Catenin delta 1 mediates epithelial–mesenchymal transition, proliferation, migration, and invasion of pancreatic cancer via the Wnt/β-catenin pathway

Xiaobin Huang
Department of Hepatobiliary Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong
Mengyi Xie
Hepatobiliary Research Institute, Affiliated Hospital of North Sichuan Medical College, Nanchong
Xingyu Liu
Department of Hepatobiliary Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong
Xiaodong Huang
North Sichuan Medical College, Nanchong
Jiayu Li
The 2nd Cadet Company, College of Basic Medicine, Naval Medical University, Shanghai
Zhihui Shu
Department of Hepatobiliary Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong
Xin Zeng
Department of Hepatobiliary Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong
Yang Yang
Department of Hepatobiliary Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong
Jun Yu
Department of Hepatobiliary Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong
Chuan Lan
Department of Hepatobiliary Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong
Dawei Deng
Department of Hepatobiliary Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong
Yong Li
Department of Hepatobiliary Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong
Guangnian Zhang
Department of Hepatobiliary Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong
Jianshui Li (✉ lijianshuinsmc@126.com)
Department of Hepatobiliary Surgery, Affiliated Hospital of North Sichuan Medical College, Nanchong

Research Article

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Abstract

Background: Catenin delta 1 (CTNND1) is upregulated in many tumors and is closely associated with poor prognosis. However, the role of CTNND1 in pancreatic cancer and its underlying mechanisms remain unclear

Methods: The expression of CTNND1 in pancreatic cancer and normal tissues in the TCGA and GTEX databases was preliminarily screened and further verified by immunohistochemistry (IHC) and qPCR. Transwell, wound healing, and cell proliferation assays were used to study the effect of CTNND1 on epithelial-mesenchymal transition (EMT), proliferation, migration, and invasion of pancreatic cancer cells. Western blot experiments verified the signaling pathway mediating the effect of CTNND1 on pancreatic cancer progression. The expression of CTNND1 in the TCGA database, clinical pancreatic cancer samples, and pancreatic cancer cells was significantly upregulated.

Results: We found that the silent CTNND1 in pancreatic cancer cells significantly inhibited the EMT, proliferation, migration, and invasion of pancreatic cancer cells. In addition, the silencing of CTNND1 in pancreatic cancer cells inhibited the Wnt/β-catenin signaling pathway. LiCl (a Wnt/β-catenin-specific activator) treatment partially restored the EMT, proliferation, migration, and invasion abilities of CTNND1-silenced pancreatic cancer cells.

Conclusion: Our research confirmed that CTNND1 can regulate the EMT, proliferation, migration, and invasion of pancreatic cancer through the WNT/β-catenin signaling pathway.

Introduction

Pancreatic cancer is a common digestive tumor with a high malignancy level and a low (10%) 5-year survival rate. Over the next 20–30 years, it is projected to be the second leading cause of cancer-related death in the U.S.(Siegel et al., 2021)As the population ages, and the westernized lifestyle spreads rapidly, the incidence of pancreatic cancer in China is expected to rise in the next few years (Sun et al., 2020) Although great progress has been made in surgical and auxiliary treatments during the past few decades (Ettrich and Seufferlein, 2021; Schizas et al., 2020; Torphy et al., 2020)approximately 80% of patients developed distant metastasis at initial diagnosis of pancreatic cancer due to the insidious onset and a lack of obvious early clinical features (Singhi et al., 2019)Therefore, understanding the molecular mechanisms underlying the early metastasis of pancreatic cancer is essential for improving the survival rate.

Tumor metastasis is an extremely complex process. Epithelial–mesenchymal transition (EMT) is a central mechanism for pancreatic cancer cells to acquire mesenchymal phenotypes and intermediary characteristics, leading to tumor cell invasion and migration(M. Liu et al., 2021; Shen et al., 2021, p. 3; Wong et al., 2022)Therefore, the regulatory factors and mechanisms driving EMT provide new directions to explore the progression, invasion, migration, and metastasis of pancreatic cancer (Luu, 2021)which may lead to new strategies for pancreatic cancer treatment.

Cell–cell adhesion junctions (AJs) initiate and maintain intercellular adhesion, regulate the organization of the underlying actin cytoskeleton, and establish hubs for cell signaling and gene transcription regulation (Garcia et al., 2018)

AJs play important roles in maintaining tissue structure and cell polarity, which limit the cell movement and proliferation. Catenin delta 1 (CTNND1), also known as p120, is a member of the cadherin-series protein complex and directly binds to the cytoplasmic tail of E-cadherin through a pre-conserved juxtamembrane domain(Ishiyama et al., 2010)Also, γ-catenin binds to the catenin-binding domain of E-cadherin. Therefore, E-cadherin, CTNND1, α-catenin, and γ-catenin are the major proteins involved in cell–cell AJ formation and maintenance.

