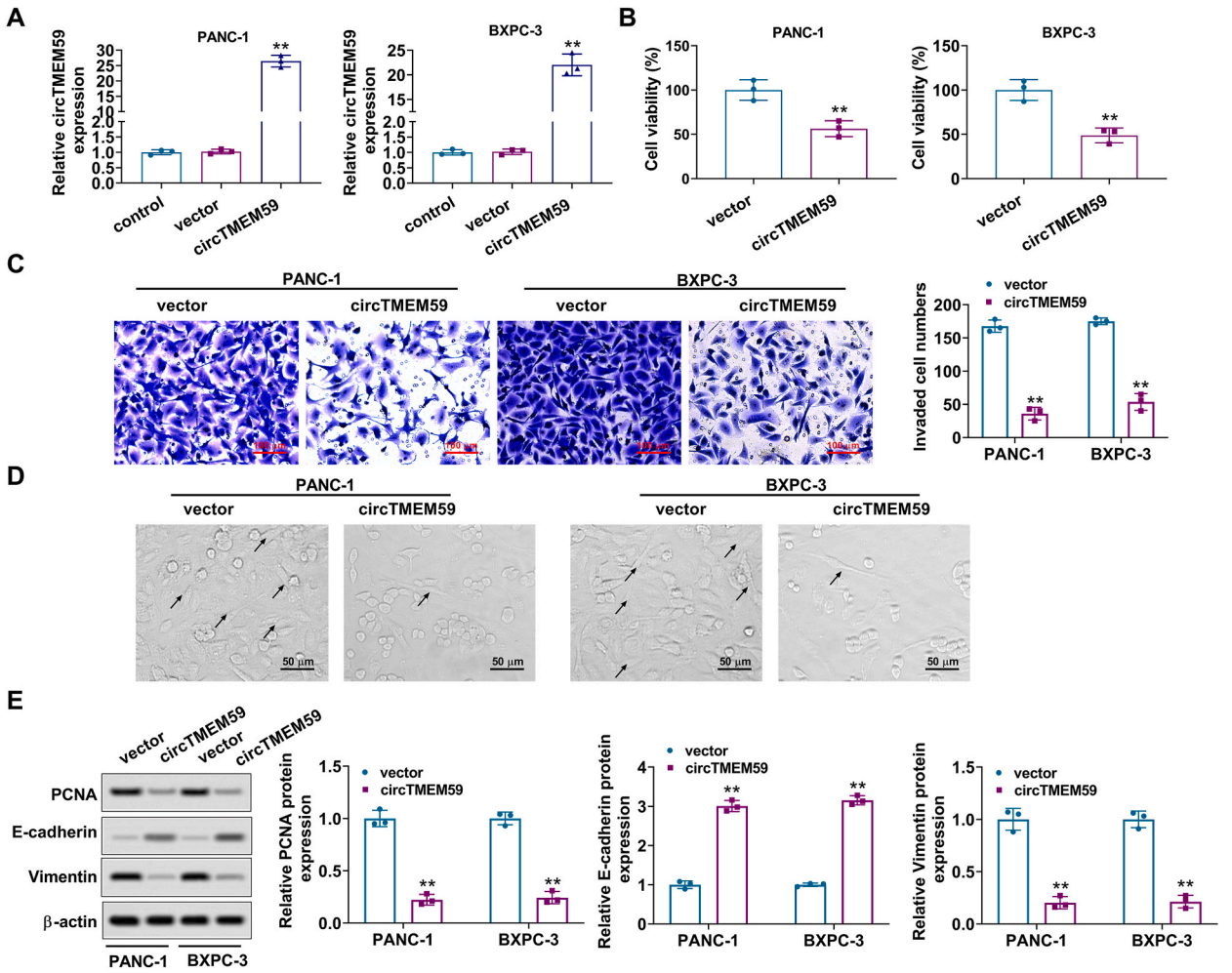

Fig. 2. Overexpression of circTMEM59 inhibited proliferation, invasion and EMT of PDAC cells. (A) After transfection with circTMEM59, the relative circTMEM59 in PANC-1 and BXPC-3 cell lines were detected by qRT-PCR; $^{**}p < 0.01$ vs. control; (B) Cell proliferation was detected by CCK8 assay in cells transfected with vector or circTMEM59; $^{**}p < 0.01$ vs. vector; (C) Cell invasion was detected by Transwell assay in cells transfected with vector or circTMEM59; $^{**}p < 0.01$ vs. vector; (D) The morphology of transfected cells were observed by a microscope; (E) The expression levels of PCNA, E-cadherin and vimentin were detected by Western blot analysis in cells transfected with vector or circTMEM59; $^{**}p < 0.01$ vs. vector. EMT, epithelial-mesenchymal transition; PDAC, pancreatic ductal adenocarcinoma; PCNA, proliferation cell nuclear antigen.

