

Expression of Catenin Delta 1 (CTNND1) in Pancreatic Cancer and Its Influence on Tumor Cell Biological Behavior

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1. Abstract

1.1. Objective: To explore the role of Catenin Delta 1 (CTNND1) in pancreatic cancer and its possible mechanism.

1.2. Methods: We selected CTNND1 as the research target using gene expression profile databases and bioinformatics analysis methods. CTNND1 expression in the pancreatic cancer cell line PANC-1 was suppressed by siRNA technology, and various cell biological experiments were used to evaluate the impact of CTNND1 on cell proliferation, migration, invasion, apoptosis, and cell cycle. Meanwhile, the protein levels of epithelial-mesenchymal transition (EMT) related biomarkers were detected to explore whether CTNND1 can regulate EMT in pancreatic cancer cells.

1.3. Results: We found that CTNND1 expression in pancreatic cancer cells was significantly increased. Knockdown of CTNND1 significantly reduced the proliferation, migration, and invasion ability of PANC-1 cells, increased the apoptosis rate, and inhibited the cell cycle progression. In addition, CTNND1 knockdown could regulate EMT in pancreatic cancer cells.

1.4. Conclusion: Our research results reveal the important role of CTNND1 in pancreatic cancer and propose its possible mechanism of action. These findings provide a new theoretical basis for the treatment of pancreatic cancer, and CTNND1 may become a new target for pancreatic cancer treatment. However, more in-depth research is still needed to clarify the specific role of CTNND1 in pancreatic cancer and its detailed molecular mechanism.

2. Introduction

Pancreatic cancer is a malignant tumor that originates from pancreatic tissue. The main treatment options include surgical resection, radiation therapy, and chemotherapy [1]. However, early symptoms of pancreatic cancer are often not obvious and can be easily overlooked. Therefore, at the time of diagnosis, most patients are already in the advanced stage, making treatment difficult and prognosis poor [2]. It is predicted that pancreatic cancer will become the second leading cause of cancer-related death in the United States within the next 20-30 years [3]. At the same time, the incidence of pancreatic cancer in China is increasing year by year [4]. Therefore, in-depth research on the pathogenesis and molecular characteristics of pancreatic cancer is crucial for early diagnosis and treatment.

Adherent junctions are protein complexes on the cell surface that connect cells to other cells, the basement membrane, or extracellular matrix substances. These proteins form a complex network structure through interactions with each other, maintaining cell morphology and stability, and have various important physiological functions. They can affect cell morphology, movement, and proliferation, and are closely related to the occurrence and development of various diseases (such as cancer, cardiovascular disease, and autoimmune diseases) [5]. Therefore, studying cell adherent junctions and their mechanisms in cell function and disease will help to deepen our understanding of the basic processes of life

and disease mechanisms, and provide new ideas and methods for disease prevention and treatment.

The CTNND1 gene is located in the chromosomal region 11q11 and encodes the p120-catenin or δ -catenin protein. p120-catenin is a structural protein that acts as a bridge in cell-cell connections. It connects proteins on the cell membrane to the cell cytoskeleton, maintaining the stability and integrity of cell-cell connections [6]. Studies have found that p120-catenin is also involved in biological processes such as signal transduction pathways, cell polarity, and metastasis [7]. Mutations and abnormal expression of the CTNND1 gene are associated with the occurrence and development of various diseases (such as tumors, neurological and cardiovascular diseases) [8]. Some studies have shown that p120-catenin plays a crucial role in the process of tumor metastasis, and its dysregulation can promote cancer cell migration and invasion [9,10].

In this study, we performed bioinformatics analysis using pancreatic cancer data from the GEO database and conducted in vitro experiments using pancreatic cancer cell lines. Our results showed that the expression level of CTNND1 was upregulated in pancreatic cancer cells. By inhibiting the expression of CTNND1, we observed a decrease in the proliferation, migration, and invasion capabilities of pancreatic cancer cells in vitro. Our research aims to explore the mechanism of CTNND1 in pancreatic cancer patients and its potential impact on pancreatic cancer prognosis. We believe that this study will help to develop new strategies for the treatment of pancreatic cancer.

