CCNI2 knockdown inhibits the proliferation, apoptosis and migration of pancreatic cancer cells

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Abstract

Background: Pancreatic cancer has been recognized as one of the most serious malignant tumors in the world, and its molecular mechanism is still not fully understood. Cyclin I-like (CCNI2) is a homolog of Cyclin I (CCNI), and at present its function is largely unknown.

Methods: In this study, we aimed to explore the role of CCNI2 in the development of pancreatic cancer. The expression levels of CCNI2 in pancreatic cancer tissues and cells were detected by immunohistochemical analysis and qPCR, respectively. Lentivirus was used to deliver shRNA to pancreatic cancer cells to construct CCNI2 knockdown cell models. MTT and colony formation assays were used to assess cell proliferation capacity, flow cytometry was used to detect apoptosis, wound-healing and Transwell assays were used to determine cell migration.

Results: Our results revealed that CCNI2 is not only highly expressed in pancreatic cancer, but also significantly correlated with pathological grade, pathological stage, and survival rate. It was confirmed that knockdown of CCNI2 inhibited the proliferation and cell migration of pancreatic cancer cells while promoting apoptosis. Furthermore, human apoptotic antibody arrays showed that CCNI2 is involved in apoptosis process by up-regulating the pro-apoptotic proteins.

Conclusions: In conclusion, CCNI2 may be a prognostic marker for pancreatic cancer and is associated with its development. Thus, CCNI2 possesses potential to act as a therapeutic target for pancreatic cancer treatment.

Background

Nowadays, pancreatic cancer is the fourth most common cause of cancer-related deaths worldwide [1]. Pancreatic cancer is a malignant disease of the digestive tract, with a high morbidity and mortality rate [1, 2]. The rapid development of pancreatic cancer disease and the lack of effective treatment in the advanced stage lead to poor prognosis for patients, and the overall 5-year survival rate is extremely low [2]. Because more than 80% of patients with pancreatic cancer have metastasized or locally advanced cancer at the time of diagnosis, only 10% -15% of patients are eligible for surgical resection [3]. Folfirinox or Gemcitabine is currently used as adjuvant therapy for pancreatic cancer [4]. Although they can improve the quality of life of patients to some extent, the improvement in overall survival rate is minimal [5]. Till now, how small molecules or signaling pathways in cells contribute to the development and progression of pancreatic cancer is still unclear. Therefore, future efforts to develop new therapies to improve survival and quality of life in patients with pancreatic cancer must be based on the exploration of new targets for effective anticancer drugs.

As an important kinase, CDK5 requires other proteins to activate its kinase activity. Several CDK5 activators have been identified, including p35, p39 and Cyclin I (CCNI) [6-8]. Cyclin I-like (CCNI2), as a novel CDK5 activator, has been shown to interact physically with CDK5 and activate its kinase activity [9]. In addition, CCNI2 possesses a higher binding affinity with CDK5 and can activate CDK5 more effectively

than previously identified CDK5 activator CCNI [9]. Sequence analysis showed that CCNI2 was not conserved during evolution [9]. Human CCNI2 has a unique long N-terminal fragment, which is responsible for binding and activating CDKs [9]. CCNI2 is considered a homolog of CCNI. Cybulski *et al.*, suggested that CCNI is associated with VEGFR-2 and cell proliferation in human epithelial ovarian cancer [10]. Recent study indicated that CCNI2 knockdown affects cell cycle progression and cell proliferation [9], and other physiological function of CCNI2 is unclear. At present, the relationship between CCNI2 and pancreatic cancer has not been reported.

This study presented the first attempt to explore the role of CCNI2 in pancreatic cancer. It was suggested that CCNI2 was highly expressed in pancreatic cancer tissues or cells. Moreover, CCNI2 was significantly correlated with the pathological grade, pathological stage, and prognosis of pancreatic cancer. We also demonstrated that CCNI2 plays an important role in the tumor development of pancreatic cancer and may be a new therapeutic target in the future.

Materials And Methods

Immunohistochemical Staining (IHC)

The 99 pairs of pancreatic cancer tissue and matched non-cancer normal tissue were purchased from Shanghai Outdo Biotech Company. First, the specimen was deparaffinized and antigen-blocked with citric acid. Subsequently, Rabbit Anti-CCNI2 (Abcam, USA, Cat. # ab97767) was added and incubated at 4°C overnight. After elution with PBS several times, Goat Anti-Rabbit IgG H&L (HRP) (Abcam, USA, Cat. # ab205718) was added and incubated at room temperature. After multiple elution with PBS again, the tissue sections were stained with DAB and hematoxylin again. Finally, images were taken under a microscope and evaluated by the German immune response score [11], the high and moderate expression parameters are determined by the median of the experimental sample in the total IHC experimental score.

Cell Culture

Human pancreatic cancer cell lines PANC-1 and SW1990 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All pancreatic cancer cell lines were incubated at 37°C under 5% CO_2 atmosphere, supplemented with Dulbecco's modified Eagle's medium (DMEM, GibcoBRL, Grand Island, NY, USA) and 10% fetal bovine serum (FBS, Gibco BRL). The medium was changed every 72 h, and 0.05% trypsin and 0.02% EDTA were passaged at a concentration of 80%.

Lentiviral shRNA Vector Construction and Cell Transfection

First, three RNA interference target sequences were designed using CCNI2 as a template. The sequence with the highest knockdown efficiency was selected and ligated to the linearized vector BR-V108. At the same time, BR-V108 linear vector (Shanghai Biological Science Co., Ltd., Shanghai, China) was obtained by digestion with restriction enzyme AgeI (NEB, Cat. # R3552L) and EcoRI (NEB, Cat. # R3101L). The clones on the medium were selected for PCR identification, and the clones were sequenced and analyzed.

