European Review for Medical and Pharmacological Sciences 2020; 24: 10462-10471

LncRNA CASC19 contributed to the progression of pancreatic cancer through modulating miR-148b/E2F7 axis

T. LU, G.-H. WEI, J. WANG, J. SHEN

Department of Anesthesia, the First Affiliated Hospital with Nanjing Medical University, Nanjing, P.R. China

Ting Lu and Guohua Wei contributed equally to this work

Abstract. – OBJECTIVE: Cancer susceptibility 19 (CASC19), a crucial lncRNA associated with multiple cancers, has been reported to play a vital role in the progression of human malignant tumors. However, the underlying mechanism of CASC19 in pancreatic cancer (PC) was still unknown. The purpose of this study was to explore the biological and clinical significance of CASC19 in PC.

PATIENTS AND METHODS: RT-qPCR assay was adopted to analyze CASC19 expression in PC tissues and cell lines. Furthermore, the correlation between the CASC19 level and the survival rate of PC patients was assessed by Kaplan-Meier analysis. Bioinformatics analysis and Luciferase reporter assay were utilized to confirm the interaction between miR-148b and CASC19 or E2F7. Cell viability, migration, invasion, and apoptosis were analyzed using MTT, transwell, and TUNEL assays.

RESULTS: The results elucidated that CASC19 expression was markedly increased in PC tissues and cell lines. Patients with high expression of CASC19 had a short survival time. Silencing of CASC19 attenuated PC cell proliferation, migration, and invasion. Moreover, we identified that miR-148b was a target of CASC19. CASC19 was negatively correlated with miR-148b and positively correlated with E2F7. The inhibitory effect of CASC19 knockdown on the progression of PC was reversed by the down-regulation of miR-148b or up-regulation of E2F7.

CONCLUSIONS: These results demonstrated that CASC19 participated in the development of PC. The CASC19/miR-148b/E2F7 axis might be a new study direction for PC treatment.

Key Words:
Pancreatic cancer, LncRNA CASC19, MiR-148b, E2F7.

Introduction

Pancreatic cancer (PC) is a kind of malignant tumor with a high mortality rate[1]. Although advances in PC therapy, containing surgery and chemotherapy, the 5-year survival rate of PC patients remains low due to the early metastasis[2]. Therefore, it is critical to identify the molecular mechanism of PC development[3].

Long noncoding RNAs (lncRNAs) are a group of no protein-coding RNAs with over 200 nts in length[4,5] and they are involved in the occurrence and metastasis of various cancers[6]. Accumulating researches have revealed that lncRNA CASC19 might serve as an oncogene to promote the progression of various cancers. LncRNA CASC19 promoted gastric cancer progression[7]. LncRNA CASC19 sponged miR-140-5p to regulate CEMIP expression in colorectal cancer[8]. However, the underlying role of CASC19 in PC remained unclear.

MicroRNAs (miRNAs) are a group of non-coding RNAs, which regulate gene expression through binding with the 3′-UTR of mRNAs[9]. Aberrant expression of miRNAs played a crucial role in the progression of cancers, including PC[10,11]. MiR-148b has been reported to be involved in the regulation of various cancers. For instance, miR-148b repressed non-small cell lung carcinoma development by suppressing ALCAM through the NF-κB pathway[12]. MiR-148b attenuated renal carcinoma cell growth and invasion by modulating FGF2[13]. Nevertheless, there are no reports on the role of miR-148b in PC.

Our study investigated the expression of CASC19 and its regulatory mechanism in PC.

Corresponding Authors: Juan Wang, MD; e-mail: wangjuan@fahnmu.com; Jian Shen, MD; e-mail: jianshen@fahnmu.com

Results indicated that CASC19 contributed to PC progression *via* regulating the miR-148b/E2F7 pathway.

Patients and Methods

Tissue Samples

20 pairs of PC tissues and paracancerous tissues were obtained from the First Affiliated Hospital with Nanjing Medical University and immediately frozen in liquid nitrogen and stored at −80°C. Patients were selected by the following inclusion criteria: (1) all patients underwent standard resection; (2) all patients did not receive chemotherapy or radiotherapy preoperatively or postoperatively. The exclusion criteria were as follows: patients receive any therapy such as radiotherapy and chemotherapy. Written consents from all patients were collected before this work. This study was approved by the Ethics Committee of the First Affiliated Hospital with Nanjing Medical University.

Cell Culture

Human PC cells (SW1990, PaCa-2, and AsPC-1) and the normal human pancreatic epithelial cells (HPDE6-C7) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, South Logan, UT, USA) at 37°C with 5% CO_2.

Cell Transfection

The short-hairpin RNA (shRNA) targeting CASC19 (shCASC19) or shNC were synthesized by GenePharma (Shanghai, China). miR-148b mimics or inhibitor, NC mimic or NC inhibitor, and pcDNA3.1/E2F7 and pcDNA3.1 were purchased from RiboBio (Guangzhou, China). Cell transfection was performed by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

RT-qPCR Assay

Total RNAs were isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The PrimeScript RT reagent kit (TaKaRa, Otsu, Shiga, Japan) was used to synthesize cDNA. Then, RT-qPCR was performed on the ABI 7500 real-time PCR System (Applied Biosystems, Foster City, CA, USA) with an SYBR-Green PCR Master Mix kit (TaKaRa, Dalian, China). The relative expression of genes was calculated using $2^{-\Delta\Delta Ct}$ method with normalization to U6 and GAPDH. The primers were presented as follows: CASC19 forward, 5′-CCATTGAAGATACCACGCTGC-3′ and reverse, 5′- GGTTGTTGCCCAGGGTAGTG-3′; miR-148b forward, 5′-TCTCTGGGCCTGTGTCTTAGGC-3′ and reverse, 5′-CAGTGCGTGTCGTGGAGT-3′; E2F7 forward, 5′-CGGCAACGCATTGTGGA-3′ and reverse, 5′-TCCTGGGCTCAGAGATTTGC; GAPDH forward, 5’- GTCAACGGATTTGGTCTGTATT-3’ and reverse, 5’- AGTCTTCTGGGTGGCAGTGAAT-3’; and U6 forward, 5’-AGAGAGATTACATGGCCCCT-3’ and reverse, 5’- CTAATGTCACGCACGATTCT-3’.