CTNND1 is overexpressed in various cancers. For example, excessive *CTNND1* expression promotes the proliferation, migration, and invasion of liver cancer cells (Tang et al., 2016) *CTNND1* overexpression is also associated with a more malignant phenotype of pancreatic cancer (Mayerle et al., 2003) Furthermore, *CTNND1* regulates cancer progression and development (Acosta et al., 2021; Liu et al., 2020; W.-W. Liu et al., 2021)These results indicate that *CTNND1* promotes certain malignant tumors. However, the role of *CTNND1* in pancreatic cancer remains unclear. Therefore, it is important to evaluate the prognostic potential of *CTNND1* in patients with pancreatic cancer.

In the present study, we aimed to elucidate the expression pattern of *CTNND1* in patients with pancreatic cancer and its possible role in the prognosis of pancreatic cancer. We obtained pancreatic cancer data from TCGA database for bioinformatics analyses and used pancreatic cancer cell lines for *in vitro* studies. We expect that this study will provide useful insights for the development of drugs to treat pancreatic cancer.

Materials And Methods

2.1 Bioinformatic analyses

The pancreatic cancer data set TCGA-PAAD (n=183 cases) was obtained from the TCGA-GDC official website (https://portal.gdc.cancer.gov/) in Count and TPM formats. The pancreatic normal tissue sample dataset (n=167 cases) was obtained from the GTEx database (http://commonfund.nih.gov/GTEx/) in Count and TPM formats. In addition, R package GDC was used to obtain the clinical data of TCGA-PAAD matched patients (n=183), including age, survival status, follow-up time, and installment. Patients with no survival information and incomplete data installment information were excluded. Data from 182 patients was used for further analysis.

RNA-seq data from TCGA and GTEx were downloaded in TPM format from UCSC Xena website (https://xenabrowser.net/datapages/). Pan-cancers were uniformly processed by a coil process for analysis. Simultaneously, the corresponding normal tissue data in the GTEX of PAAD (pancreatic cancer)

and TCGA were extracted to analyze the difference between the disease and normal tissue samples.RNA-seq data from the TCGA-PAAD project were obtained in Level III htseq-count format. According to the median expression level of *CTNND1*, the samples were divided into a high-expression group and a low-expression group. The genes in the different groups were analyzed using the R-package deseq2. We set $|\log_2 FC|>1$, and the threshold for differential genes was adjusted to $P<0.05$. The results were displayed by a volcano map created by the R package ggplot2.

The Kaplan–Meier survival curve was used to compare the survival rate of the *CTNND1* high- and low-expression groups. The median survival time represents the survival time corresponding to 50% survival rate. The log-rank test was used to test the significance of survival between groups, and the R survival package was used for statistical analysis. Moreover, time-dependent ROC curves were created using the R package "timeROC" to evaluate the predictive accuracy of the prognostic features of *CTNND1*.

To determine the independent prognostic factors, univariate combined with multivariate Cox regression was used to analyze the relationship between *CTNND1* and OS (significance cutoff: $p<0.05$). The associations between the expression of *CTNND1* and clinical pathological characteristics (age, sex, stage, etc.) were analyzed with Wilcoxon test and logistic regression. GO annotation analysis and KEGG pathway enrichment analysis of differential genes were performed using R software package clusterprofiler. A cutoff value of FDR<0.05 was considered statistically significant.

We used the string database to construct the protein–protein interaction (PPI) network of the *CTNND1* gene and set the parameters as a correlation coefficient of 0.5. In addition, single-sample gene set enrichment analysis (ssGSEA) was used to analyze the extent of immune cell infiltration. The GSVA package in R software was used for quantitative tumor infiltration of immune cells. Spearman's tests were used to measure the interrelationship between *CTNND1* and the 24 immune cells involved in tumor immunity.

2.2 Clinical sample collection

We collected adjacent pancreatic tissues from 10 patients diagnosed with pancreatic cancer and treated with radical resection for the first time at the Affiliated Hospital of North Sichuan Medical College from October 1, 2020 to October 1, 2021. The inclusion criteria were as follows: 1) The patient never received any antineoplastic therapy before; 2) The patient had no other serious underlying disease; 3) The specimen was pathologically proven to be pancreatic cancer. Each tissue sample was divided into two groups. One group was placed in a -80 ℃ freezer for qRT-PCR analysis, and the other group was fixed with formaldehyde for immunohistochemical analysis (IHC). Informed consent was obtained for sample collection, which was approved by the Ethics Committee of the Affiliated Hospital of the North Sichuan Medical College (2022 ER032-1).

2.3 Immunohistochemical staining

Ten pairs of pancreatic cancer tissue samples and the matched paracancerous tissues were obtained from patients at the Affiliated Hospital of the North Sichuan Medical College. CTNND1 antibody (1:100 dilution, Abcam, USA) was used to stain the CTNND1 positive cells. The percentage and staining intensity of the positive tumor cells were evaluated. The scores were assigned to the percentage of positive cells as follows: 0<10%, 1=10-20%, 2=21-50%, and 3>50%. Staining intensity was rated as follows: 0=no staining, 1=weak staining, 2=moderate staining, and 3=strong staining. The staining index (SI) was calculated as follows: SI = (intensity score) × (positive staining score). SI<3 indicates low expression level, and SI≥4 indicates high expression level.