Culture positive clones and extract plasmids according to the kit instructions (Endo Free midi Plasmid Kit, TIANGEN, Cat. # DP118-2). After that, 293T cells were co-transfected with three plasmids (BRV-108, Helper 1.0 and Helper 2.0). According to different experimental requirements, it was determined to adopt the corresponding concentration and purification method to obtain high titer lentivirus preservation solution. Based on quality standards, various indicators of lentivirus are tested, such as sterility, viscosity, color and titer. After 72 h, the expression of green fluorescent protein was observed with a fluorescence microscope to evaluate the transfection efficiency.

Target sequence (Number)	Sequence (5'-3')
Human-CCNI2-1 (Pbr00138)	TACCTGCATTGCGCCACAATT
Human-CCNI2-2 (Pbr00164)	ATCTGCGACGCCTTCGAGGAA
Human-CCNI2-3 (Pbr00165)	CCTGGAAGGCGACCTGGACGA

Quantitative Real-Time -PCR (qPCR)

First, follow the manufacturer's instructions (Thermo Fisher Scientific, Cat. # 204211) to extract RNA from human pancreatic cancer cell lines PANC-1 and SW1990. The Nanodrop 2000/2000C spectrophotometer was used to determine the concentration and quality of the extracted RNA. Promega M-MLV kit was then used to obtain cDNA by reverse transcription. Finally, the cDNA was used as a template, and the primer information was shown below, GAPDH was used as a reference control, AceQ qPCR SYBR Green Master Mix (Vazyme, Nanjing, China) was added to perform qPCR and the fusion curve was drawn.

Primer	Upstream Primer Sequence (5'-3')	Downstream Primer Sequence (5'-3')
GAPDH	TGACTTCAACAGCGACACCCA	CACCCTGTTGCTGTAGCCAAA
CCNI2	CCAGGGAGTATGAATGAATGTT	TTGGGATAAGCCTGGGAAGTT

Western Blot

The BCA protein assay kit (Thermo Fisher Scientific, catalog number A53227) extracted and quantified the total cellular proteins of human pancreatic cancer cell lines PANC-1 and SW1990. Thereafter, proteins were separated by 10% SDS-polyacrylamide gel (SDS-PAGE) electrophoresis. Next, the sample was transferred to polyvinylidene fluoride (PVDF) membrane at 4°C. After blocking, the membrane was first incubated with Anti CCNI2, Akt, p-Akt, CDK6, MAPK9, PIK3CA and GAPDH (internal reference), then incubated with Goat Anti-Rabbit IgG H&L (HRP) (1: 3000, Beyotime, Cat. # A0208). Finally, the blots were imaged using the reagent Amersham ECL and luminescent image analyzer.

Antibody Name	Band Size (Kda)	Diluted Multiples	Antibody Source	Company	Number
CCNI2	41/50	1:1000	Rabbit	Abcam	ab97767
Akt	60	1:1000	Rabbit	CST	4685
p-Akt	60	1:1000	Rabbit	Bioss	BS5193R
CDK6	37	1:1000	Rabbit	Abcam	ab15127
MAPK9	48	1:1000	Rabbit	Abcam	ab76125
PIK3CA	110	1:1000	Rabbit	Abcam	ab40776
GAPDH	37	1:3000	Rabbit	Bioworld	AP0063

MTT Assay

ShCCNI2 and the negative control group shCtrl human pancreatic cancer cell lines PANC-1 and SW1990 cells (2×10^3 cells/well) were seeded at 100 µL/well in 96-well plates for 120 h. After trypsinization, the medium was completely resuspended. Subsequently, 20 mg of 3 (4,5-dimethylthiazole 2yl) 2,2,5 (diphenyltetrazole bromide) (MTT) (5 mg/mL) (Genview, Beijing, China; catalog number JT343) was added. After the medium is completely removed, 100 µL of dimethyl sulfoxide (DMSO) was added and mixed solution. Microplate reader was used to detect the OD value (the value of OD490 reflects the number of living cells), and the data for analysis.

Colony Formation Assay

PANC-1 and SW1990 cells with CCNI2 knockdown and the negative control group shCtrl are trypsinized, resuspended in complete medium to make a cell suspension, and counted. Cells were continuously cultured for 8 days, which were seeded in 6-well plates (2 mL/well) with 600 cells/well. Then 1 mL of 4% paraformaldehyde was added to each well, fixed for 60 min, and the cells were washed once with PBS. 500 µL of GIEMSA staining solution was stained the cells for 20 min. Finally, the cells were washed several times, and cell clones were photographed under a fluorescent microscope after being dried.

Flow Cytometry Apoptotic Assay

PANC-1 and SW1990 cells with CCNI2 knockdown and the negative control group shCtrl were seeded in 6-well plates (2 mL/well). After the cells were continuously cultured for 5 days, they were trypsinized and suspended. Annexin V-APC was added and stained in the dark for 15 min. The percentage of cell phase was determined by FACScan to evaluate the apoptotic rate, and the results were analyzed.

Transwell Assay

The chamber was placed in an empty 24-well plate, and 100 µL of serum-free medium was added to the chamber. After that PANC-1 and SW1990 cells with CCNI2 knockdown and the negative control group

shCtrl were cultured in 24-well plates for 24 h. The number of seeded cells was 5×10^4 cells/well, of which the inner chamber was 100 μL/well and the outer chamber was 600 μL/well. These cells were trypsinized and resuspended in low serum medium. Subsequently, the Transwell chamber was removed and washed with PBS. Next, it was fixed in methanol for 30 min, and stained with 0.1% crystal violet for 20min. Finally, the cells were observed under a microscope, photographed and counted.