Dimethyl Hiazolyl Diphenyl Tetrazolium (MTT) Assay

Transfected cells were seeded in a 96-well plate cultured overnight. Then, 10 μL of MTT solution (Bioswamp, Wuhan, China) was added and cells were cultured at 37°C for 4 hours. The absorbance was measured at 450 nm utilizing a microplate reader (Bio-Rad, Hercules, CA, USA).

TUNEL Assay

TUNEL apoptosis kit (Roche, Mannheim, Germany) was employed to assess the number of apoptotic cells. After ethanol dehydration, SW1990 and PaCa-2 cells were dyed and incubated with TUNEL reaction mixture (Roche, Mannheim, Germany). The nuclear staining was performed with DAPI. A microscope (BX41, Olympus, Japan) was utilized to observe TUNEL-positive cells.

Transwell Assay

Cell migration and invasion abilities were evaluated using transwell chambers (pore size of 8 μm; Corning, Lowell, MA, USA). For the migration assay, transfected cells were suspended in serum-free medium and added into the upper chamber. The bottom chambers were covered with complete RPMI-1640 medium (RPMI-1640; HyClone, South Logan, UT, USA). After 48 h, cells in the lower chamber were fixed and stained using crystal violet. Cell invasion was assessed similarly with cell migration, except that the insert membranes were pre-coated with Matrigel (BD, Franklin Lakes, NJ, USA). Finally, migrated and invaded cells were counted under a microscope (Olympus Corporation, Tokyo, Japan).

Luciferase Reporter Assay

The CASC19-WT/Mut and E2F7-WT/Mut were synthesized and cloned into the pmirGLO vectors (Promega, Madison, WI, USA). MiR-148b mimics or NC mimics were co-transfected with these above reporter vectors into 293T cells. After 48 h co-transfection, the relative luciferase activity was monitored using the dual-luciferase reporter system (Promega, Madison, WI, USA).

Pull-Down Assay

Cells were transfected with biotin-labeled CASC19 (Bio-CASC19 -Wt or Bio-CASC19-Mut) and their NC (Bio-NC) were purchased from RiboBio (Guangzhou, China). After 48 h, the cells were lysed in lysis buffer (Invitrogen, Carlsbad, CA, USA) and the lysate was incubated with streptavidin magnetic beads (Sigma-Aldrich, St. Louis, MO, USA) pre-treated with RNase-free BSA. After elution, RT-qPCR analysis was employed to analyze miR-148b level.

Statistical Analysis

Data were processed through SPSS 19.0 (SPSS IBM, Armonk, NY, USA), and presented as the mean ± standard deviation (SD) from three groups of independent data. Comparisons between two groups were performed by a Student's *t*-test. Comparisons among the three groups were analyzed using one-way ANOVA followed by Tukey's test. Pearson's correlation analysis was used to conduct the correlations among genes. The overall survival was used estimated by Kaplan-Meier analysis and log-rank test. $p < 0.05$ was defined as statistically significant.

Results

CASC19 Expression Was Up-Regulated in PC Tissues and Cells

To determine whether CASC19 was abnormally expressed in PC tissues and cell lines, RT-qPCR was employed to detect CASC19 expression. Results revealed that CASC19 level was remarkably elevated in PC tissues and cell lines (SW1990, PaCa-2, and AsPC-1) (Figure 1A and B). Besides, Kaplan-Meier analysis demonstrated that patients with high expression of CASC19 had a shorter overall survival time compared with those with low expression of CASC19 (Figure 1C). Taken together, these results suggested that CASC19 was dysregulated in PC, and the up-regulation of CASC19 predicted poor prognosis in PC.

CASC19 Deletion Suppressed PC Development

To further explore the potential biological role of CASC19 in PC progression, SW1990 and PaCa-2 cells were transfected with shCASC19 or shNC. RT-qPCR indicated the expression of CASC19 was significantly decreased in PC cells transfected with shCASC19 (Figure 2A). Subsequently, MTT assay revealed that CASC19 knockdown inhibited the proliferation of PC cells (Figure 2B). Meanwhile, the apoptosis of SW1990 and PaCa-2 cells was promoted by the silence of CASC19 (Figure 2C). Moreover, down-regulated CASC19 extremely suppressed the migration and invasion of SW1990 and PaCa-2 cells (Figure 2D and E). All the above