2.4 Cell culture and reagents

Two pancreatic cancer cell lines (PANC-1 and SW1990) and normal pancreatic epithelial cells (HPNE) were purchased from the bacterial storage committee of the Chinese Academy of Sciences (Shanghai, China). SW1990 and HPNE cell lines were cultured in RPMI-1640 (Invitrogen) with 10% FBS (fetal bovine serum, Invitrogen). PANC-1 cell line was cultured in DMEM medium (Invitrogen) with 10% FBS. The cell culture was performed according to the manufacturer's protocols. All cell lines were grown in a 5% carbon dioxide cell incubator at 37 °C and revived every 3 to 4 months. LiCl (L9650, β-catenin activator) was purchased from Sigma-Aldrich (USA).

2.5 SiRNA and lentiviral vector transfection

When the cells grew in six-well plates reached to an appropriate density, they were transfected with CTNND1-siRNA using Lipofectamine 3000 (Invitrogen, USA) and CTNND1-shRNA (Shanghai Genechem Co., Ltd.) according to the manufacturer's instructions. These cells with an unstable or stable knockdown of CTNND1 were used in the subsequent experiments. The CTNND1 siRNA sequence was synthesized by Integrated Biotech Solutions Company (Shanghai, China), as shown in Table 1.

Table 1 The CTNND1 siRNA sequence

□□	□□□□	□□□□
Si-CTNND1-1	5′- GCAUGAGCGAGGAAGUUUAGC-3′	5′- UAAACUUCCUCGCUCAUGCUG -3′
Si-CTNND1-2	5′- CGGUCAAGAAAGUAGUGAAGA -3′	5′- UAGAAGGUGACUGUGAUCCUG -3′
Si-CTNND1-3	5′- CGGUCAAGAAAGUAGUGAAGA -3′	5′- UUCACUACUUUCUUGACCGUG -3′
Si-CTNND1-NC	5′- UUCUCCGAACGUGUCACGUTT-3′	5′- ACGUGACACGUUCGGAGAATT-3′

2.6 qRT-PCR

Total RNA was extracted with TRIzol reagent and reverse transcribed with Hiscript III RT Supermix for qPCR (+gDNA wiper) (r323-01, Novozan, China) according to the manufacturer's instructions. qRT-PCR was performed under light cycling using the Chamq Universal SYBR qPCR Master Mix (Q711, Novozan, China) in a Real-Time PCR System (Roche, USA). Expression level was calculated using the 2−ΔΔCt method. The primer sequences used are listed in Table 2.

Table 2 Primer sequence

□□	F	R
CTNND1	5'-ACCACGGTCAAGAAAGTAG-3'	5'-GAAATCACGACCCAAAGT-3'
GAPDH	5'- GCACCGTCAAGGCTGAGAAC -3'	5'- TGGTGAAGACGCCAGTGGA -3'

2.7 Western blotting

Total protein was extracted from human pancreatic cancer cells using a RIPA lysis buffer (Solarbio, Beijing, China). Equal amounts of denatured protein samples were separated by 10% SDS-PAGE (Solarbio, Beijing, China) prior to transfer to PVDF membranes (Millipore, Billerica, USA). The membranes were then blocked in 5% skim milk (Solarbio, Beijing, China) for 2 h at room temperature, followed by an overnight incubation at 4 ℃ in primary antibodies. Subsequently, the membranes were incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody (Wuhan Finn Biotechnology Co., Ltd.) for 1 h at 37 ℃. After 1 min of incubation with the enhanced chemiluminescence solution, protein bands were imaged using a WB exposure meter (Viber, FX4, France).

The following primary antibodies were obtained from Fine Test (Wuhan Finn Biotechnology Co., Ltd.): β-Catenin, c-Myc, E-cadherin, vimentin, GAPDH-specific primary antibody (rabbit anti-human).

The CTNND1-specific primary antibody was purchased from Abcam (USA). The secondary antibody (goat anti-rabbit IgG-HRP) was purchased from Fine Test (Wuhan Finn Biotechnology Co., Ltd.).

2.8 Cell counting kit (CCK-8) assay

After cell transfection, equivalent amounts of pancreatic cancer cells were seeded into each well of 96-well plates and cultured for 3 days. Subsequently, 10 μL of CCK-8 solution (A311-02, Huaxin Biotechnology Co., Ltd., Nanjing) was added to each well and incubated at 37 ℃ for 1 h. The OD values were measured at 450 nm.

2.9 Wound healing assay

After cell transfection, the pancreatic cancer cells were seeded in six-well plates. When the cells reached approximately 90% confluency, the cells were scratched with a 100 μL pipette tip. Cell debris was washed away with PBS, followed by 48 h of incubation in 2% low serum medium. The cells were imaged under a microscope.

2.10 Invasion and migration assay

Before cell seeding, the upper chamber membrane of Transwell was pre-coated or not coated with an equal amount of Matrigel (Corning, NY, USA). The group coated with Matrigel was allowed to stand at 37 °C for 2 h. Serum-free DMEM or RPMI 1640 cell suspension was added to the upper chamber containing

approximately 2 × 100 µL of 104 cells, and 600 µL of DMEM or 1640 medium containing 20% FBS was added to the lower chamber. The incubation was then continued for 24 h at 37 °C and in 5% CO2. After 24 h, the cells were fixed with 4% paraformaldehyde for 30 min. Next, impermeable cells and Matrigel were wiped off with a wet cotton swab, followed by staining the fixed cells with 1% crystal violet for 20 min. After washing with PBS and drying, cells were counted and photographed under a microscope.