Wound-healing Assay

PANC-1 and SW1990 cells with CCNI2 knockdown and the negative control group shCtrl at 1×10^5 cells/well were seed in a 96-well plate (100 μL/well) and cultured 120 h, then serum medium with a low concentration was replaced. Align the scraper at the lower part of the 96-well plate and push it up slightly to form a scratch. Pictures were taken at preset time points (8 h and 24 h for PANC-1; 24 h and 30 h for SW1990), and cell migration rates were then calculated for each group.

Animal Xenograft Model

Animal experiments have been approved by the Ethics Committee of Shanghai Tongji University and conducted in accordance with guidelines and protocols for animal care and protection. BALB/c female nude mice (4 weeks old) were purchased from Shanghai Jiesijie Experimental Animal Co., Ltd. (Shanghai, China). Twenty mice were randomly divided into two groups, shCtrl and shCCNI2. Subsequent subcutaneous injection of trypsinized human pancreatic cancer cells SW1990 was performed in the right arm of the mouse. After 18 days, tumor size was measured every other week. Subsequently, mice were anesthetized intraperitoneally with 0.7% sodium pentobarbital (dose, 10 μL/g), tumor burden was assessed by bioluminescence imaging, and analyzed by IVIS spectral imaging system (emission wavelength of 510 nm). After 46 days, the experimental mice were sacrificed at the cervical spine and the tumors were removed. Finally, tumors were measured for volume and weight and photographed.

Ki67 Staining

Tumor tissue removed from the mice was sectioned, and the antigen was repaired and blocked with citrate. Antigen was eluted multiple times with PBS before and after antibody addition. Subsequently, antibody Ki67 (1: 200), (Abcam, USA, Cat. # ab16667) was added to the groups of shCCNI2 or shCtrl, respectively, and incubated overnight at 4°C. Thereafter, Goat Anti-Rabbit IgG H&L (HRP) (1: 400, Abcam, USA, Cat. # ab6721) was added and incubated for 30 min at room temperature. Tissue sections were stained with DAB first and then with hematoxylin. Finally, the images were collected with an optical microscope and analyzed.

Human Apoptosis Antibody Array

The human apoptotic antibody array kit (Cat. #AB134001) was used to examine the intracellular apoptotic signaling pathway. PANC-1 cells with CCNI2 knockdown and the negative control group shCtrl were collected, washed with PBS, lysed with lysis buffer at 4°C for 30 min, and then shaken gently to mix.

The total protein extracted was diluted to 0.5 mg/mL with the array dilution buffer kit. Each array antibody membrane was blocked with blocking buffer for 30 min at room temperature, incubated at 4°C and gently shaken overnight. Biotin-conjugated anti-cytokine was incubated overnight at 4°C and then gently shaken. After adding HRP-linked streptavidin to the membrane, the protein was visualized using ChemiDoc XRS chemiluminescence detection and imaging system. Finally, the density of spots was quantified using Quantity One software and normalized to alpha-tubulin levels.

Statistical Analysis

The data were expressed as mean ± SD (n ≥ 3) and analyzed using GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA). The qPCR was analyzed by $2^{-\Delta\Delta CT}$ method. T-test were used to compare the difference. P values less than 0.05 were considered statistically significant.

Results

CCNI2 is abundantly expressed in pancreatic cancer

Through IHC analysis, it was found that CCNI2 expression in pancreatic cancer tissues was significantly higher than that in adjacent normal tissues (P < 0.001) (Table 1, Figure 1A). Subsequently, according to Mann-Whitney U test, there was a significant correlation between CCNI2 expression and pathological characteristics, such as pathological grade and pathological stage (Table 2). Spearman rank correlation analysis also confirmed this conclusion (Table 3). According to Kaplan-Meier survival analysis (Figure 1B), the expression of CCNI2 was significantly negatively correlated with the overall survival of pancreatic cancer. To sum up, CCNI2 may be related to the development and prognosis of pancreatic cancer.

Construction of CCNI2 knockdown pancreatic cancer cell models

As shown in the qPCR results of Figure 1C, the mRNA expression levels of CCNI2 in cells PANC-1 and SW1990 were higher than those of the other two pancreatic cancer cells. Furthermore, the siRNA screening results in Figure 1D showed that shCCNI2 (RNAi-00138) conducted the highest knockdown efficiency (P < 0.001). Therefore, PANC-1 and SW1990 cells and RNAi-00138 sequences were used to construct CCNI2 knockdown pancreatic cancer cell lines for all the subsequent experiments. Moreover, as shown in Figure2A, after infection of PANC-1 and SW1990 cells with shCtrl and shCCNI2, the fluorescence of cells was observed under microscope to confirm a > 80% efficiency of infection. Further analysis by qPCR and Western Blot confirmed that CCNI2 knockdown was successful. Specifically, knockdown efficiencies of CCNI2 in PANC-1 and SW1990 cells were 72.8% (P < 0.001) and 62.2% (P < 0.01) in shCCNI2 group, compared to shCtrl group as indicated by qPCR (Figure 2B). Results of Western Blot showed that the protein level of the shCCNI2 group was down-regulated compared to the shCtrl group (Figure 2C). The above data fully proved that the CCNI2 knockdown of PANC-1 and SW1990 cells models were successfully established.

Knockdown of CCNI2 inhibits cell proliferation of pancreatic cancer cells

The effects of knockdown of CCNI2 on the growth of pancreatic cancer cells were examined by MTT and colony formation assays. As shown in Figure 3A, knockdown of CCNI2 significantly inhibited the proliferation ability. In addition, the colony formation ability of pancreatic cancer cells PANC-1 and SW1990 after CCNI2 knockdown was reduced by 8.5 times and 2.03 times, respectively ($P < 0.001$) (Figure 3B). The comprehensive results proved that CCNI2 knockdown can inhibit the cell proliferation of PANC-1 and SW1990 in human pancreatic cancer.