upregulation miR-147b could revert the functions of circTMEM59 overexpression on the elevation of SOCS1 and E-cadherin, and the depletion of PCNA and vimentin (Fig. 6D–E). si-SOCS1 exhibited a similar effect (Fig. 6D–E). Thus, the above results demonstrated that the upregulation of circTMEM59 could suppress cell proliferation, invasion and EMT by miR-147b/SOCS1 axis in PDAC.

4. Discussion

Downregulation of circTMEM59 has been found in CRC previously [12,13]. It has been reported that circTMEM59 could inhibit CRC cell growth and metastasis [12,13]. In this study, an anti-tumor role of circTMEM59 in PDAC was also found. To our best knowledge, we demonstrated for the first time that circTMEM59 expression was decreased in PDAC, a difficult to treat tumor. Low circTMEM59 expression was correlated with shorter survival time in PDAC patients. For the underlying mechanisms, circTMEM59 was found to repress the proliferation, invasion and EMT of PDAC cells by regulating miR-147b/SOCS1 axis. Thus, circTMEM59 was a potential effective therapeutic target for PDAC.

CircRNAs are widely reported to be related to the malignant progression of tumor [7]. In the former studies of PDAC, some circRNAs were found to play a carcinogenic role in PDAC, such as circEYA3 [15], circFOXK2 [16] and circRTN4 [17]. Some circRNAs were found to have anti-cancer effects against PDAC, such as circNFIB1 [18] and circRNA_0047744 [19]. Using the bioinformatics tools, we found that circTMEM59 was a potential circRNA participating in PDAC progression. Downregulated expression of circTMEM59 was verified in PDAC tissues and cell lines. Furthermore, high expressed circTMEM59 were found to have better clinicopathological characteristics and longer survival time in PDAC patients. The above data indicated that circTMEM59 could act as a tumor