2.11 Flow cytometry analysis

We used an apoptosis detection kit (KGA1030-100, KGI) and a cell cycle detection kit (KGA511, KGI) to evaluate the effects of different interventions on the apoptosis rate and cell cycle of pancreatic cancer cells. To detect apoptotic cells, 5 × 105 cells were centrifuged at 500 ×g for 5 min and resuspended in 500 µL of binding buffer. Cells were then incubated with 5 µL Annexin V-APC for 10 min, followed by an incubation in 5 µL propidium iodide (PI) for 5 min at 37 °C in the dark. To detect the cell cycle, 5×105 cells were centrifuged at 500 ×g for 5 min. The cells were then mixed with 500 µL pre-chilled 70% ethanol, fixed at 4 °C for 2 h, and centrifuged at 500 ×g for 5 min. After washing the cells twice with cold PBS, 500 µL PI/RNase A staining working solution was added, followed by incubating in dark for 30 min at room temperature. The cells were analyzed using a flow cytometer (ACEA Biosciences, USA).

2.12 Statistical analysis

All statistical analyses were performed using R (version 4.1.1) or GraphPad Prism (version 9.3). The expression of CTNND1 in unpaired samples was analyzed using the Wilcoxon rank-sum test. Chi-square test or Fisher's exact test was used to assess the relationship between clinicopathological features and CTNND1 expression. Cox regression analysis and the Kaplan–Meier method were used to assess prognostic factors. The median expression of CTNND1 was considered a critical value. All results were considered to be statistically significant at $P<0.05$.

Results

3.1 CTNND1 is highly expressed in pancreatic cancer and associated with poor prognosis

The mRNA levels of CTNND1 in different types of tumor tissues in TCGA combined with the GTEX database were analyzed. Compared with the normal tissues, a variety of tumors had upregulated mRNA levels of CTNND1, including pancreatic cancer ($P<0.001$, Figure 1a, Online Resource Online Resource figure s1a). We further mapped a box diagram of mRNA expression between pancreatic cancer and normal pancreatic tissue and found that the expression of CTNND1 in pancreatic cancer was significantly higher than that in normal pancreatic tissues ($P<0.001$, Figure 1b). In addition, we identified 2999 differentially expressed mRNA (327 upregulated genes and 2672 downregulated genes) by analyzing the PAAD data from TCGA (Figure 1c). We showed the distribution of genetic differences in

pancreatic cancer chromosomes (Online Resource figure s1b). We also showed the relationship between the gene copy number and CTNND1 mRNA expression in Online Resource figure s1c.

We analyzed the relationship between CTNND1 expression and survival in 33 tumors and found that the expression of CTNND1 in varied tumors was associated with poor prognosis such as BRCA (Breast invasive carcinoma), PAAD (Pancreatic adenocarcinoma), and LGG (Lower grade glioma) (Online Resource figure s2a). Survival analysis showed that the pancreatic cancer patients with high CTNND1 expression levels had a lower survival rate than patients with low CTNND1 expression levels (P=0.005, Figure 1d).

The diagnostic efficacy of CTNND1 was evaluated using time-dependent ROC analysis. The AUC values at 1, 3, and 5 years in the TCGA-lihc cohort were 0.602, 0.719, and 0.788, respectively (Figure 1e). In addition, we explored CTNND1 with protein-protein interaction, transcription factor-target gene, miRNA-target gene, and chemical-gene interaction (Online Resource figure s1e).

We further detected the CTNND1 levels in pancreatic cancer tissue samples using immunohistochemistry (Figure 1f). We observed that the number of CTNND1-positive cells in pancreatic cancer was significantly higher than that in normal pancreatic tissues (Figure 1g). We then measured the mRNA levels of CTNND1 in 10 pancreatic cancer tissue samples and their matched normal adjacent tissues using qRT-PCR. Compared with normal tissues, pancreatic cancer specimens showed overexpression of CTNND1 (P<0.001, Figure 1h). Similarly, we further verified the CTNND1 levels in pancreatic cancer cell lines (PANC-1 and SW1990) and normal pancreatic epithelial cells (HPNE) by qRT-PCR and western blotting. Results showed that both mRNA and protein levels were significantly increased (P<0.05, Figures 1i-1k), suggesting that CTNND1 is upregulated in pancreatic cancer and strongly associated with poor prognosis.

3.2 High expression level of CTNND1 affects the prognosis of patients with pancreatic cancer in different clinicopathological states

To better understand the correlation and potential mechanisms of CTNND1 expression in pancreatic cancer, we studied the relationship between CTNND1 expression and clinical characteristics of patients with pancreatic cancer using Cox regression analysis. Univariate Cox analysis showed that high expression level of CTNND1 was significantly correlated with T stage, N stage, pathological and histological grade, initial treatment results, tumor anatomical location, and overall survival time of patients with pancreatic cancer (all P<0.05, Figure 2a). We analyzed the association between CTNND1 expression and tumor staging and found that CTNND1 expression was positively related to tumor staging (Online Resource figure s1d).