Knockdown of CCNI2 induces apoptosis of pancreatic cancer cells

To examine the effects of CCNI2 knockdown on apoptosis of pancreatic cancer cells, Annexin V staining was used for flow cytometry analysis. As shown in Figure 3C, the percentage of PANC-1 and SW1990 cells apoptosis in shCCNI2 increased by 28.55% and 5.85% compared to the shCtrl group ($P < 0.001$). The above data proved that CCNI2 knockdown promotes the apoptosis of human pancreatic cancer cells.

Knockdown of CCNI2 inhibits migration of pancreatic cancer cells

To assess the effects of CCNI2 knockdown on the migration ability of pancreatic cancer cells, Transwell and wound healing experiments were performed. Transwell analysis showed that the migration rates of PANC-1 and SW1990 cells were reduced by 2.13-fold and 2.48-fold, respectively in shCCNI2 group, compared to shCtrl group ($P < 0.001$) (Figure 4A). In addition, a similar trend was observed in wound healing experiments. The mobility of PANC-1 in the shCCNI2 group decreased by 1.26 times at 24 h ($P < 0.01$), while that of SW1990 decreased by 2.46 times at 30 h ($P < 0.001$) (Figure 4B). Therefore, it can be concluded that the knockdown of CCNI2 inhibits the motility of human pancreatic cancer cells to some extent.

Knockdown of CCNI2 in pancreatic cancer cells impaired tumorigenesis in vivo

Although *in vitro* experimental data confirmed that down-regulation of CCNI2 inhibited cell proliferation, migration, and promote apoptosis, whether knockdown CCNI2 has consistent effects *in vivo* still needs to be further explored. Therefore, PANC-1 cells with or without CCNI2 knockdown were injected subcutaneously into nude mice to establish mouse xenograft model. The data showed that the volume of tumor in shCCNI2 group was significantly smaller than that of shCtrl group ($P < 0.01$) (Figure 5A). Furthermore, the average tumor weight in shCCNI2 group was 0.127 ± 0.014 g, which was significantly lower than that in the shCtrl group ($P < 0.01$) (Figure 5B, 5C). In addition, bioluminescence imaging showed that tumor growth in the shCCNI2 group was slower than that in the shCtrl group ($P < 0.01$) (Figure 5D, 5E). Moreover, results of Ki67 staining showed that the tumor tissue proliferation index of the shCCNI2 group was significantly lower than that of the shCtrl group (Figure 5F, 5G). In conclusion, the results of *in vivo* experiments confirmed the correctness of the conclusions of *in vitro* experiments, indicating that CCNI2 plays a regulatory role in pancreatic cancer cells and impairs tumorigenicity.

Exploration of CCNI2 downstream molecular mechanism of pancreatic cancer cells

To explore the potential regulatory mechanism of CCNI2 in pancreatic cancer cells, human apoptosis antibody array analysis was performed to analyze the differential expression of 43 proteins in PANC-1 cells between the shCCNI2 and shCtrl groups. As shown in Figure 6A, among the tested proteins, the expression levels of pro-apoptotic proteins including Bax, BIM, Caspase3, cytoC, FasL, p21, p27 and p53 were up-regulated ($P < 0.05$). These results are consistent with cell experiments, especially apoptosis assays. In addition, the protein expression of typical tumor signaling pathway was deduced by apoptosis pathway as shown in Figure 6B. Compared with shCtrl, the expressions of p-Akt, CDK6 and PIK3CA were down-regulated, MAPK9 were up-regulated, while no significant changes in the expression of Akt. Based on these experimental data, it is speculated that knockdown of CCNI2 may affect the function of pancreatic cancer cells through the p53 and the PI3K/Akt pathways.

Discussion

This study first proposed the role and mechanism of CCNI2 in pancreatic cancer cells.

CCNI2 is significantly associated with the clinicopathological grade and the overall survival of pancreatic cancer patients. Furthermore, *in vitro* experiments demonstrated that knocking down CCNI2 inhibited growth, colony formation, migration, and induced apoptosis. At the same time, *in vitro* experiments have also confirmed that CCNI2 knockdown impair tumorigenicity.

Induction of apoptosis in cancer cells has been considered as an innovative drug discovery strategy for cancer treatment [12]. Therefore, understanding the mechanisms of apoptosis and designing treatments to trigger cell death in cancer cells is essential for effectively treating cancer [13]. Treatment of pancreatic cancer remains a major challenge due to the poor efficacy and severe toxicity of standard chemotherapy. There is an increased interest in seeking new therapy for pancreatic cancer from inducing apoptosis agents [12]. In this study, we found that knockdown of CCNI2 promotes apoptosis of pancreatic cancer cells by activating the expression of pro-apoptotic proteins, such as Bax, BIM, Caspase3, cytoC, FasL, p21, p27 and p53. Previous studies have shown that Bax is an important regulator of cell proliferation and apoptosis, which activates both endogenous and exogenous apoptotic pathways [14]. Burmi *et al.*, proposed that combined inhibition of the PI3K/mTOR/MEK pathway induce apoptosis in pancreatic cancer cells regulated by BIM [15]. Additionally, FasL (CD95L), as a transmembrane protein, is a pro-apoptotic member of the tumor necrosis factor (TNF) superfamily and plays an important role in inducing cell apoptosis of pancreatic cancer [16, 17]. The p53 tumor suppressor inhibits tumor formation by inducing apoptosis, which has been identified to regulate the transcription and expression of pro-apoptotic proteins, including Bax and Caspase3, leading to apoptosis [18]. Therefore, we speculated that knockdown of CCNI2 promotes apoptosis process of pancreatic cancer cells is achieved through a series of cascades of apoptosis-related proteins.