3. Materials and Methods

3.1. GEO Database

The Gene Expression Omnibus (GEO) database is a public bioinformatics database that stores large amounts of high-throughput biological data uploaded by researchers from around the world, such as gene expression and DNA methylation data [11]. The database contains various types of data, including microarrays, RNA sequencing, proteomics, and chips. These data are available free of charge to scientists and researchers for bioinformatics analysis and mining, which helps better understand biological issues such as genomics, transcriptomics, and proteomics [12,13].

3.2. DAVID Database

The DAVID Bioinformatics Database is a widely used bioinformatics tool library that contains various resources for biologists to analyze gene function, genomics, and proteomics data [14]. This database was developed by the US National Institutes of Health (NIH) to assist researchers in gaining a deeper understanding of genes and their roles in the organism. The DAVID database contains a large number of gene annotations and biological pathway information that helps users identify the function of a specific gene, explore its association with specific diseases, and confirm its involvement in particular biological processes. Additionally, the

database includes a large amount of gene expression data, which helps researchers compare gene expression patterns across different tissues and cell types [15-17].

3.3. Cytoscape

Cytoscape is widely used software in bioinformatics, primarily for analyzing and visualizing molecular and gene networks [18]. It offers a rich set of tools and plugins that enable researchers to extract biological insights from large-scale molecular interaction data, particularly in the fields of systems biology and genomics. The software supports the import of various network data formats, including biological pathways, gene regulation, and protein-protein interactions, and provides various layout algorithms to make the network structure more clear and understandable. Cytoscape is also equipped with numerous analysis tools and visualization effects, such as clustering analysis, GO enrichment analysis, and network analysis, to help users gain a deeper understanding and interpretation of network data [19,20].

3.4. GEPIA2 Database

GEPIA2 is an online, interactive, and free bioinformatics database dedicated to gene expression data analysis and visualization. It brings together gene expression profile data from TCGA and GTEx databases and provides multiple tools to support gene expression analysis and bioinformatics research. GEPIA2 can compare the expression levels of different genes in numerous tumor and normal tissues and provides functions such as differential analysis, survival analysis, and gene correlation analysis. Through interactive visualization tools, users can intuitively understand and interpret large amounts of gene expression data, explore gene expression patterns and correlations in various tumor types and tissues. Additionally, GEPIA2 provides easy-to-read and display charts and visualization results, making it easier for biological researchers to analyze and present data [21,22].

3.5. Cell Culture and Reagents

The pancreatic cancer cell line (PANC-1) and normal pancreatic epithelial cells (HPNE) were obtained from the Shanghai Cell Bank of the Chinese Academy of Sciences. HPNE cells were grown in RPMI-1640 medium containing 10% FBS (Invitrogen), while PANC-1 cells were grown in DMEM medium containing 10% FBS (Invitrogen), following the manufacturer's protocol. All cells were grown in a cell incubator at 37°C with 5% CO₂ and were reseeded every 3-4 months.

3.6. siRNA Transfection

When the cells reached appropriate density in a six-well plate, CTNND1-siRNA was transfected using Lipofectamine 3000 (Invitrogen, USA) according to the manufacturer's instructions. These cells were used for subsequent experiments. The CTNND1 siRNA sequence was synthesized by Integrative Biotechnology Solutions in Shanghai, China, and the Si-CTNND1 sequence is as follows:

Forward sequence: 5'- GCAUGAGCGAGGAAGUUUAGC-3',

Reverse sequence: 5'- UAAACUCCUCGCUCAUGCUG -3'.

3.7. qRT-PCR

Total RNA was extracted from the samples using TRIzol reagent and reverse transcription was performed using the Hiscript III RT Supermix (r323-01, Novazyme). qRT-PCR was performed using Chamq Universal SYBR qPCR Master Mix (Q711, Novazyme) and carried out on a Roche real-time PCR system. The expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method and the following primer sequences: CTNND1 forward primer: 5'-AC-CACGGTCAAGAAAGTAG-3', reverse primer: 5'-GAAAT-CACGACCCAAAGT-3'; GAPDH forward primer: 5'-GCAC-CGTC AAGGCTGAGAAC-3', reverse primer: 5'-TGGTGAA-GACGCCAGTGGA-3'.