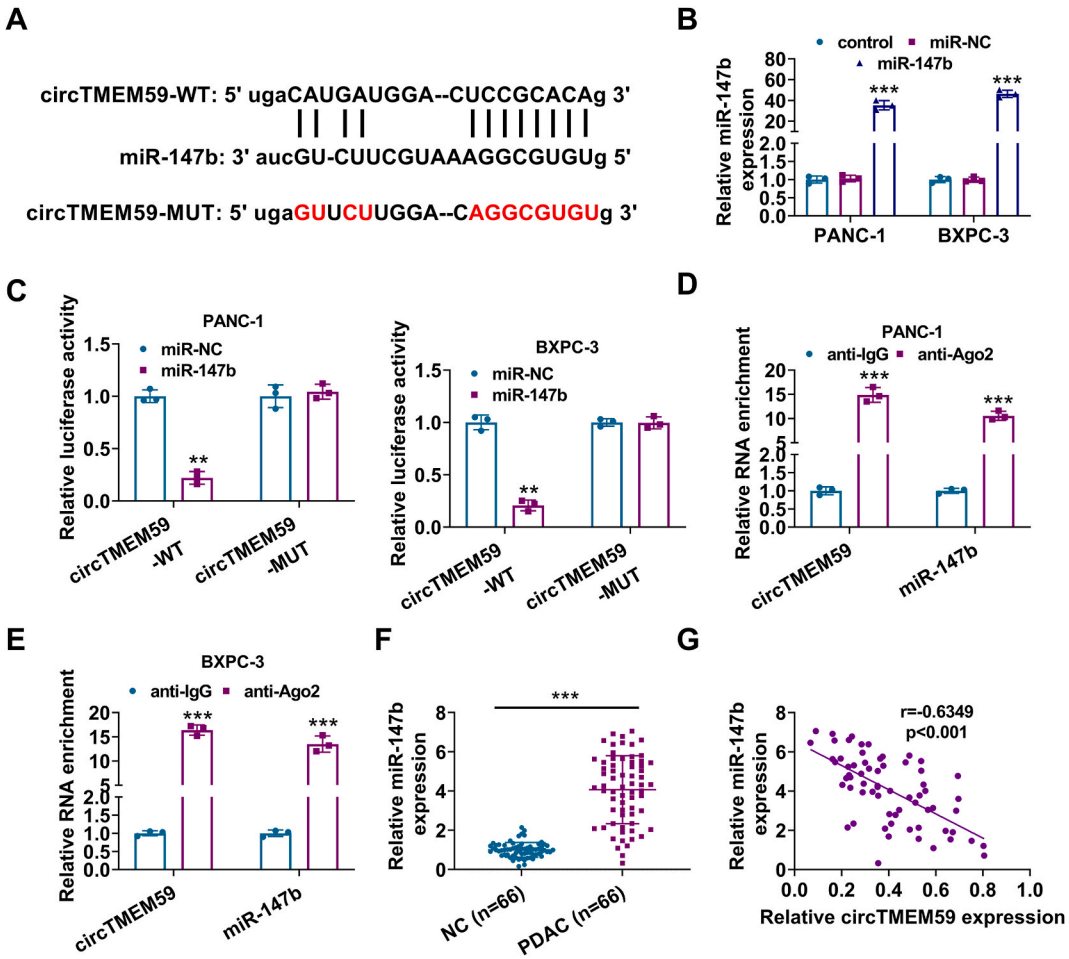

Fig. 3. CircTMEM59 inhibited PDAC progression by acting as a molecular sponge of miR-147b. (A) StarBase predicted miR-147b binding to circTMEM59; (B) After transfection with miR-147b, the relative miR-147b in PANC-1 and BXPC-3 cell lines were detected by qRT-PCR; ***$p < 0.001$ vs. control; (C) Luciferase assay confirmed that circTMEM59 targeted miR-147b; **$p < 0.01$ vs. miR-NC; (D,E) RIP assay indicated that circTMEM59 and miR-147b were both in the same RNA-induced silencing complex; ***$p < 0.001$ vs. anti-IgG; (F) The miR-147b expression in PDAC tumor tissues and adjacent normal tissues was measured by qRT-PCR; ***$p < 0.001$ vs. NC; (G) Correlation analysis of the expression of circTMEM59 and miR-147b in 66 PDAC tissues. DAC, pancreatic ductal adenocarcinoma.

suppressor like other noncoding RNAs [20,21]. Uncontrolled proliferation and increased invasion are the major causes of the development cancer [22]. Our results further revealed that overexpressed circTMEM59 significantly inhibited proliferation and invasion of PDAC cells, consistent with the results obtained in CRC [12,13]. In line with the above results, the expression of PCNA, a marker of DNA replication [23], was also decreased in PDAC cells following circTMEM59 overexpression. Furthermore, overexpressed circTMEM59 was found to repress EMT, a highly conserved cellular process defined by the loss of epithelial markers (eg E-cadherin) and the gain of mesenchymal markers (eg vimentin) [24]. Thus, our data indicated that circTMEM59 could inhibit the progression of PDAC.

The major role of circRNAs is to act as miRNA sponges to affect tumor progression [12,13]. Previously, Liu et al. found that circTMEM59 could act as a miR-410-3p sponge to regulate CRC progression [13]. Feng et al. revealed that circTMEM59 could function as a miR-668-3p sponge to inhibit proliferation and metastatic behaviors of CRC cells [12]. Through bioinformatics analysis and luciferase reporter assay, our study found that circTMEM59 might act as a miR-147b sponge in PDAC. MiR-147b is a miRNA that has been reported to be upregulated in various solid tumors [25,26]. Inhibition of miR-147b could suppress tumor cell proliferation and invasion [25,26]. Like other miRNAs, previous studies have also demonstrated that miR-147b is regulated by circRNAs and long non-coding RNAs (lncRNAs) [27,28]. As expected, our data indicated that miR-147b was overexpressed in PDAC tissues and cell lines. And miR-147b′s expression was significantly negatively correlated with circTMEM59. Furthermore, in line with overexpression of circTMEM59, knockdown of miR-147b also inhibited the proliferation, invasion and EMT of PDAC cells.