Moreover, exploration of the molecular mechanism downstream of pancreatic cancer cells CCNI2 revealed that the expressions of p-Akt, CDK6 and PIK3CA were downregulated, MAPK9 were upregulated, while no significant changes in the expression of Akt. Activation of the PI3K/Akt pathway plays an

important role in the occurrence and development of pancreatic cancer [19]. Bondar *et al*., previously suggested that inhibition of the PI3K/Akt pathway induces apoptosis and reduces proliferation of pancreatic cancer cells [20]. Similarly, Mao *et al*., found that the expression level of Akt was closely related to the pathological grade of pancreatic cancer, and inhibition of the PI3K/Akt pathway may be a potential therapeutic target for pancreatic cancer [21]. Moreover, CDK6 as a member of the cell cycle regulated kinase family is an important and operable target in pancreatic cancer. Franco, *et al*., proposed that CDK4/6 inhibitors have strong activity in combination with pathway selective therapy drugs in pancreatic cancer models [22]. Furthermore, MAPK9 participates in MAPK signaling pathway, and MAPK signaling exerts important roles in tumorigenesis [23, 24]. For example, Qun *et al*., suggested that YY1 could target proteins to promote microtubulin aggregation through p38/MAPK and PI3K/AKT pathways inhibit the migration, invasion and angiogenesis of pancreatic cancer [25]. Based on these experimental data, it is speculated that knockdown of CCNI2 may affect the function of pancreatic cancer cells through p53 and PI3K/Akt pathway.

Conclusions

Overall, the data provided by this study indicated that CCNI2 knockdown can indeed promote the apoptosis of pancreatic cancer cells by activating the apoptotic pathway and regulating the p53 and PI3K/Akt signaling pathway, inhibiting its proliferation and metastasis. This will provide new strategies for developing clinical applications for the treatment of pancreatic cancer. Therefore, our research suggested that CCNI2 may be a new therapeutic target for pancreatic cancer.

Declarations

Ethics approval and consent to participate

The research was approved by the Ethics Committee of Shanghai Tongji University and conducted in accordance with guidelines and protocols for animal care and protection.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Competing interests

The authors have no conflicts of interest.

Funding

Not applicable.

Author contributions

The program was designed by TW and WZ; BH, CZ, KP and XG operated the cell and animal experiments; BH and KP conducted the data collection and analysis; BH, TW, CL and NZ produced the manuscript, which was checked by CL and TW.

Acknowledgements

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Tables

Table 1. Expression patterns in pancreatic cancer tissues and para-carcinoma tissues revealed in immunohistochemistry analysis

CCNI2 expression	Tumor tissue		Para-carcinoma tissue		P value
	Cases	Percentage	Cases	Percentage	< 0.0001
Low	31	50.8%	53	96.4%	
High	30	49.2%	2	3.6%	

Table 2. Relationship between CCNI2 expression and tumor characteristics in patients with pancreatic cancer

Features	No. of patients	CCNI2 expression		P value
		low	high	
All patients	61	31	30	
Age (years)				0.254
< 67	30	13	17	
≥ 67	31	18	13	
Gender				0.879
Male	36	18	18	
Female	25	13	12	
Lymph node positive				0.520
≤ 0	33	18	15	
> 0	24	11	13	
Tumor size				0.870
< 4 cm	23	12	11	
≥ 4 cm	38	19	19	
Grade				0.007
I	1	0	1	
II	31	22	9	
III	29	9	20	
Stage				0.021
1	5	5	0	
2	43	22	21	
4	4	1	3	
T Infiltrate				0.163
T1	2	1	1	
T2	7	6	1	
T3	36	18	18	
lymphatic metastasis(N)				0.624
N0	33	18	15	
N1	25	12	13	

Table 3. Relationship between CCNI2 expression and tumor characteristics in patients with pancreatic cancer