To further explore the factors related to survival, multivariate Cox regression analysis was performed using TNM stage, pathological and histological grade, and initial treatment results. Results showed that the high expression of CTNND1 was still an independent prognostic factor for poor overall survival (Figure 2b). We evaluated CTNND1 expression level differences in six clinical parameters (DSS event, PFI

event, OS event, T stage, histologic grade, and primary therapy outcome) in patients with pancreatic cancer. The expression of CTNND1 was higher in patients with disease progression and death from pancreatic cancer. In addition, the mRNA expression level of CTNND1 was higher in patients with a late T stage, a pathological stage, and a poor initial treatment effect (Figures 2c-2h). Most of the 33 tumors had higher TPM (transcripts per million) of CTNND1 than normal tissues (Online Resource figure s3a).Therefore, a high CTNND1 expression level may lead to a poor prognosis by promoting the malignant progression of pancreatic cancer.

3.3 GO, KEGG, PPI, drugs, CNV and methylation Analysis in pancreatic cancer

To better understand the function of CTNND1 in PAAD, the cluster Profiler software package was used to analyze the functional enrichment of 2999 differentially expressed genes between the high and low CTNND1 groups using GO and KEGG enrichment analyses. GO analysis showed that the functions of CTNND1 co-expressed genes were mainly concentrated in cell adhesion molecule binding, cadherin binding, actin binding, cell-matrix binding, cell matrix adhesion binding, focus adhesion, cell junction tissue, protein localization to the plasma membrane, cell–cell junction, and other biological functions (Figure 3a). KEGG analysis mainly focused on the endocytosis, regulation of actin cytoskeleton, focal adhesions, adhesive junctions, Wnt signaling pathway, tight junctions, PD-L1 expression and PD-1 checkpoint, apoptosis, T cell receptor signaling pathway, leukocyte trans-endothelial migration, cell cycle, and sphingolipid signaling pathway (Figure 3b). GSEA showed that CTNND1 co-expressed genes in pancreatic cancer were mainly related to drug metabolism cytochrome p450, cytochrome p450 metabolism of xenobiotics, retinol metabolism, steroid hormone biosynthesis, and NABA_ECM regulators (Online Resource figure s1f).

We generated a map of CTNND1 methylation sites (Online Resource figure s3b) and a correlation analysis of methylation with CTNND1 expression ((Online Resource figure s3c). To find the potential relationship between CTNND1 and other genes in pancreatic cancer, PPI network analysis was performed using STRING database (Figure 3c). CTNND1 interacts with MLLT4, SRC, RHOA, CDH2, CDH5, CDH17,

CTNNB1, ZBTB33, CDH1, and PLEKHA7. The survival analysis of CTNND1 interacting genes in PAAD is shown in Online Resource figure s3d.

3.4 Correlation between CTNND1 expression and immune cell infiltration in pancreatic cancer

Spearman’s correlation was used to analyze the correlation between the expression level of CTNND1 (TPM) in the pancreatic cancer tumor microenvironment and the level of immune cell infiltration quantified by ssGSEA. We found that the T cell enrichment score of patients with high CTNND1 expression levels was lower than that of the low-expression group ($P<0.05$, Figure 3d). In addition, CTNND1 expression was positively correlated with the abundance of Th2 cells, TCM, and T helper cells and negatively correlated with the abundance of dendritic cells, NK cells, B cells cytotoxic cells, and CD8+ T cells in plasma (Figure 3e and 3f). These results suggest that CTNND1 may play a specific role in immune cell infiltration in pancreatic cancer.

3.5 CTNND1 enhances the biological function of pancreatic cancer cells in vitro

To evaluate the effect of CTNND1 on pancreatic cancer cell proliferation, migration, and invasion, we inhibited CTNND1 expression in PANC-1 and SW1990 cells using siRNAs. The knockdown efficiency of CTNND1 was confirmed by qRT-PCR and western blotting (Figures 4a and 4b). We selected the S1 (Si-CTNND1-S1) and S2 (Si-CTNND1-S2) sequences for subsequent experiments as they had better knockdown efficiency than S3 (Si-CTNND1-S3). We found that CTNND1 knockdown significantly reduced the growth rate of PANC-1 and SW1990 cells using CCK-8 analysis (Figures 4c). In addition, we found that CTNND1 knockdown significantly inhibited the wound closure of PANC-1 and SW1990 cells after 48 h compared with the control group (Figures 4d). Furthermore, transwell analysis showed that CTNND1 knockdown significantly reduced the invasive and migratory abilities of PANC-1 and SW1990 cells compared with the control group (Figures 4 E). Flow cytometry analysis showed that the apoptotic rate of PANC-1 and SW1990 cells in the CTNND1-knockdown group was significantly higher than that in the control group (Figures 4f). Cell cycle analysis of PANC-1 and SW1990 cells showed that CTNND1 knockdown increased the proportion of cells in the G1 phase and decreased the proportion of cells in the S phase, indicating that CTNND1 knockdown inhibited cells from G1 to S phase (Figures 4g). Taken together, these results

suggest that CTNND1 plays a crucial role in pancreatic cancer cell proliferation, migration, and invasion.