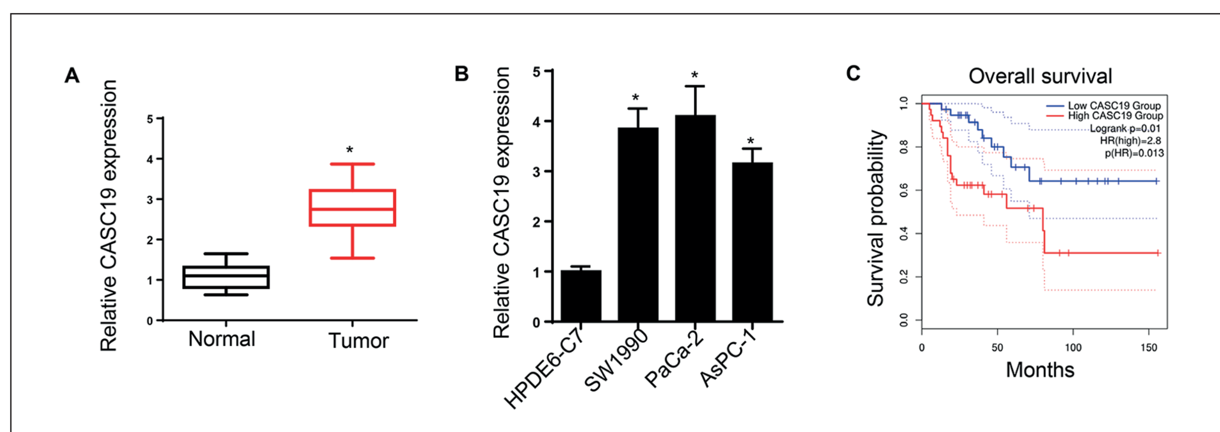

Figure 1. CASC19 expression was enhanced in PC tissues and cells. **A, B,** RT-qPCR showed the relative CASC19 expression in PC tissues and PC cell lines (SW1990, PaCa-2 and AsPC-1) compared with normal tissues and normal pancreatic duct epithelial cell line (HPDE6-C7). **C**, Kaplan-Meier survival analysis was employed to analyze the correlation between CASC19 expression and overall survival. $*p<0.05$.

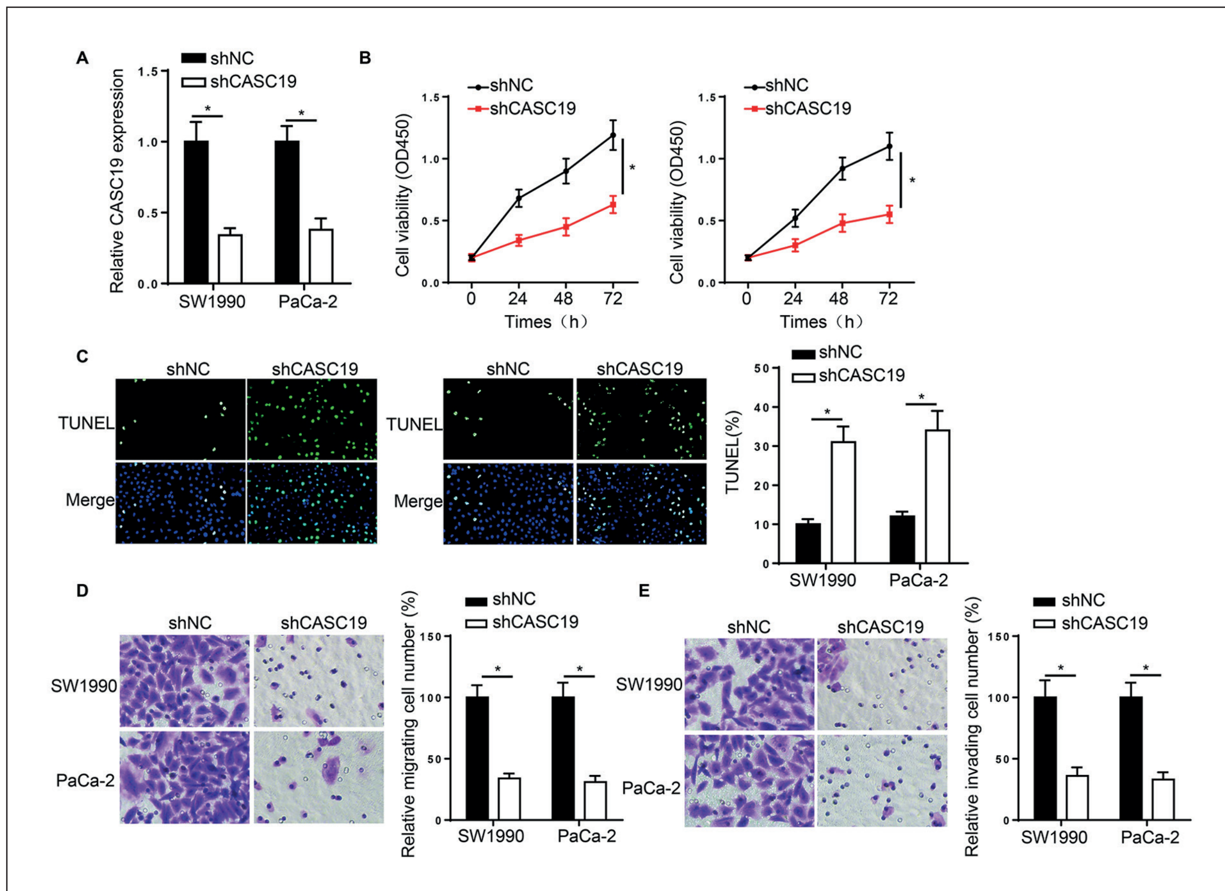

Figure 2. CASC19 deletion suppressed PC development. **A**, RT-qPCR was used to determine CASC19 expression in SW1990 and PaCa-2 cells transfected with shCASC19 or shNC. **B**, Cell viability was performed using MTT assay in SW1990 and PaCa-2 cells transfected with shCASC19 or shNC. **C**, TUNEL assay was conducted to verify cell apoptosis in SW1990 and PaCa-2 cells after CASC19 silence (magnification x 40). **D, E,** Transwell assay was performed to assess cell migration and invasion in SW1990 and PaCa-2 cells after suppressing CASC19 (magnification × 40). *$p<0.05$.

results demonstrated that CASC19 knockdown attenuated PC progression.

CASC19 Directly Interacted With MiR-148b in PC

Through using StarBase, we found that there was a binding site between miR-148b and CASC19 (Figure 3A). Luciferase reporter assay demonstrated that miR-148b overexpression decreased the Luciferase activity of CASC19-Wt, but had no effect on the Luciferase activity of CASC19-Mut reporter in 293T cells (Figure 3B). Moreover, CASC19 pull-down in SW1990 and PaCa-2 cells was remarkably enriched for miR-148b compared with the Beads and CASC19-Mut (Figure 3C and D). Then, RT-qPCR assay showed that the expression of miR-148b was reduced in PC tissues and cells (Figure 3E and F). Meanwhile, we confirm that CASC19 was negatively correlated with miR-148b in PC tissues (Figure 3G). In addition, knockdown of CASC19 enhanced miR-148b expression, and overexpression of miR-148b reduced CASC19 level (Figure 3H and I). Based on these results, we clarified that CASC19 directly bound with miR-148b and negatively regulated miR-148b expression in PC.