3.8. Western Blotting Experiment

Total protein from human pancreatic cancer cells was extracted using RIPA lysis buffer produced by Solarbio. Denatured protein samples of equal quantity were separated by 10% SDS-PAGE gel electrophoresis and transferred to PVDF membranes. Before transfer, the PVDF membranes were blocked for 2 hours at room temperature in 5% skimmed milk. Next, the PVDF membranes were incubated overnight at 4°C with the corresponding primary specific antibodies. Afterwards, the membrane was incubated with secondary antibodies (goat anti-rabbit IgG-HRP) containing horseradish peroxidase (HRP) (Finnzymes Oy, China) at 37°C for 1 hour. After incubating with enhanced chemiluminescence solution for 1 minute, protein bands were visualized using the WB exposure instrument from Viber (France). The primary antibodies (rabbit anti-human) used in this experiment, including β -Catenin, c-Myc, GAPDH, were purchased from Finnzymes Oy, and the primary antibody for CTNND1 (rabbit anti-human) was purchased from Abcam (USA).

3.9. Cell Proliferation Assay

After cell transfection, equal numbers of pancreatic cancer cells were seeded into each well of a 96-well plate. At 0, 24, 48, and 72 hours, 10 μ L of CCK-8 reagent (A311-01, Novozymes, Nanjing, China) was added to each well, followed by incubation at 37°C for 1 hour. The OD value was measured using a microplate reader at a wavelength of 450 nm.

3.10. Wound Healing Assay

After completing cell transfection, equal amounts of pancreatic cancer cells were seeded into a 6-well plate until the cell density reached approximately 90%. Next, we used a 100 μ L pipette tip to create a scratch on the cell layer and gently washed away the cell debris with PBS. Then, the cells were incubated in 2% low serum medium for 48 hours. Finally, cell migration ability was evaluated by microscopy imaging.

3.11. Transwell Assay

Before cell seeding, an equal amount of Matrigel gel was evenly spread onto the upper chamber of the Transwell and incubated at 37°C for 2 hours to solidify. Next, 100 μ L of serum-free DMEM or RPMI 1640 cell suspension containing about 2×10^4 cells was added to the upper chamber, while 600 μ L of DMEM or 1640 medium containing 20% FBS was added to the lower chamber. After cell seeding, the Transwell was placed in a cell culture incubator at 37°C and 5% CO₂ for 24 hours. After 24 hours, cells were fixed with 4% paraformaldehyde for 30 minutes, and then non-invaded cells and matrix gel were removed with a wet cotton swab. The cells were then stained with 1% crystal violet for 20 minutes, washed with PBS, and allowed to dry. Finally, cells were counted and photographed under a microscope. The Matrigel gel was provided by Corning, New York, USA.

3.12. Flow Cytometry Analysis

We used the cell cycle detection kit (model: KGA511, manufacturer: KGI) to evaluate the effects of different interventions on the cell cycle of pancreatic cancer cells. First, we mixed the cells with 500 μ L of pre-cooled 70% ethanol and fixed them for 2 hours at 4°C, followed by centrifugation at $500 \times g$ for 5 minutes. Next, we added 500 μ L of PI/RNase A staining solution and incubated the cells at room temperature in the dark for 30 minutes. Finally, we analyzed the results using a flow cytometer from ACEA Biosciences in the United States.

3.13. Statistical Analysis

We used GraphPad Prism (version 9.3) for all statistical analyses. Student's t-test was used for comparisons between groups, and analysis of variance (ANOVA) was used for data involving more than two groups. A P-value less than 0.05 was considered statistically significant.

4. Results

4.1. Gene and Pathway Analysis Results

We downloaded two datasets, GSE16515 and GSE15471, from the GEO database and used the GEO2R tool to identify up-regulated and down-regulated genes. By using a Venn diagram, we found that there were 656 up-regulated genes and 135 down-regulated genes in the intersection of GSE16515 and GSE15471 (Figure 1A). We input these commonly up-regulated and down-regulated genes into the DAVID database for GO analysis, and the results showed that these genes were mainly involved in the role of proteoglycans in cancer, viral carcinogenesis, cancer pathways, herpes simplex infection, HTLV-I infection, influenza A, hepatitis B, regulation of cytoskeleton and cell adhesion molecules, leukocyte transendothelial migration, adhesive junctions, cell adhesion molecules (CAMs), ECM-receptor interaction, Hippo signaling pathway, PI3K-Akt signaling pathway, phagosomes, endocrine cell proliferation, cell cycle, and cytokine-cytokine receptor interaction pathways (Figure 1B).