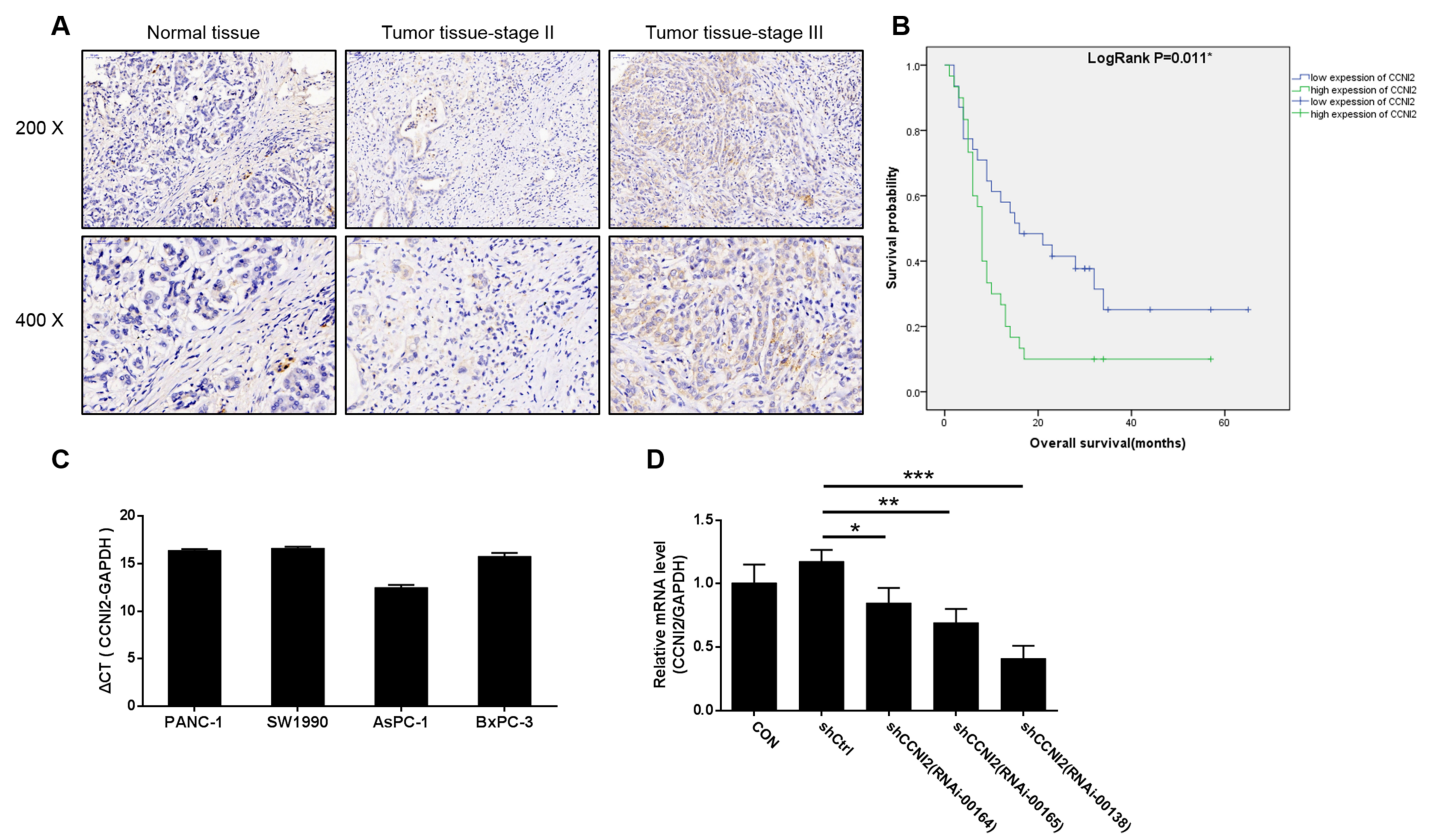|		CCNI2
Grade	Pearson Correlation	0.350
	Significance (double-tail)	0.006
	N	61
Stage	Pearson Correlation	0.322
	Significance (double-tail)	0.020
	N	52

Figures

Figure 1

CCNI2 is highly expressed in pancreatic cancer. (A) The expression of CCNI2 in the normal and tumor samples detected by IHC. (B) Kaplan-Meier survival analysis of overall survival for pancreatic cancer. (C) The mRNA expression of CCNI2 in pancreatic cancer cell line detected by qPCR. (D) Effective interference targets were screened by qPCR.
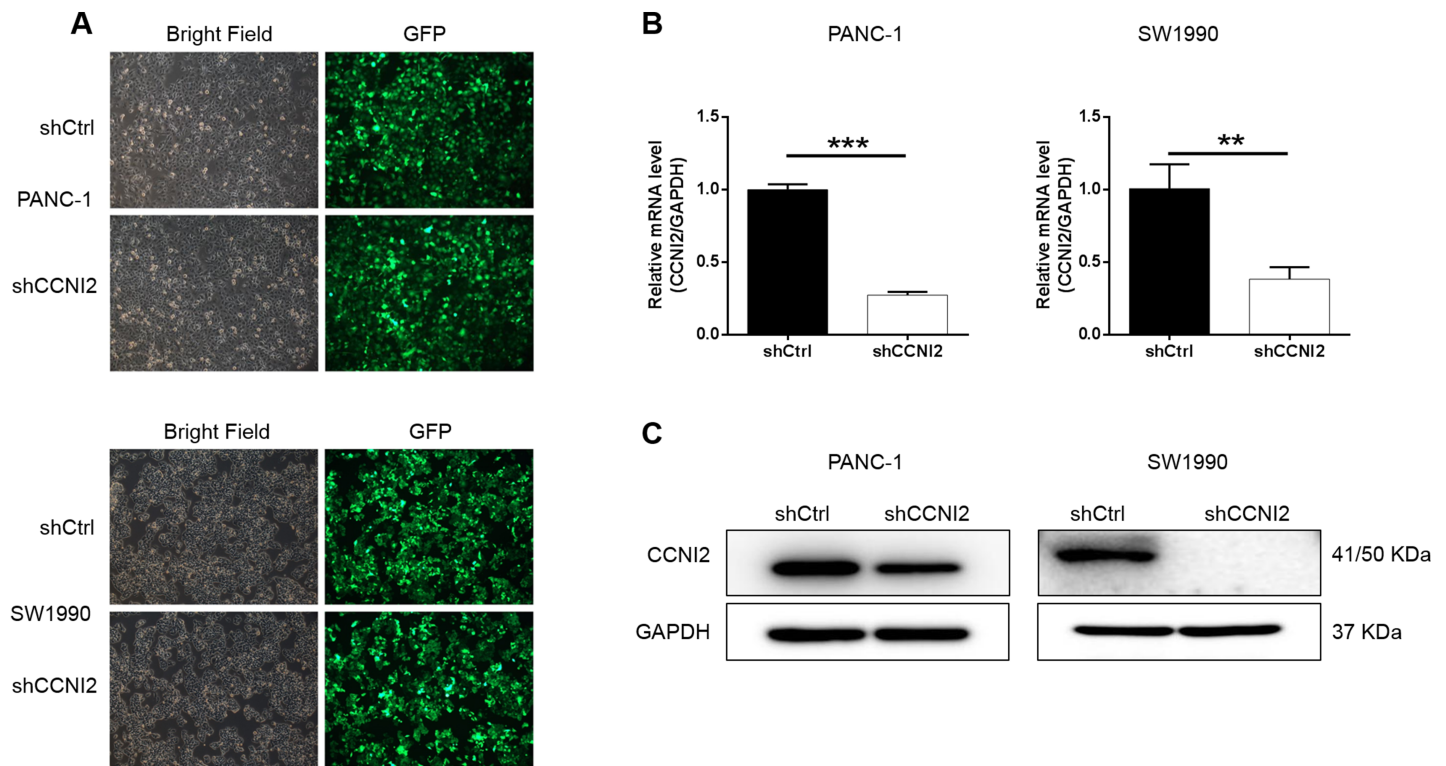
Figure 2