SOCS1 is an important protein that regulates the release of a number of cytokines involving in the progression of human cancers [29]. Previously, SOCS1 has been regarded as a tumor suppressor. Downregulation of SOCS1 has been found in several solid tumors including PDAC [30,31]. Previous studies have shown that several miRNAs could regulate the expression levels of SOCS1. For example,

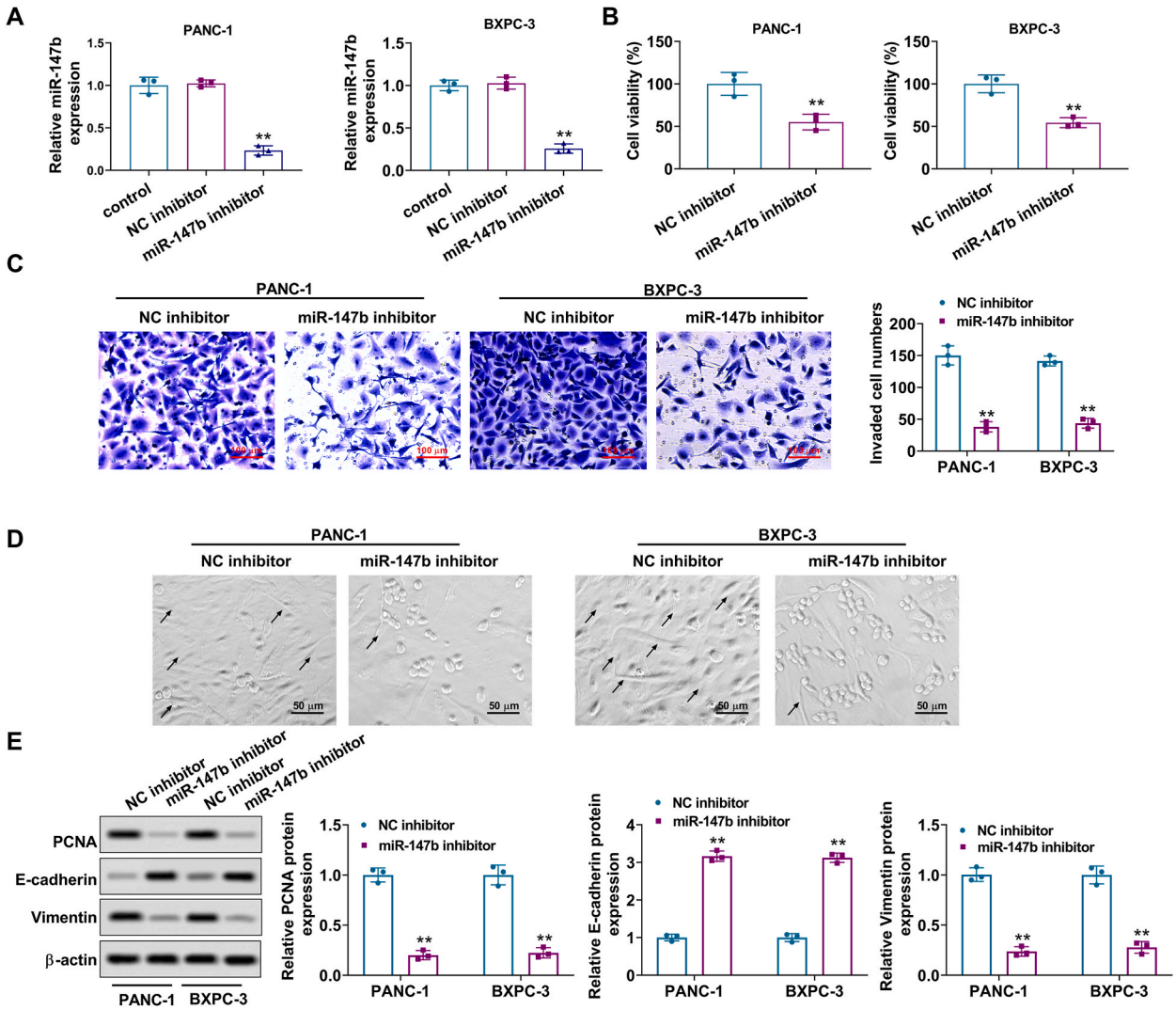

Fig. 4. Knockdown of miR-147b inhibited proliferation, invasion and EMT of PDAC cells. (A) After transfection with miR-147b inhibitors, the relative miR-147b in PANC-1 and BXPC-3 cell lines were detected by qRT-PCR; $^{**}p < 0.01$ vs. control; (B) Cell proliferation was detected by CCK8 assay in cells transfected with NC inhibitors or miR-147b inhibitors; $^{**}p < 0.01$ vs. NC inhibitor; (C) Cell invasion was detected by Transwell assay in cells transfected with NC inhibitors or miR-147b inhibitors; $^{**}p < 0.01$ vs. NC inhibitor; (D) The morphology of transfected cells were observed by a microscope; (E) The expression levels of PCNA, E-cadherin and vimentin were detected by Western blot analysis in cells transfected with NC inhibitors or miR-147b inhibitors; $^{**}p < 0.01$ vs. NC inhibitor. EMT, epithelial-mesenchymal transition; PDAC, pancreatic ductal adenocarcinoma; PCNA, proliferation cell nuclear antigen. $^{**}p < 0.01$.