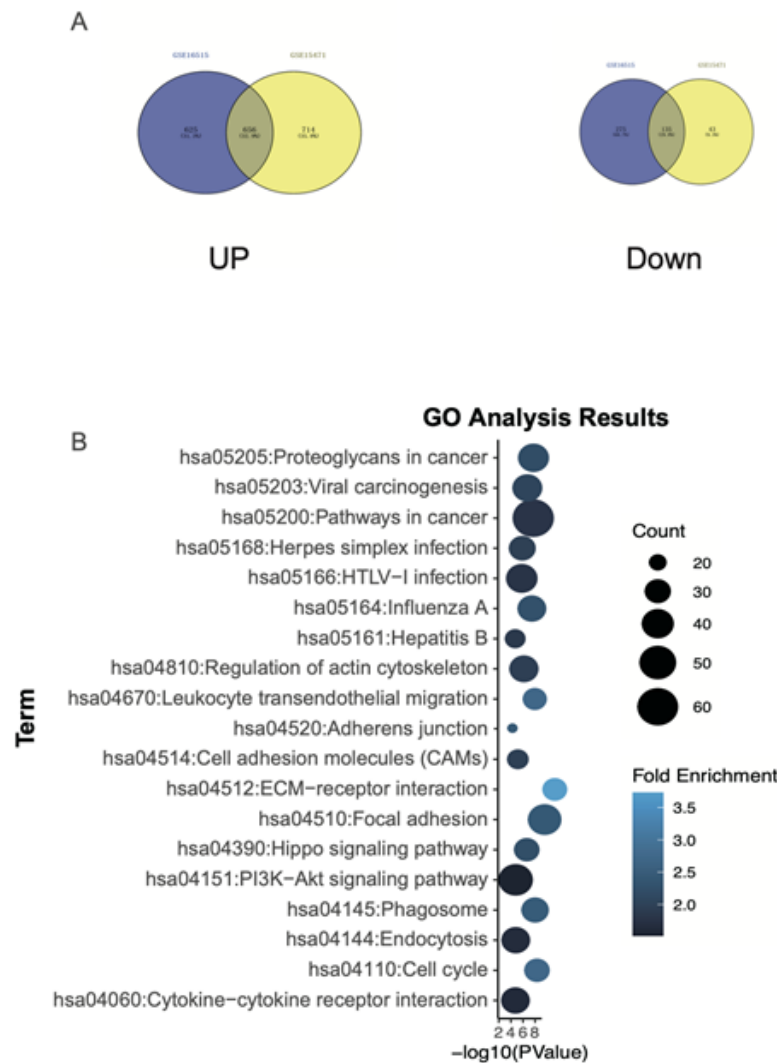


Figure 1: Gene analysis and pathway analysis results; A. VENN maps of the Up and Down genes of GSE16515 and GSE15471; B. GO analysis results.

4.2. Gene Screening and Expression Analysis

We imported the genes related to adhesion and connection into the Cytoscape software and calculated their hub genes using Degree, MCC, and Closeness methods, and then obtained their intersection. The results showed that the intersection genes included ACTB, CTNNA1, ERBB2, RAC1, VCL, CTNNA1, IQGAP1, ACTB, and CTNND1 (Figure 2A). We queried the expression levels of these 8 genes in pancreatic cancer using the Gepia2 database, and the results showed that all of these genes were highly expressed in pancreatic cancer (Figure 2B). Further survival analysis showed that the expression levels of CTNNA1, CTNNA1, CTNND1, IQGAP1, RAC1, and VCL were significantly correlated with patients' survival ($P < 0.05$) (Figure 2C).

4.3. Impact of CTNND1 Knockdown on the Biological Functions of Pancreatic Cancer Cells

Given the relatively limited research on CTNND1 in pancreatic cancer, we chose to investigate it and validated the expression