3.6 CTNND1 induces EMT in pancreatic cancer cells

Tumor cell EMT promotes malignant progression and metastasis of tumor cells (Luu, 2021)，therefore, we investigated whether CTNND1 regulates EMT in pancreatic cancer cells by examining the protein levels of EMT-related biomarkers. CTNND1 knockdown significantly upregulated the expression of the epithelioid marker E-cadherin and downregulated the expression of the mesenchymal marker vimentin (Figure 5a). These results suggest that CTNND1 drives the EMT phenotype in pancreatic cancer cells.

3.7 CTNND1 regulates the Wnt/β-catenin signaling pathway

The Wnt/β-catenin signaling pathway plays a crucial role in EMT of cancer cells (18). KEGG enrichment analysis of *CTNND1* co-expressed genes in pancreatic cancer suggested that *CTNND1* may be involved in the Wnt signaling pathway (Figure 3b). Therefore, we investigated whether *CTNND1* mediates the canonical Wnt/β-catenin signaling pathway. As shown in Figure 5a, the protein levels of β-catenin and c-Myc were significantly reduced in *CTNND1*-knockdown cells compared with those in control cells, indicating that *CTNND1* regulates the Wnt/β-catenin pathway in pancreatic cancer cells.

3.8 CTNND1 induces the EMT, proliferation, migration, and invasion of pancreatic cancer cells via the Wnt/β-catenin signaling pathway

To confirm that *CTNND1* plays a role in EMT, proliferation, migration, and invasion of pancreatic cancer through the Wnt/β-catenin signaling pathway, we treated *CTNND1*-knockdown pancreatic cancer cells with LiCl, a specific activator of the Wnt/β-catenin signaling pathway. We assessed whether it could

reverse the effects of *CTNND1* knockdown on pancreatic cancer cells. We transfected siRNA with the S1 sequence into PANC-1 and SW1990 cells and treated them with 20 mM LiCl for 24 h. We found that β-catenin and c-Myc levels significantly increased (Figure 5b). The levels of EMT-related proteins E-cadherin and vimentin showed opposite effects after treatment of *CTNND1*-knockdown cells with LiCl compared to knockdown of *CTNND1* alone (Figure 5b). These results suggest that LiCl partially alleviates the inhibitory effect of *CTNND1* knockdown on EMT progression. Moreover, LiCl significantly increased the proliferation, migration, and invasion of *CTNND1*-knockdown cells (Figures 5c – 5e). Together, *CTNND1* induces EMT, proliferation, migration, and invasion of pancreatic cancer cells through the Wnt/β-catenin signaling pathway.

Discussion

This study first elucidated the clinical significance of CTNND1 in pancreatic cancer and its role in regulating EMT, proliferation, and migration of pancreatic cancer cells. Specifically, we confirmed CTNND1 overexpression in pancreatic cancer. In addition, in vitro silencing of CTNND1 inhibited EMT, proliferation, migration, and invasion of pancreatic cancer cells, as well as Wnt/β-catenin signaling. These results suggest that CTNND1 regulates EMT, proliferation, migration, and invasion in pancreatic cancer cells by regulating the Wnt/β-catenin signaling pathway.

CTNND1 and β-catenin jointly bind to E-cadherin (Ishiyama et al., 2010)The different degrees of loss of E-cadherin and CTNND1 leads to different roles of CTNND1 in tumors (Thoreson and Reynolds, 2002) The initial loss of CTNND1 leads to the instability of the E-cadherin complex, resulting in the reduction of E-cadherin expression level. However, the first loss of E-cadherin leads to an increase in CTNND1 in the cytoplasm(Soto et al., 2008; Thoreson and Reynolds, 2002)CTNND1 binds the transcription repressor kaiso in the cytoplasm (Daniel and Reynolds, 1999)resulting in the inhibited translocation of kaiso to the nucleus and promoted β-catenin expression (Ogden et al., 2008; van Roy and McCrea, 2005; Xue et al., 2017)Therefore, CTNND1 accumulation leads to an increased level of β-catenin in the cytoplasm (Jiang et al., 2012; Liu et al., 2014; Spring et al., 2005)and activated Wnt/β-catenin signaling pathway, which plays a key role in the apoptosis, proliferation, and metastasis of cancer cells (Li et al., 2021)EMT can regulate a variety of cancers, including pancreatic cancer (Liu et al., 2019)EMT is a key step in metastasis in patients with pancreatic cancer and associated with poor prognosis (Nachiyappan et al., 2022, p. 1)Our study showed that CTNND1 knockdown significantly inhibited the Wnt/β-catenin pathway, as well as the proliferation, migration, invasion, and EMT phenotype of pancreatic cancer cells. In addition, LiCl, a specific activator of the Wnt/β-catenin signaling pathway, partially reversed the changes in the biological function and EMT phenotype of CTNND1-knockdown pancreatic cancer cells. Therefore, our results show that CTNND1 regulates EMT, proliferation, migration, and invasion of pancreatic cancer cells via the Wnt/β-catenin signaling pathway. Although further research is needed, our study provides comprehensive evidence to support the role of CTNND1 in EMT, proliferation, migration, and invasion. CTNND1 is a valuable therapeutic target in pancreatic cancer. Our study has several limitations. First, the cohort of patients with pancreatic cancer considered in this study from our hospital was relatively small. Second, we did not conduct animal experiments. Furthermore, we did not

overexpress CTNND1 in different pancreatic cancer cell lines. These issues need to be solved in future surveys.