Tang et al. found that miR-155 could regulate SOCS1 to control cytokines expression [32]. Similarly, Zhang et al. revealed that miR-155 inhibited SOCS1 expression to regulate cervical cancer progression [33]. In this study, our results demonstrated that SOCS1 was located at the downstream of miR-147b. As expected, SOCS1 was found to be decreased in PDAC tissues and be negatively related to miR-147b expression and positively related to circTMEM59 expression. In addition, miR-147b inhibited and circTMEM59 facilitated the expression of SOCS1 protein in PDAC cells. The rescue studies found that upregulated miR-147b and downregulated SOCS1 rescued the effects of circTMEM59 on cell proliferation, invasion and EMT. Taken together, all data suggested that overexpressed circTMEM59 inhibited PDAC progression, at least in part, by regulating a miR-147b/SOCS1 axis.

5. Conclusion

Our data indicated that circTMEM59 inhibited cell proliferation, invasion and EMT of PDAC by regulating miR-147b/SOCS1 axis. Our study validated for the first time that circTMEM59 played a tumor suppressor role in PDAC. This study improves our understanding of the mechanism of regulation of PDAC, more importantly, it provides a potential treatment target for PDAC management. However, this study also has several limitations. For example, the role of circTMEM59, miR-147b and SOCS1 should be confirmed *in*