Construction of CCNI2 knockdown cell model. (A) Transfection efficiency for PANC-1 and SW1990 cells are evaluated by expression of green fluorescent protein 72 h post-infection. (B, C) The specificity and validity of the lentivirus-mediated shRNA knockdown of CCNI2 expression are verified by qPCR (B) and Western Blot (C). The data are presented as the mean ± SD (n = 3), *P<0.05, **P<0.01, ***P<0.001.
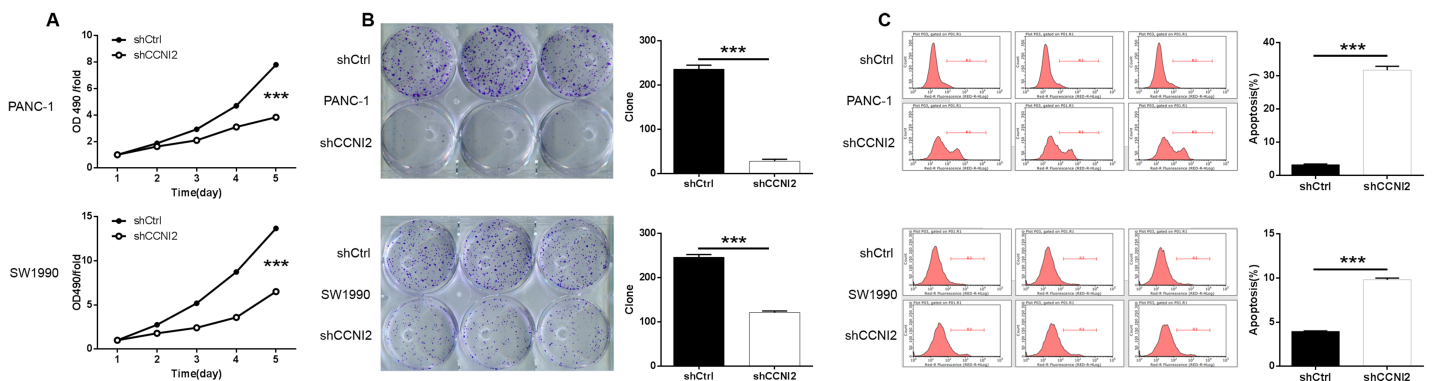

Figure 3

Knockdown of CCNI2 inhibits cell proliferation, arrests cell cycle and promotes apoptosis in pancreatic cancer cells. (A) Cell proliferation of PANC-1 and SW1990 cells with or without knockdown of CCNI2 is evaluated by MTT assay. (B) Cell colony formation of PANC-1 and SW1990 cells with or without knockdown of CCNI2 is measured. (C) Flow Cytometry analysis based on Annexin V-APC staining is utilized to detect the percentage of early apoptotic cell for PANC-1 and SW1990 cells.
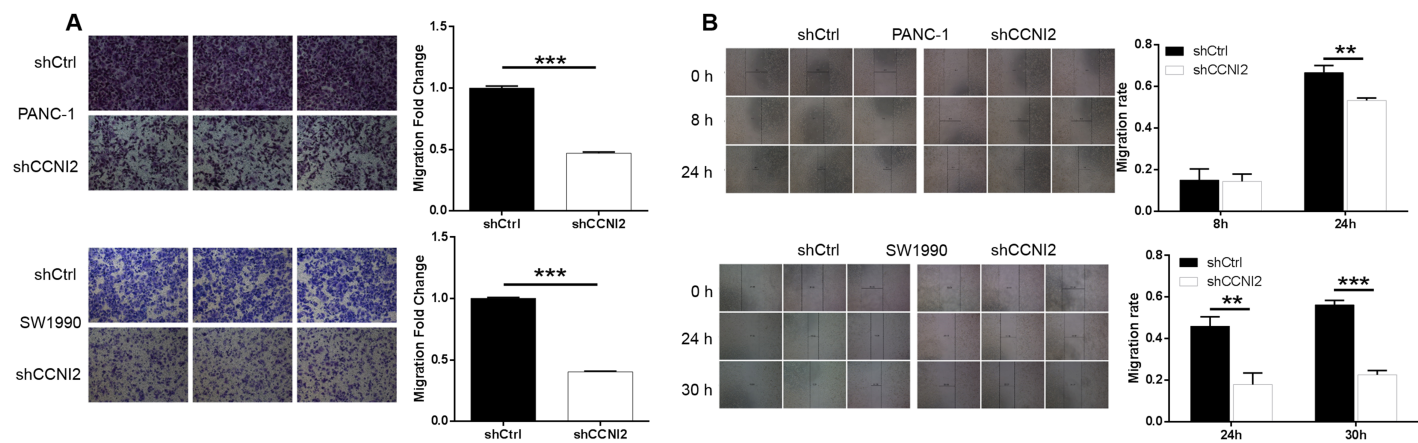
Figure 4