CASC19 Knockdown Inhibited Metastasis and Promoted Apoptosis of PC Cells Through Sponging MiR-148b

Initially, miR-148b expression was markedly downregulated after transfected with miR-148b inhibitor in SW1990 cells (Figure 4A). Then, to verify whether miR-148b participated in CASC19-mediated PC progression, sh-NC, shCASC19, and shCASC19+miR-148b inhibitor were transfected into SW1990 cells. Functional assays revealed that CASC19 knockdown-in-

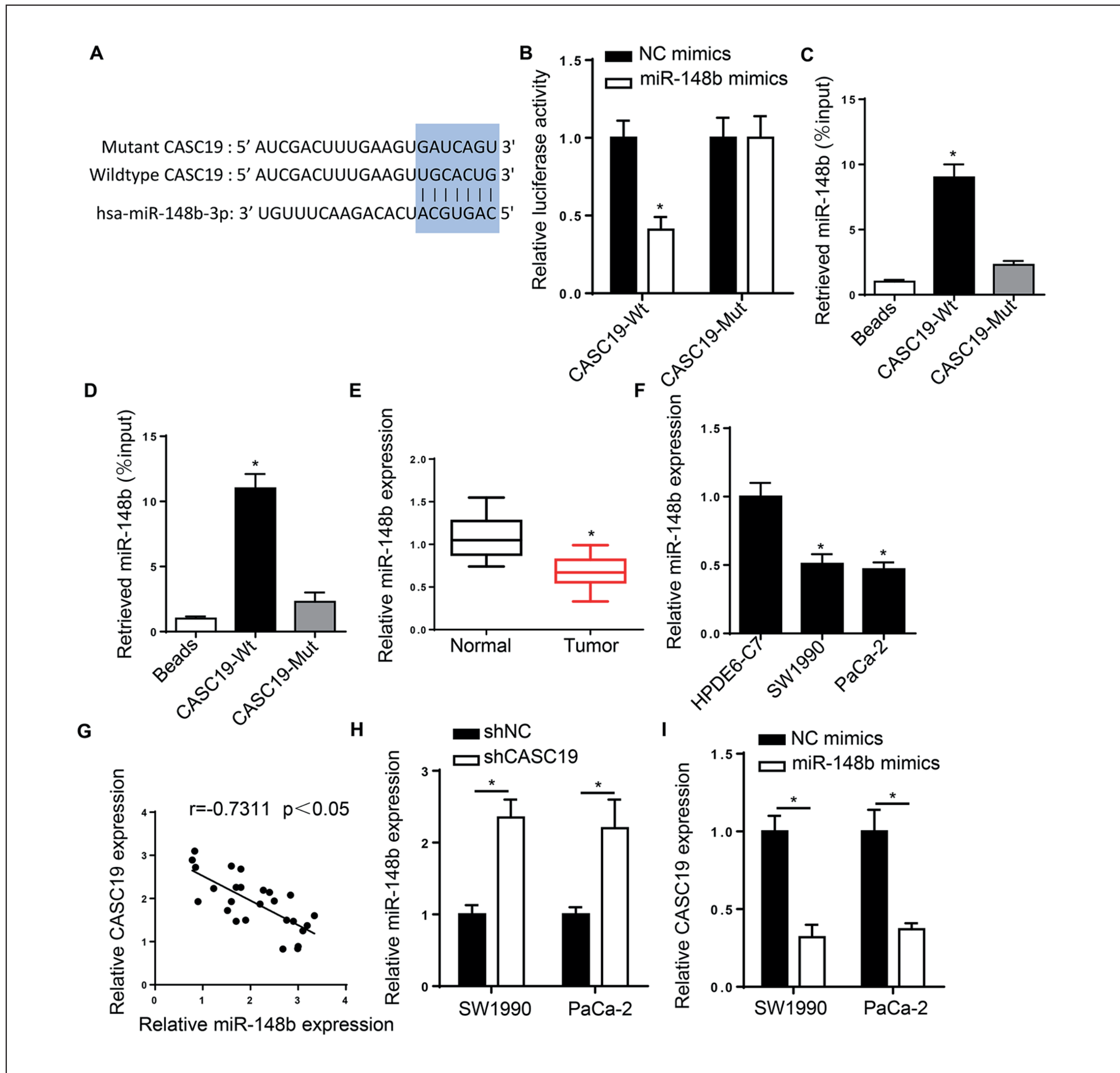

Figure 3. CASC19 was a sponge of miR-148b in PC cells. **A**, Binding sequences between CASC19 and miR-148b were predicted by starBase bioinformatic analysis website. **B**, Dual-Luciferase reporter assay showed Luciferase activity of CASC19-Wt or CASC19-Mut in 293T cells transfected with NC mimics or miR-148b mimics. **C, D**, RT-qPCR assay was applied to assess the enrichment of miR-148b in samples pulled down by bio-labelled CASC19 or negative control in SW1990 and PaCa-2 cells. **E, F**, RT-qPCR assay was employed to assess miR-148b expression in PC tissues and cell lines. **G**, Pearson correlation analysis of the correlation of CASC19 with miR-148b expression in PC tissues. **H, I**, RT-qPCR showed the relative expression of miR-148b in SW1990 and PaCa-2 cells transfected with shCASC19, and the relative expression of CASC19 transfected with miR-148b mimics. *$p<0.05$.

duced inhibition of cell viability, migration, and invasion was neutralized by down-regulated miR-148b (Figure 4B-D). TUNEL assay showed that miR-148b inhibition reversed the promoting effect of CASC19 silence on apoptosis of SW1990 cells (Figure 4E). In sum, CASC19 deletion suppressed PC progression via sponging miR-148b.