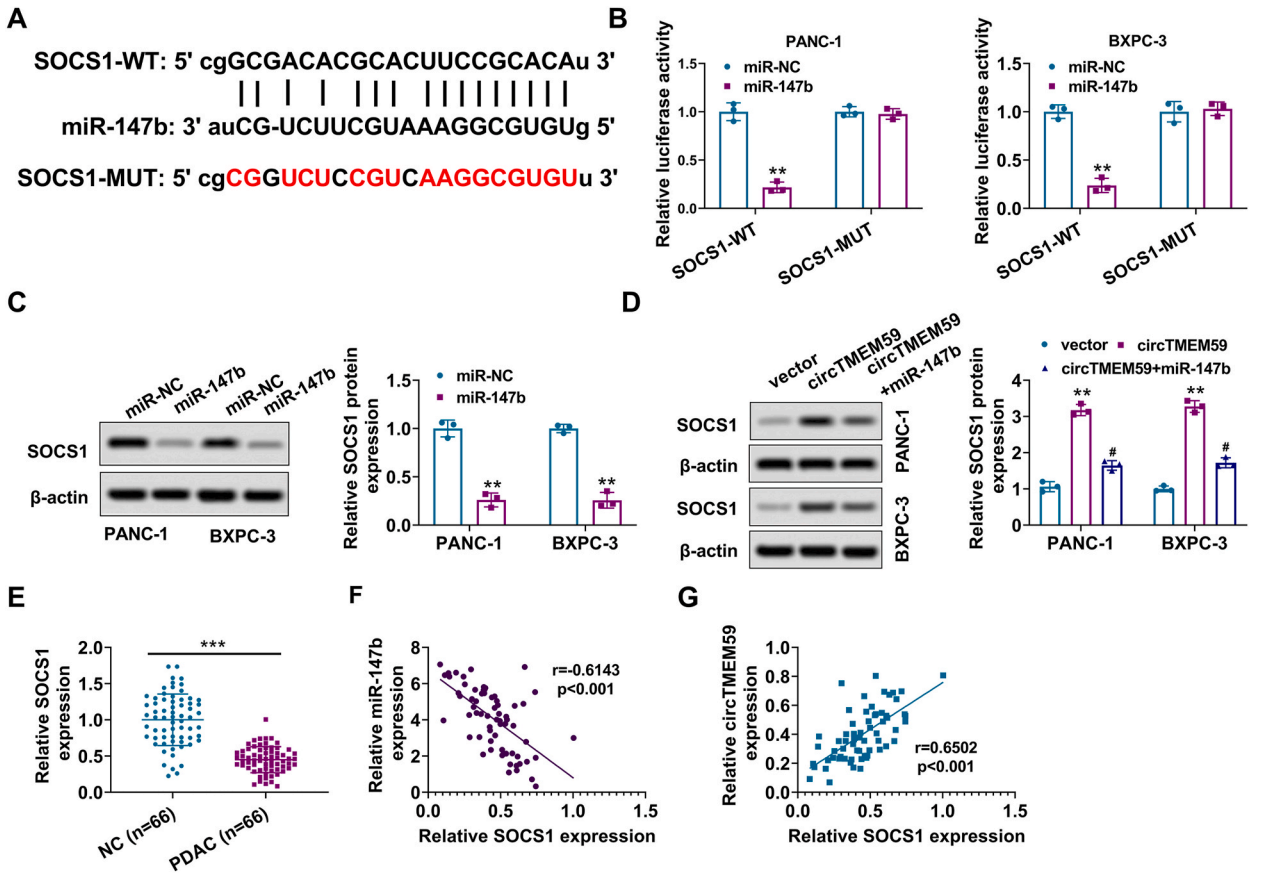

Fig. 5. SOCS1 was a target of miR-147b. (A) TargetScan predicted that SOCS1 was the potential target of miR-147b; (B) Luciferase assay confirmed that SOCS1 targeted miR-147b; $^{**}p < 0.01$ vs. miR NC; (C) After transfection with miR-147b, the relative SOCS1 expression in PANC-1 and BXPC-3 cell lines were detected by Western blot analysis; $^{**}p < 0.01$ vs. miR NC; (D) After transfection with circTMEM59 or circTMEM59 + miR-147b, the relative SOCS1 expression in PANC-1 and BXPC-3 cell lines were detected by Western blot analysis. $^{**}p < 0.01$ vs. vector, $^{\#}p < 0.05$ vs. circTMEM59; (E) The SOCS1 expression in PDAC tumor tissues and adjacent normal tissues was measured by qRT-PCR; $^{***}p < 0.001$ vs. NC; (F) Correlation analysis of the expression of SOCS1 and miR-147b in 66 PDAC tissues; (G) Correlation analysis of the expression of SOCS1 and circTMEM59 in 66 PDAC tissues. SOCS1, suppressor of cytokine signaling 1; PDAC, pancreatic ductal adenocarcinoma.

vivo. The regulated network with circRNA, miRNA and genes should be constructed. Thus, further studies are needed to strengthen our findings.

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CRediT authorship contribution statement

Tieliu Cao: Writing – original draft, Methodology. **Liang Hong:** Writing – review & editing, Resources, Data curation. **Dan Yu:** Writing – review & editing, Resources, Methodology. **Jie Shen:** Writing – review & editing, Software, Methodology. **Liwen Jiang:** Writing – review & editing, Software, Methodology. **Nanhua Hu:** Writing – review & editing, Supervision, Investigation. **Shengli He:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