levels of CTNND1 in pancreatic cancer cell line (PANC-1) and normal pancreatic epithelial cells (HPNE) using Western blotting. The results showed a significant increase in the protein expression levels of CTNND1 in PANC-1 cells ($P < 0.05$, (Figure 3A). To evaluate the impact of CTNND1 on the proliferation, migration, and invasion capabilities of pancreatic cancer cells, we used siRNA technology to knock down CTNND1 expression in PANC-1 cells. qRT-PCR and Western blotting experiments confirmed the effective knockdown of CTNND1 (Figure 2B, C). The CCK-8 assay results showed that CTNND1 knockdown significantly decreased the proliferation capability of PANC-1 cells (Figure 2D), and in the scratch healing assay at 48 hours, CTNND1 knockdown significantly inhibited the wound healing capability of PANC-1 cells (Figure 3A). The Transwell assay further confirmed that CTNND1 knockdown significantly reduced the migration and invasion capabilities of PANC-1 cells compared to the control group (Figure 3B, C). Flow cytometry analysis showed that CTNND1 knockdown significantly increased the apoptosis rate of PANC-1 cells

(Figure 3D), increased the proportion of cells in the G1 phase, and decreased the proportion of cells in the S phase. This suggests that CTNND1 knockdown promotes the apoptosis of pancreatic cancer cells and inhibits their progression from the G1 phase to the S

phase (Figure 4E). Overall, these results clearly demonstrate that CTNND1 plays a critical role in the proliferation, migration, invasion, apoptosis, and cell cycle of pancreatic cancer cells.

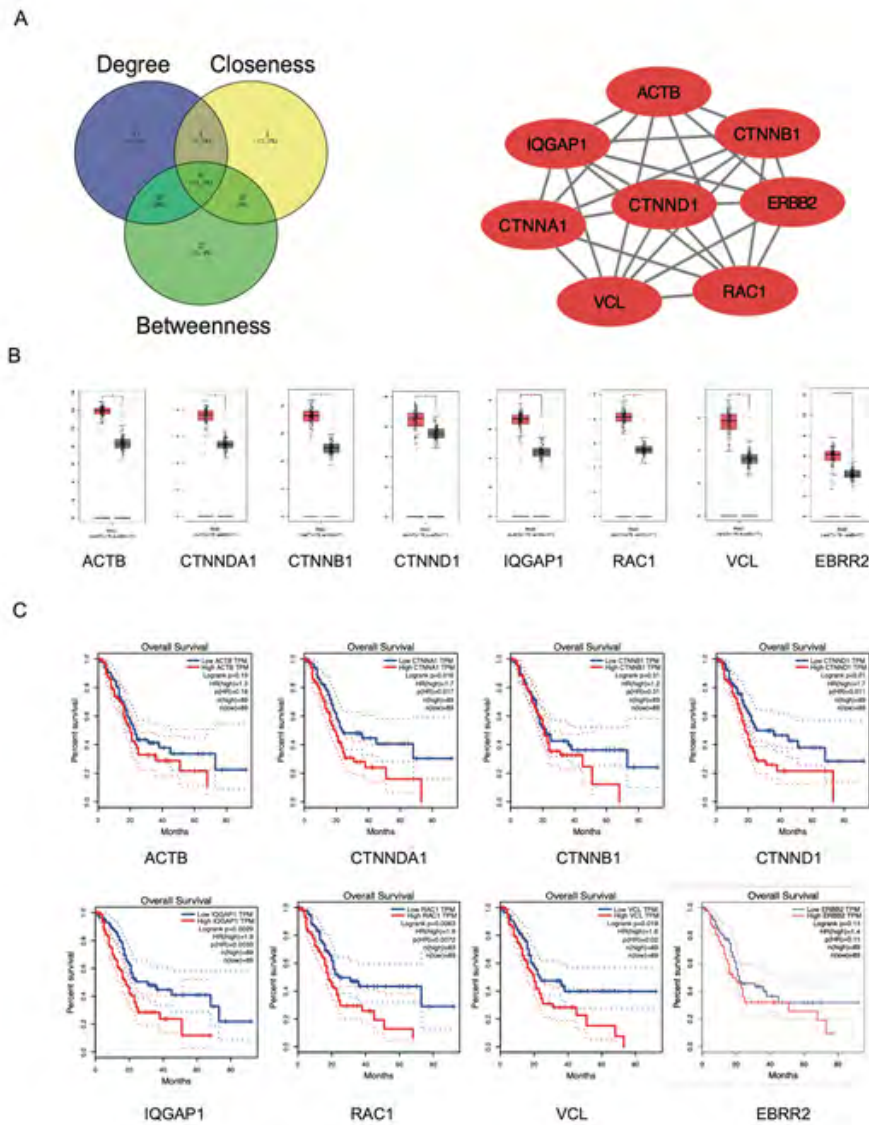


Figure 2: Gene screening and expression analysis: Hub gene related to adhesion and connection is highly expressed in pancreatic cancer; A. VENN maps of HUB genes obtained by Degree, MCC, and Closeness methods; B. Expression of HUB gene in GEPIA2 database; C. Survival analysis results of HUB gene in GEPIA2 database. $P < 0.05$, the difference was statistically significant.