E2F7 Targeted MiR-148b in PC Cells

With the assistance of starBase, E2F7 was predicted to be a potential target of miR-148b in PC cells (Figure 5A). Dual-Luciferase reporter assay discovered that miR-148b mimics markedly reduced the Luciferase activity of E2F7-Wt, while no effect on the Luciferase activity

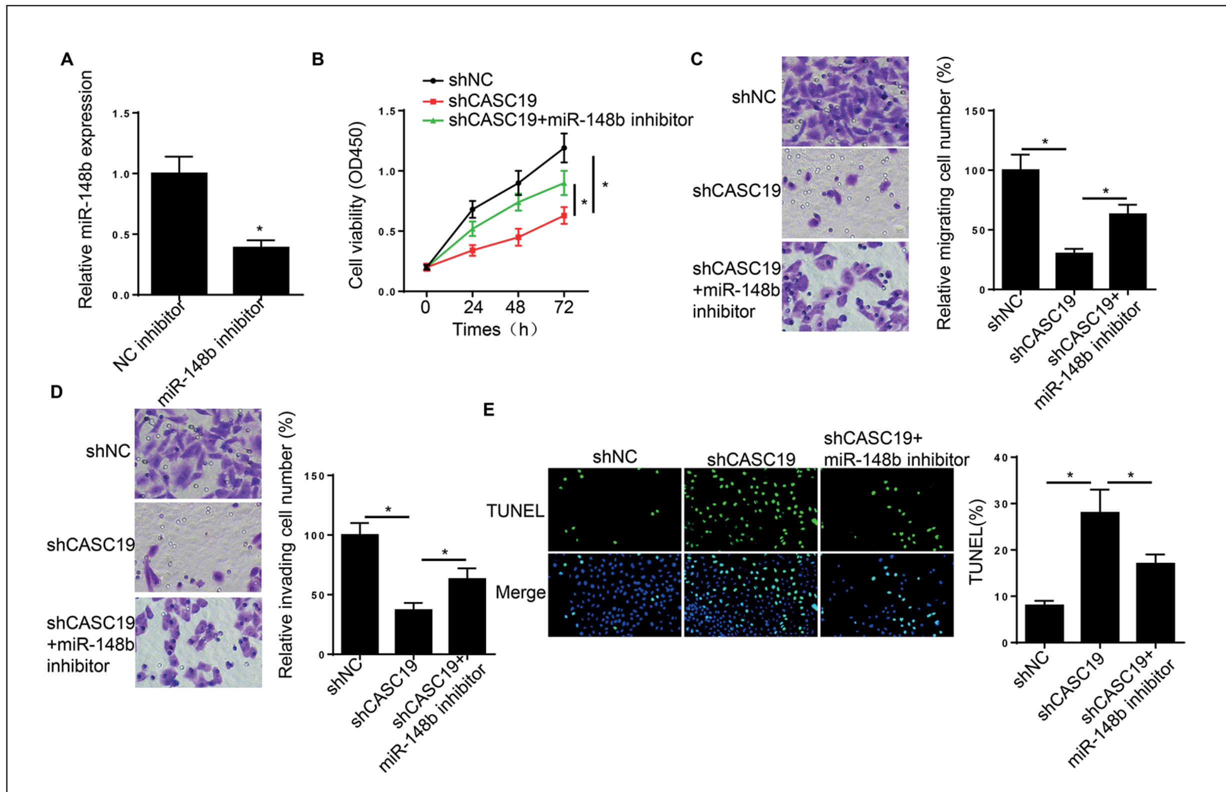

Figure 4. CASC19 knockdown inhibited cell metastasis and apoptosis of PC through sponging miR-148b. **A**, RT-qPCR assay showed miR-148b expression transfected with miR-148b inhibitor. **B-D**, MTT and transwell assays (magnification × 40) showed cell proliferation, migration and invasion ability of SW1990 cells transfected with shNC, shCASC19 and shCASC19+miR-148b inhibitor. **E**, TUNEL assay was conducted to verify cell apoptosis in SW1990 cells after transfected with sh-NC, shCASC19 and shCASC19+miR-148b inhibitor (magnification × 40). $^*p<0.05$.

of cells with E2F7-Mut (Figure 5B), suggesting that miR-148b directly interacted with E2F7. Furthermore, RT-qPCR results revealed that E2F7 expression was significantly increased in PC tissues and cell lines (Figure 5C and D). In addition, we observed that miR-148b expression was negatively correlated with E2F7 expression in PC tissues (Figure 5E). In addition, it was demonstrated that the expression of E2F7 was repressed by overexpression of miR-148b mimics, which could be restored by E2F7 overexpression in SW1990 cells (Figure 5F), implying that miR-148b inhibited the expression of E2F7 through direct interaction.

Silence of CASC19 Repressed PC Cell Progression Through Modulating E2F7

To explore whether CASC19 regulated E2F7 through miR-148b, SW1990 cells were transfected with shNC, shCASC19, shCASC19+E2F7. First, we found that E2F7 expression was increased in SW1990 cells transfected with pcDNA3.1/E2F7 (Figure 6A). Subsequently, we discovered that E2F7 expression was suppressed by CASC19 silence, while the suppression was neutralized by E2F7 overexpression in SW1990 cells (Figure 6B). Furthermore, rescue experiments showed that E2F7 overexpression reversed the inhibitory effects of CASC19 knockdown-induced on viability, migration, and invasion of SW1990 cells (Figure 6C-E). Furthermore, the up-regulation of E2F7 eliminated the promoting effect of CASC19 deletion on cell apoptosis (Figure 6F). Therefore, we illustrated that CASC19 knockdown inhibited PC progression by down-regulated E2F7.