Knockdown of CCNI2 inhibits cell migration in pancreatic cancer cells. (A) Cell migration of PANC-1 and SW1990 cells with or without knockdown of CCNI2 is evaluated by Transwell assay. (B) Cell migration of PANC-1 and SW1990 cells with or without knockdown of CCNI2 is evaluated by wound-healing assay. The data are expressed as mean ± SD (n = 3), *P<0.05, **P<0.01, ***P<0.001.
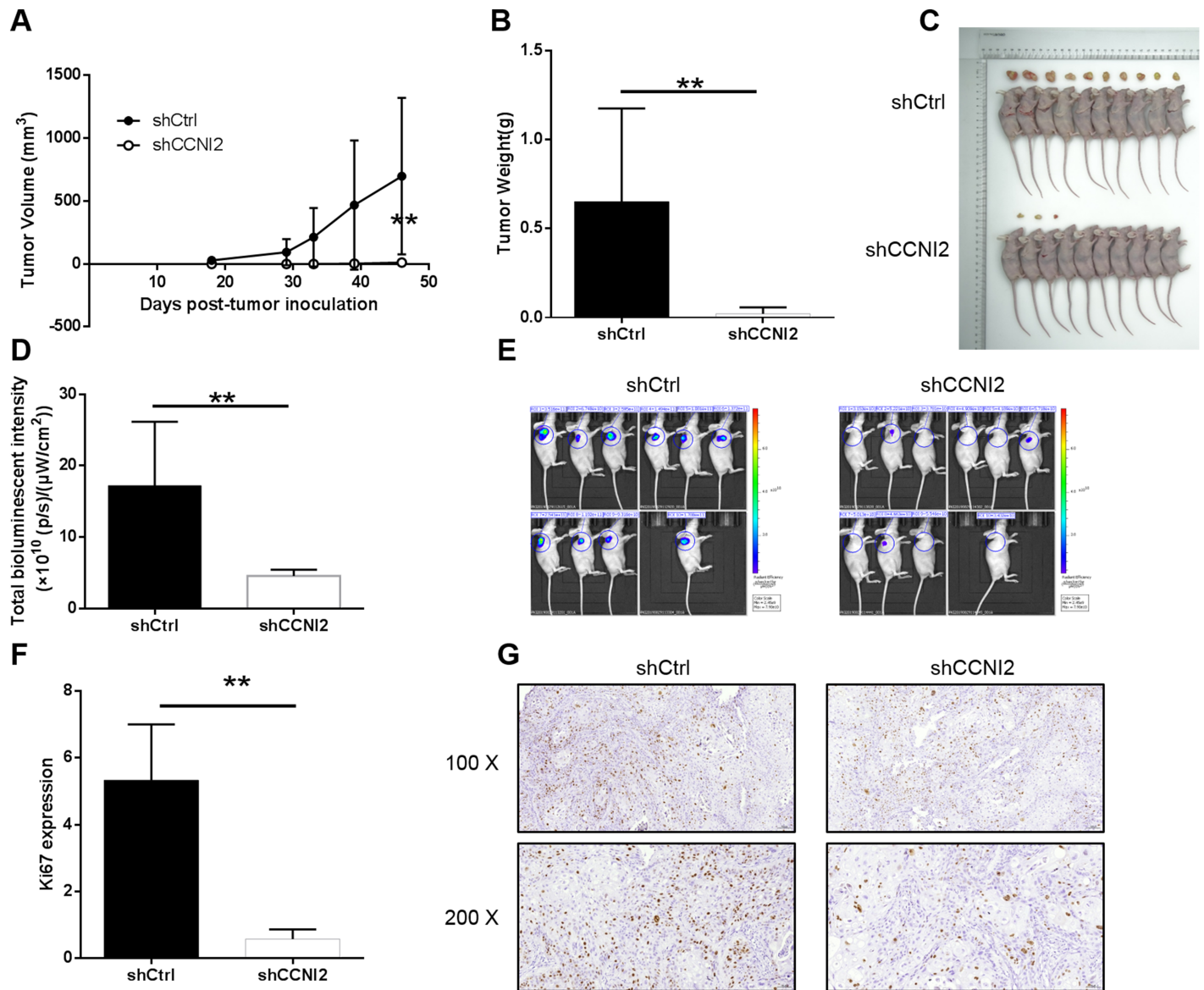
Figure 5

Knockdown of CCNI2 inhibits tumor growth in mice xenograft models. (A) The volume of tumors in shCtrl group and shCCNI2 group was measured post-injection. (B) The average weight of tumors in shCtrl group and shCCNI2 group. (C) Images of mice and tumors in shCtrl group and shCCNI2 group. (D) The total bioluminescent intensity of tumors in shCtrl group and shCCNI2 group. (E) The bioluminescence imaging of tumors in shCtrl group and shCCNI2 group. (F) The Ki67 expression of tumor tissues in shCtrl group and shCCNI2 group. (G) The Ki67 staining of tumor tissues in shCtrl group and shCCNI2 group. The data were expressed as mean ± SD (n = 3), *P<0.05, **P<0.01, ***P<0.001.

Figure 6

Exploration of downstream molecular mechanism of CCNI2 in pancreatic cancer cells (A) Human apoptosis antibody array analysis was performed in PANC-1 cells with or without CCNI2 knockdown; Densitometry analysis was performed and the gray values of differentially expressed proteins were shown. (B) The protein expression of downstream signaling pathway was observed by Western Blot in PANC-1. The data were expressed as mean ± SD (n = 3), *P<0.05, **P<0.01, ***P<0.001.