In conclusion, we investigated the role of CTNND1 in pancreatic cancer. CTNND1 mRNA and protein levels were significantly higher in pancreatic cancer tissues than that in normal pancreatic tissues. High CTNND1 expression level in patients with pancreatic cancer was associated with a poor prognosis. CTNND1 mRNA levels negatively correlated with E-cadherin mRNA levels and positively correlated with vimentin mRNA levels. This finding suggests that CTNND1 regulates EMT. The expression level of CTNND1 in pancreatic cancer cells (PANC-1 and SW1990) was significantly higher than that in normal pancreatic epithelial cells (HPDE). Moreover, CTNND1 knockdown in PANC-1 and SW1990 cells reduced their proliferation, migration, and invasion abilities compared to control cells. Reduced levels of proteins (β-catenin and c-Myc) involved in the Wnt/β-catenin signaling pathway were also observed in CTNND1-knockdown cells. Furthermore, proliferation, migration, and invasion of pancreatic cancer cells were restored to a certain extent after the activation of Wnt/β-catenin signaling pathway in the CTNND1-knockdown pancreatic cancer cells. Therefore, CTNND1 appears to regulate EMT, migration, and invasion through the Wnt/β-catenin signaling pathway. CTNND1 may be a potential prognostic biomarker and therapeutic target in patients with pancreatic cancer.

Declarations

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without

undue reservation.

Ethics Statement

This study was reviewed and approved by the ethics committee of the Affiliated Hospital of North Sichuan Medical College (2022ER032-1).

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Huang Xiaobin and Xie Mengyi wrote manuscripts and conducted experimental work and data collection and interpretation; Liu Xingyu, Huang Xiaodong and Li Jiayu performed experiments, collected clinical specimen, and analyzed data; Shu Zhihui, Yu Jun, Zeng Xin,and Yang Yang performed experiments, wrote the manuscript, and prepared figures; Lan Chuan, Deng Dawei, Zhang Guangnian and Li Yong discussed

the manuscript and proofread the manuscript; Li Jianshui designed experiments and supervised the research. All authors agree to be accountable for the content of the work.

Abbreviations

CTNND1: Catenin delta 1

PBS: Phosphate Buffered Saline

PPI: Protein–Protein Interaction

ROC: Receiver Operating Characteristic

OD: Optical Density

ssGSEA: Single Sample Gene Set Enrichment Analysis

IHC: Immunohistochemical Analysis

DSS: Disease Special Survival

PFI: Progression Free Interval

OS: Overall Survival

BRCA: Breast invasive carcinoma

PAAD: Pancreatic adenocarcinoma

LGG: Lower grade glioma

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References

Acosta, A.M., Barletta, J., Sonpavde, G., Schnitt, S., Hirsch, M.S., 2021. p-120 Catenin is a Useful Diagnostic Biomarker for Distinguishing Plasmacytoid and Sarcomatoid Variants From Conventional Urothelial Carcinoma. Arch Pathol Lab Med 145, 1000–1008. https://doi.org/10.5858/arpa.2020-0262-OA

Daniel, J.M., Reynolds, A.B., 1999. The catenin p120(ctn) interacts with Kaiso, a novel BTB/POZ domain zinc finger transcription factor. Mol Cell Biol 19, 3614–3623. https://doi.org/10.1128/MCB.19.5.3614

Ettrich, T.J., Seufferlein, T., 2021. Systemic Therapy for Metastatic Pancreatic Cancer. Curr Treat Options Oncol 22, 106. https://doi.org/10.1007/s11864-021-00895-4

Garcia, M.A., Nelson, W.J., Chavez, N., 2018. Cell-Cell Junctions Organize Structural and Signaling Networks. Cold Spring Harb Perspect Biol 10, a029181. https://doi.org/10.1101/cshperspect.a029181

Ishiyama, N., Lee, S.-H., Liu, S., Li, G.-Y., Smith, M.J., Reichardt, L.F., Ikura, M., 2010. Dynamic and static interactions between p120 catenin and E-cadherin regulate the stability of cell-cell adhesion. Cell 141, 117–128. https://doi.org/10.1016/j.cell.2010.01.017

Jiang, G., Wang, Y., Dai, S., Liu, Y., Stoecker, M., Wang, Endi, Wang, Enhua, 2012. P120-catenin isoforms 1 and 3 regulate proliferation and cell cycle of lung cancer cells via β-catenin and Kaiso respectively. PLoS One 7, e30303. https://doi.org/10.1371/journal.pone.0030303