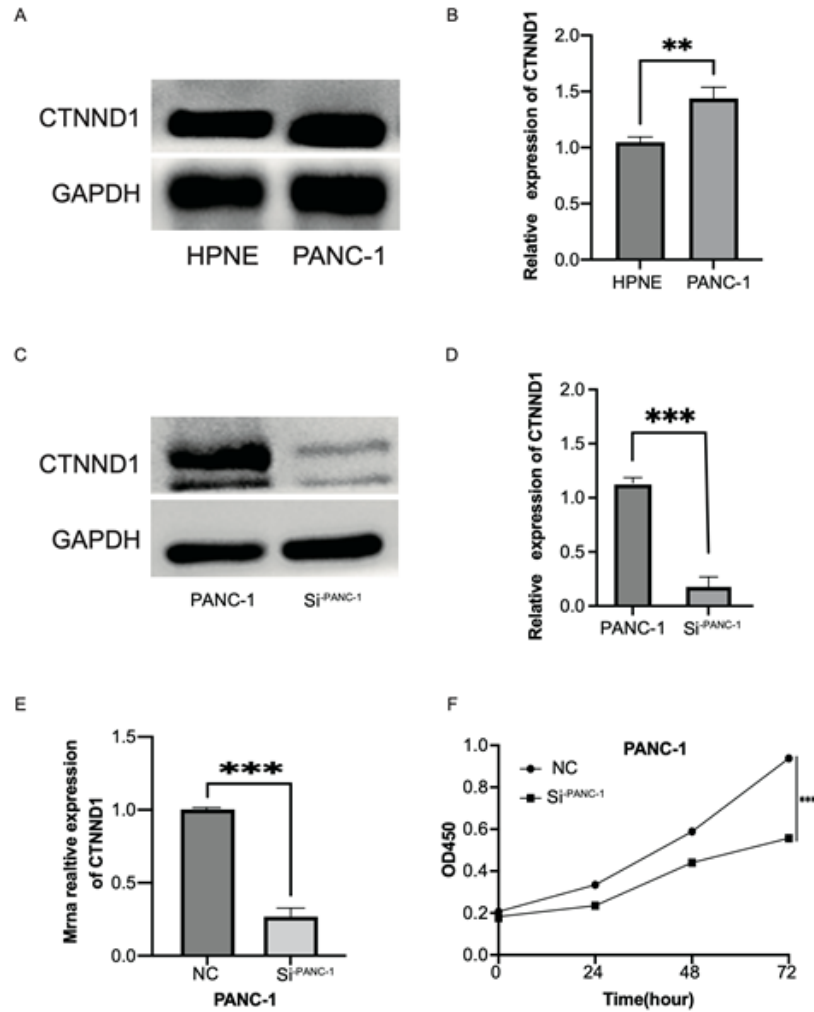


Figure 3: Effect of knockdown CTNND1 on biological function of pancreatic cancer cells A. Expression and analysis of CTNND1 in HPNE and PANC-1 cells; B. Western blot results and analysis of knocking down the efficiency of CTNND1 in PANC-1 cells; C. QRT PCR results of knocking down the efficiency of CTNND1 in PANC-1 cells; D. Knocking down CTNND1 in PANC-1 cells inhibits its ability to increase proliferation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