Discussion

LncRNAs participate in the regulation of tumorigenesis and development[14]. Accumulating

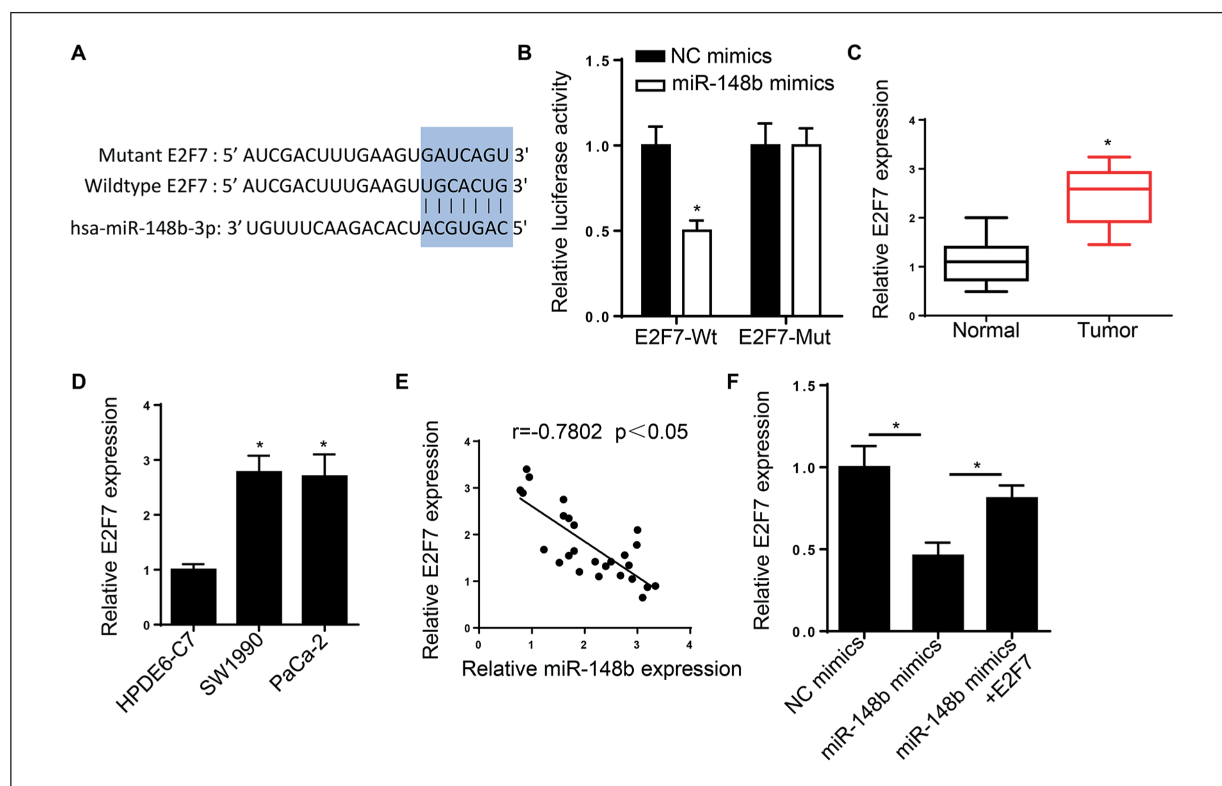

Figure 5. E2F7 targeted miR-148b in PC cells. **A**, Binding sequences between E2F7 and miR-148b were predicted by starBase bioinformatic analysis website. **B**, Dual-Luciferase reporter assay showed Luciferase activity of E2F7-Wt or E2F7-Mut in 293T cells transfected with NC mimics or miR-148b mimics. **C, D**, RT-qPCR assay showed E2F7 expression in PC tissues and cell lines. **E**, Pearson correlation analysis of the correlation of E2F7 with miR-148b expression in PC tissues. **F**, RT-qPCR showed the relative expression of E2F7 in SW1990 cells transfected with NC mimics, miR-148b mimics, and miR-148b mimics + E2F7. *p<0.05.

lncRNAs have been found to implicate in PC progression by acting as an oncogene or tumor suppressor. Of note, lncRNA SNHG16 acted as an oncogene and promoted cell metastasis via sponging miR-200a-3p in PC[15]. LncRNA NEAT1 contributed to cell proliferation and metastasis via stabilizing ELF3 in PC[16]. LNC00673 inhibited proliferation and migration through regulating the miR-504/HNF1A axis in PC[17]. All the data manifested that lncRNA played an essential regulatory role in PC development.

CASC19 was reported to be a tumor-related lncRNA. For example, CASC19 regulated miR-449b-5p expression on the proliferation and apoptosis of cervical cancer cells[18]. LncRNA CASC19 sponged miR-532 and promoted clear cell renal cell carcinoma development via up-regulating ETS1[19]. Nevertheless, the molecular mechanism of CASC19 in PC needs to be further explored. In the current study, we demonstrated that the expression of CASC19 was enhanced in PC tissues and cells. CASC19 knockdown attenuated PC cell viability, migration, invasion, and induced apoptosis. Taken together, CASC19 could facilitate PC development.

Competing endogenous RNA (ceRNA) network exhibits its regulatory function in human cancers[20,21]. So, lncRNA NCK1-AS1 acted as a ceRNA to promote glioma development via sponging miR-138-2 and up-regulating TRIM24[22]. LncRNA DGUOK-AS1 functioned as a ceRNA of miR-653-5p to facilitate cell proliferation in cervical cancer[23]. In this study, our results identified that miR-148b directly targeted CASC19. Silence of CASC19 elevated the expression of miR-148b in SW1990 and Pa-Ca-2 cells, and transfection of miR-148b mimics down-regulated CASC19, which indicated that CASC19 was negatively correlated with miR-148b. Rescue experiments displayed that the inhibitory effect of silencing CASC19 on cell viability, migration, and invasion was offset by