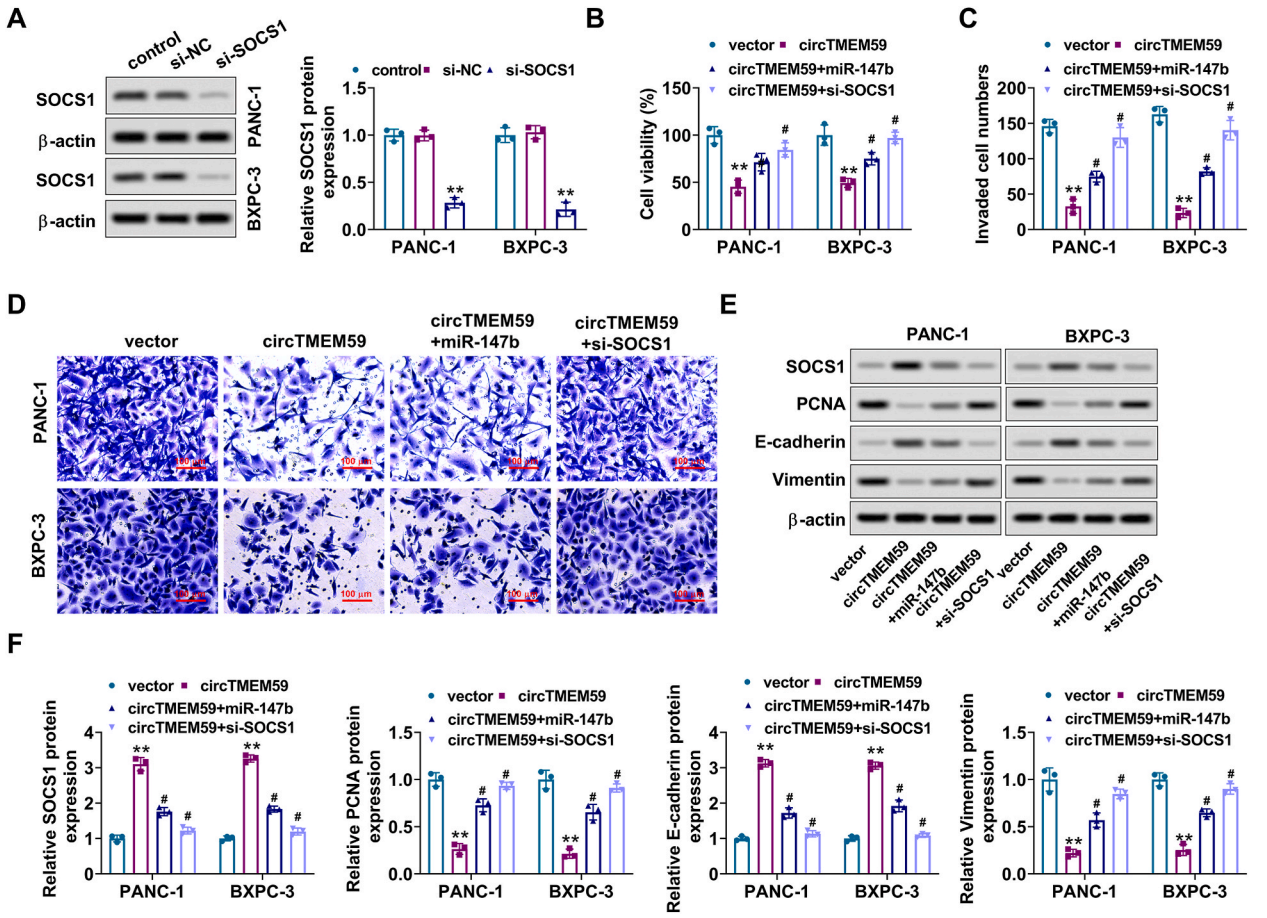

Fig. 6. CircTMEM59 inhibited PDAC progression by regulating miR-147b/SOCS1 axis. (A) After transfection with si-SOCS1, the relative SOCS1 expression in PANC-1 and BXPC-3 cell lines were detected by Western blot analysis; $^{**}p < 0.01$ vs. control, (B) Cell proliferation was detected by CCK8 assay in cells transfected with vector, circTMEM59, circTMEM59+ miR-147b or circTMEM59+ si-SOCS1; $^{**}p < 0.01$ vs. vector group, $^{\#}p < 0.05$ vs. circTMEM59 group; (C) Cell invasion was detected by Transwell assay in cells transfected with vector, circTMEM59, circTMEM59+ miR-147b or circTMEM59+ si-SOCS1; $^{**}p < 0.01$ vs. vector group, $^{\#}p < 0.05$ vs. circTMEM59 group; (D, E) The expression levels of SOCS1, PCNA, E-cadherin and vimentin were detected by Western blot analysis in cells transfected with vector, circTMEM59, circTMEM59+ miR-147b or circTMEM59+ si-SOCS1. PDAC, pancreatic ductal adenocarcinoma; $^{**}p < 0.01$ vs. vector group, $^{\#}p < 0.05$ vs. circTMEM59 group. SOCS1, suppressor of cytokine signaling 1; PCNA, proliferation cell nuclear antigen.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24402.

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