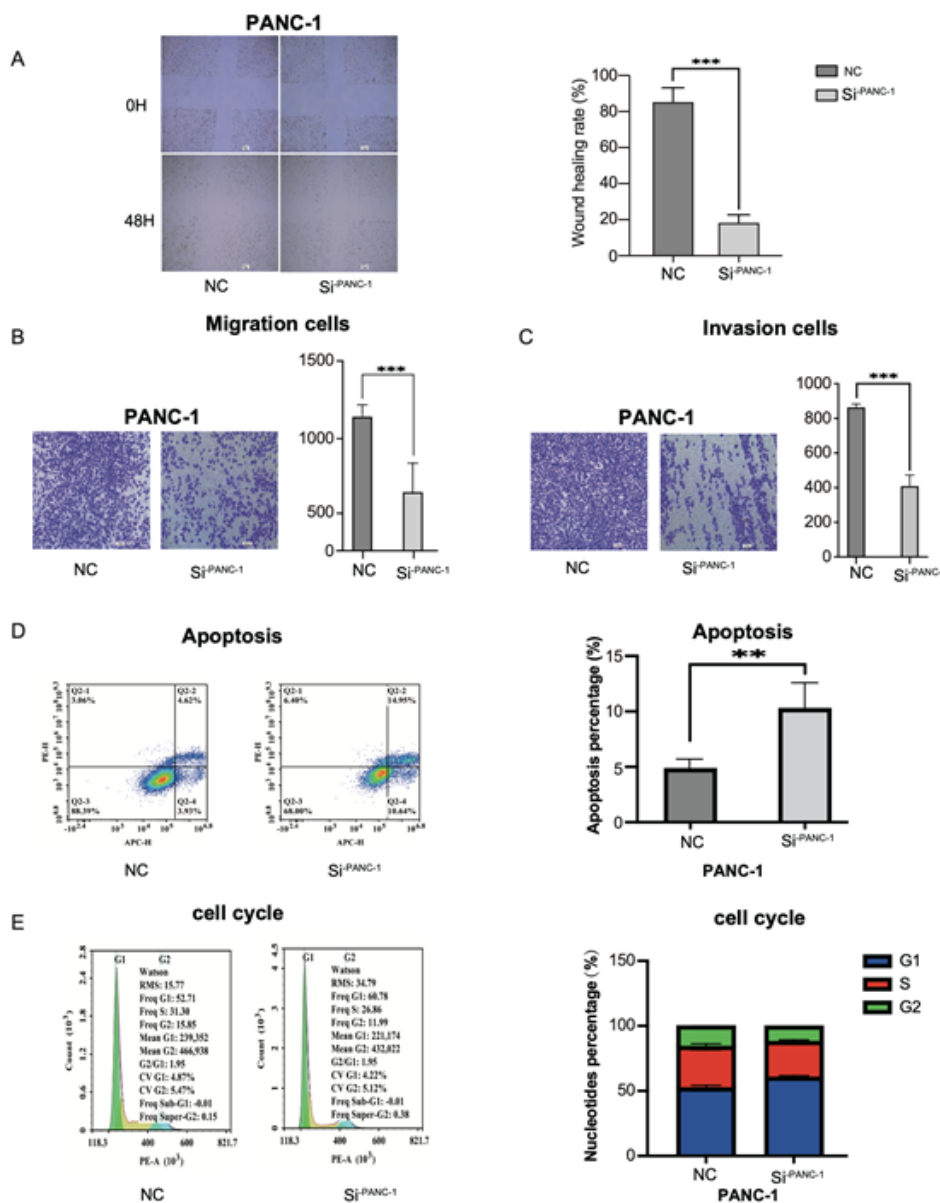


Figure 4: The impact of CTNND1 knockdown on the biological functions of pancreatic cancer cells; A. Scratch assay showed that CTNND1 knockdown reduced the migration ability of pancreatic cancer cells ($\times 100$); (B,C) Transwell assay demonstrated that knockdown of CTNND1 decreased the migration and invasion ability of pancreatic cancer cells ($\times 100$); D. Apoptosis rate increased after CTNND1 knockdown in PANC-1 cells; E. Cell cycle analysis suggested that CTNND1 knockdown resulted in G1 phase arrest of pancreatic cancer cells. (Scale bar = $100\mu\text{m}$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

4.4. Knockdown of CTNND1 Inhibits EMT in Pancreatic Cancer Cells

Epithelial mesenchymal transition (EMT) plays a crucial role in many physiological and pathological processes, including embryonic development, organ formation, wound healing, and cancer metastasis [23]. In cancer, EMT is considered as one of the important mechanisms by which cancer cells acquire invasiveness and metastatic potential [24]. EMT can cause cancer cells to detach from the primary tumor and migrate to other sites via the circulatory or lymphatic system, forming distant metastases. Moreover, EMT can make cancer cells resistant to treatment modalities such as chemotherapy and radiation therapy, thereby increasing

the difficulty of treatment [25-26]. Therefore, EMT has important clinical significance in cancer treatment and has become one of the current research hotspots.