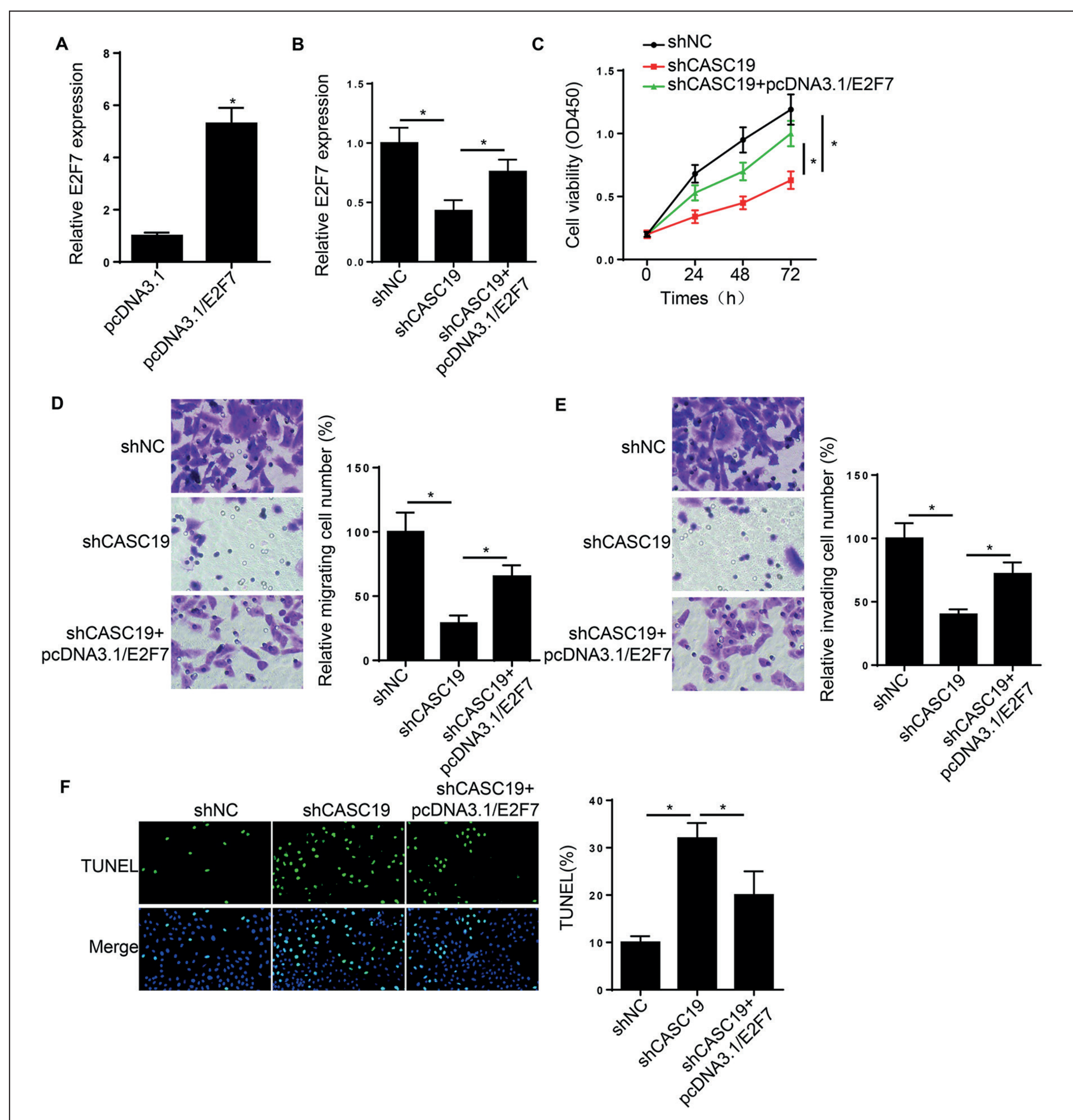

Figure 6. Silence of CASC19 repressed PC progression through modulating E2F7. **A**, RT-qPCR assay showed E2F7 expression in SW1990 cells transfected with pcDNA3.1/E2F7. **B**, RT-qPCR assay showed E2F7 expression in SW1990 cells transfected with shNC, shCASC19, and shCASC19+pcDNA3.1/E2F7. **C-E**, MTT and transwell assays (magnification x 40) showed cell proliferation, migration and invasion ability of SW1990 cells transfected with shNC, shCASC19, and shCASC19+pcDNA3.1/E2F7. F, TUNEL assay was conducted to verify cell apoptosis in SW1990 cells after transfected with shNC, shCASC19, and shCASC19+pcDNA3.1/E2F7 (magnification × 40). *p<0.05.

miR-126 inhibitor. Furthermore, cell apoptosis was promoted by CASC19 knockdown but reversed by miR-148b inhibitor. All these findings demonstrated that CASC19 modulated PC progression via sponging miR-148b.

E2F transcription factor 7 (E2F7) has been reported to participate in the progression of malignancies[24]. Ye et al[25] revealed that MiR-30a-5p attenuated cell proliferation and metastasis of gallbladder cancer through targeting

E2F7. Guo et al[26] elucidated that MiR-30a functioned as a tumor suppressor by targeting E2F7 to inhibit papillary thyroid cancer cell metastasis. In the present study, we found that miR-148 negatively regulated E2F7 expression by direct interaction, and CASC19 positively regulated the E2F7 expression, indicating the CASC19 could act as a ceRNA for miR-148b to upregulate E2F7 expression. Furthermore, gain-of-function assays revealed that E2F7 overexpression rescued the inhibitory effects on PC progression caused by CASC19 knockdown. Taken together, the results demonstrated that CASC19 accelerated development and progression of PC by positively regulating E2F7 through sponging miR-148b.