We further investigated whether CTNND1 could regulate the epithelial-mesenchymal transition (EMT) process in pancreatic cancer cells by evaluating the expression levels of EMT-related biomarker proteins. The experimental results showed that knockdown of CTNND1 significantly upregulated the expression of the epithelial marker E-cadherin and downregulated the expression of the mesenchymal marker vimentin (Figure 5A). These findings suggest that knockdown of CTNND1 expression can effectively regulate the EMT phenotype of pancreatic cancer cells.

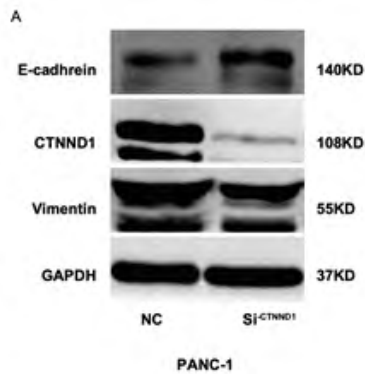


Figure 5: Western blot results of CTNND1 knockdown in PANC-1 cells; A. Upregulation of E-cadherin and downregulation of Vimentin can be observed in the Si^{CTNND1} group.

5. Discussion

In this study, we focused on revealing the function and potential mechanisms of CTNND1 in pancreatic cancer. Although CTNND1 has been extensively studied in various types of cancer [27-29], its role in pancreatic cancer has not been fully elucidated. Our experimental data showed a significant increase in CTNND1 expression in pancreatic cancer cells, suggesting that CTNND1 may play an important role in the development and progression of pancreatic cancer. We effectively suppressed CTNND1 expression in the pancreatic cancer cell line PANC-1 using siRNA technology and observed a significant decrease in the cell proliferation, migration, and invasion ability of pancreatic cancer cells after CTNND1 knockdown. These results suggest that CTNND1 may further affect the development of pancreatic cancer by influencing cell proliferation and migration. In addition, our study also found that CTNND1 knockdown significantly increased the apoptosis rate of PANC-1 cells and inhibited the progress of the cell cycle, further highlighting the critical role of CTNND1 in pancreatic cancer.

It is worth noting that we also found that CTNND1 knockdown significantly upregulated the expression of the epithelial marker E-cadherin and downregulated the expression of the mesenchymal marker vimentin. This finding suggests that CTNND1 may affect the biological characteristics of pancreatic cancer by regulating the epithelial-mesenchymal transition (EMT) process. Regarding the mechanism of CTNND1 in pancreatic cancer, we proposed several possible theories. First, CTNND1 may regulate the progression of the cell cycle by directly affecting the expression or activity of cell cycle-related proteins. As our experimental data showed, CTNND1 knockdown significantly increased the proportion of cells in the G1 phase and decreased the proportion of cells in the S phase, which may be due to CTNND1 affecting some key cell cycle regulatory proteins.

Second, CTNND1 may regulate cell survival by affecting apoptosis-related signaling pathways, such as the Bcl-2/Bax pathway. Experimental results showed that CTNND1 knockdown significantly

increased the apoptosis rate of PANC-1 cells, which may be due to the important role of CTNND1 in the cell apoptosis signaling pathway. However, further research is needed to confirm this. Overall, our study provides new evidence revealing the significant role of CTNND1 in pancreatic cancer and its potential mechanisms. These findings not only deepen our understanding of the mechanisms underlying the occurrence and development of pancreatic cancer but also provide a theoretical basis for the development of new therapeutic strategies targeting CTNND1. However, further research is needed to clarify the precise role of CTNND1 and its detailed molecular mechanisms in pancreatic cancer. Although our study has limitations such as the lack of in vivo experiments to validate the role of CTNND1 and the incomplete exploration of its clinical significance in pancreatic cancer patients, our preliminary research results provide an important theoretical basis for further study. Nevertheless, our study also has some limitations, such as the lack of in vivo experiments to validate the role of CTNND1 and the incomplete exploration of its clinical significance in pancreatic cancer patients. Therefore, future research needs to further explore the role and mechanisms of CTNND1 in pancreatic cancer.

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