Conclusions

Our study first reported the potential molecular mechanisms of CASC19 in PC progression. The results illustrated that CASC19 promoted the tumorigenesis of PC by acting as a sponge for miR-148b and upregulating E2F7 expression, which might serve as a potential therapeutic target for PC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Kikuyama M, Kamisawa T, Kuruma S, Chiba K, Kawaguchi S, Terada S, Satoh T. Early Diagnosis to Improve the Poor Prognosis of Pancreatic Cancer. Cancers (Basel) 2018; 10: 48.
2) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.
3) Neuzillet C, Tijeras-Raballand A, Bourget P, Cros J, Couvelard A, Sauvanet A, Vullierme MP, Tournigand C, Hammel P. State of the art and future directions of pancreatic ductal adenocarcinoma therapy. Pharmacol Ther 2015; 155: 80-104.
4) Schmitt AM, Chang HY. Long Noncoding RNAs in Cancer Pathways. Cancer Cell 2016; 29: 452-463.
5) Gibb EA, Brown CJ, Lam WL. The functional role of long non-coding RNA in human carcinomas. Mol Cancer 2011; 10: 38.
6) Bartonicek N, Maag JL, Dinger ME. Long noncoding RNAs in cancer: mechanisms of action and technological advancements. Mol Cancer 2016; 15: 43.
7) Wang WJ, Guo CA, Li R, Xu ZP, Yu JP, Ye Y, Zhao J, Wang J, Wang WA, Zhang A, Li HT, Wang C, Liu HB. Long non-coding RNA CASC19 is associated with the progression and prognosis of advanced gastric cancer. Aging (Albany NY) 2019; 11: 5829-5847.
8) Wang XD, Lu J, Lin YS, Gao C, Qi F. Functional role of long non-coding RNA CASC19/miR-140-5p/CEMIP axis in colorectal cancer progression in vitro. World J Gastroenterol 2019; 25: 1697-1714.
9) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-97.
10) Chen S, Xu J, Su Y, Hua L, Feng C, Lin Z, Huang H, Li Y. MicroRNA-145 suppresses epithelial to mesenchymal transition in pancreatic cancer cells by inhibiting TGF-beta signaling pathway. J Cancer 2020; 11: 2716-2723.
11) Liu CS, Zhou Q, Zhang YD, Fu Y. Long noncoding RNA SOX2OT maintains the stemness of pancreatic cancer cells by regulating DEK via interacting with miR-200a/141. Eur Rev Med Pharmacol Sci 2020; 24: 2368-2379.
12) Jiang Z, Zhang J, Chen F, Sun Y. MiR-148b suppressed non-small cell lung cancer progression via inhibiting ALCAM through the NF-kappaB signaling pathway. Thorac Cancer 2020; 11: 415-425.
13) Zhang H, Ye Q, Du Z, Huang M, Zhang M, Tan H. MiR-148b-3p inhibits renal carcinoma cell growth and pro-angiogenic phenotype of endothelial cell potentially by modulating FGF2. Biomed Pharmacother 2018; 107: 359-367.
14) Prensner JR, Chinnaiyan AM. The emergence of lncRNAs in cancer biology. Cancer Discov 2011; 1: 391-407.
15) Guo JQ, Yang ZJ, Wang S, Wu ZZ, Yin LL, Wang DC. LncRNA SNHG16 functions as an oncogene by sponging miR-200a-3p in pancreatic cancer. Eur Rev Med Pharmacol Sci 2020; 24: 1718-1724.
16) Feng Y, Gao L, Cui G, Cao Y. LncRNA NEAT1 facilitates pancreatic cancer growth and metastasis through stabilizing ELF3 mRNA. Am J Cancer Res 2020; 10: 237-248.
17) Gong Y, Dai HS, Shu JJ, Liu W, Bie P, Zhang LD. LNC00673 suppresses proliferation and metastasis of pancreatic cancer via target miR-504/HNF1A. J Cancer 2020; 11: 940-948.
18) Liu YJ, Guo RX, Han LP, Gu H, Liu MZ. [Effect of CASC19 on proliferation, apoptosis and radiation sensitivity of cervical cancer cells by regulating miR-449b-5p expression]. Zhonghua Fu Chan Ke Za Zhi 2020; 55: 36-44.
19) Luo Y, Liu F, Yan C, Qu W, Zhu L, Guo Z, Zhou F, Zhang W. Long non-coding RNA CASC19 sponges microRNA-532 and promotes oncogenicity of clear cell renal cell carcinoma by increasing ETS1 expression. Cancer Manag Res 2020; 12: 2195-2207.
20) Wang LX, Wan C, Dong ZB, Wang BH, Liu HY, Li Y. Integrative Analysis of long noncoding RNA

(lncRNA), microRNA (miRNA) and mRNA Expression and construction of a competing endogenous RNA (ceRNA) network in metastatic melanoma. Med Sci Monit 2019; 25: 2896-2907.

21) Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. Nature 2014; 505: 344-52.

22) Huang L, Li X, Ye H, Liu Y, Liang X, Yang C, Hua L, Yan Z, Zhang X. Long non-coding RNA NCK1-AS1 promotes the tumorigenesis of glioma through sponging microRNA-138-2-3p and activating the TRIM24/Wnt/beta-catenin axis. J Exp Clin Cancer Res 2020; 39: 63.

23) Wu N, Song H, Ren Y, Tao S, Li S. DGUOK-AS1 promotes cell proliferation in cervical cancer via acting as a ceRNA of miR-653-5p. Cell Biochem. Funct. 2020.

24) Conte A, Pierantoni GM. Update on the regulation of HIPK1, HIPK2 and HIPK3 protein kinases by microRNAs. MicroRNA 2018; 7: 178-186.

25) Ye YY, Mei JW, Xiang SS, Li HF, Ma Q, Song XL, Wang Z, Zhang YC, Liu YC, Jin YP, Hu YP, Jiang L, Liu FT, Zhang YJ, Hao YJ, Liu YB. MicroRNA-30a-5p inhibits gallbladder cancer cell proliferation, migration and metastasis by targeting E2F7. Cell Death Dis 2018; 9: 410.

26) Guo H, Zhang L. MicroRNA-30a suppresses papillary thyroid cancer cell proliferation, migration and invasion by directly targeting E2F7. Exp Ther Med 2019; 18: 209-215.