Li, T.-H., Zhao, B.-B., Qin, C., Wang, Y.-Y., Li, Z.-R., Cao, H.-T., Yang, X.-Y., Zhou, X.-T., Wang, W.-B., 2021. IFIT1 modulates the proliferation, migration and invasion of pancreatic cancer cells via Wnt/β-catenin signaling. Cell Oncol. 44, 1425–1437. https://doi.org/10.1007/s13402-021-00651-8

Liu, D., Zhang, H., Cui, M., Chen, C., Feng, Y., 2020. Hsa-miR-425-5p promotes tumor growth and metastasis by activating the CTNND1-mediated β-catenin pathway and EMT in colorectal cancer. Cell Cycle 19, 1917–1927. https://doi.org/10.1080/15384101.2020.1783058

Liu, F., Xia, Z., Zhang, M., Ding, J., Feng, Y., Wu, J., Dong, Y., Gao, W., Han, Z., Liu, Y., Yao, Y., Li, D., 2019. SMARCAD1 Promotes Pancreatic Cancer Cell Growth and Metastasis through Wnt/β-catenin-Mediated EMT. Int. J. Biol. Sci. 15, 636–646. https://doi.org/10.7150/ijbs.29562

Liu, M., Zhang, Y., Yang, J., Zhan, H., Zhou, Z., Jiang, Y., Shi, X., Fan, X., Zhang, J., Luo, W., Fung, K.-M.A., Xu, C., Bronze, M.S., Houchen, C.W., Li, M., 2021. Zinc-Dependent Regulation of ZEB1 and YAP1 Coactivation Promotes Epithelial-Mesenchymal Transition Plasticity and Metastasis in Pancreatic Cancer. Gastroenterology 160, 1771-1783.e1. https://doi.org/10.1053/j.gastro.2020.12.077

Liu, W.-W., Hu, J., Wang, R., Han, Q., Liu, Y., Wang, S., 2021. Cytoplasmic P120ctn Promotes Gefitinib Resistance in Lung Cancer Cells by Activating PAK1 and ERK Pathway. Appl Immunohistochem Mol Morphol 29, 750–758. https://doi.org/10.1097/PAI.0000000000000965

Liu, Y., Dong, Q.-Z., Wang, S., Xu, H.-T., Miao, Y., Wang, L., Wang, E.-H., 2014. Kaiso interacts with p120-catenin to regulate β-catenin expression at the transcriptional level. PLoS One 9, e87537. https://doi.org/10.1371/journal.pone.0087537

Luu, T., 2021. Epithelial-Mesenchymal Transition and Its Regulation Mechanisms in Pancreatic Cancer. Front Oncol 11, 646399. https://doi.org/10.3389/fonc.2021.646399

Mayerle, J., Friess, H., Büchler, M.W., Schnekenburger, J., Weiss, F.U., Zimmer, K.-P., Domschke, W., Lerch, M.M., 2003. Up-regulation, nuclear import, and tumor growth stimulation of the adhesion protein p120 in

pancreatic cancer. Gastroenterology 124, 949–960. https://doi.org/10.1053/gast.2003.50142

Nachiyappan, A., Gupta, N., Taneja, R., 2022. EHMT1/EHMT2 in EMT, cancer stemness and drug resistance: emerging evidence and mechanisms. FEBS J 289, 1329–1351. https://doi.org/10.1111/febs.16334

Ogden, S.R., Wroblewski, L.E., Weydig, C., Romero-Gallo, J., O'Brien, D.P., Israel, D.A., Krishna, U.S., Fingleton, B., Reynolds, A.B., Wessler, S., Peek, R.M.J., 2008. p120 and Kaiso regulate Helicobacter pylori-induced expression of matrix metalloproteinase-7. Mol Biol Cell 19, 4110–4121. https://doi.org/10.1091/mbc.e08-03-0283

Schizas, D., Charalampakis, N., Kole, C., Economopoulou, P., Koustas, E., Gkotsis, E., Ziogas, D., Psyrri, A., Karamouzis, M.V., 2020. Immunotherapy for pancreatic cancer: A 2020 update. Cancer Treat Rev 86, 102016. https://doi.org/10.1016/j.ctrv.2020.102016

Shen, P., Yang, T., Chen, Q., Yuan, H., Wu, P., Cai, B., Meng, L., Huang, X., Liu, J., Zhang, Y., Hu, W., Miao, Y., Lu, Z., Jiang, K., 2021. CircNEIL3 regulatory loop promotes pancreatic ductal adenocarcinoma progression via miRNA sponging and A-to-I RNA-editing. Mol Cancer 20, 51. https://doi.org/10.1186/s12943-021-01333-7

Siegel, R.L., Miller, K.D., Fuchs, H.E., Jemal, A., 2021. Cancer Statistics, 2021. CA: A Cancer Journal for Clinicians 71, 7–33. https://doi.org/10.3322/caac.21654

Singhi, A.D., Koay, E.J., Chari, S.T., Maitra, A., 2019. Early Detection of Pancreatic Cancer: Opportunities and Challenges. Gastroenterology 156, 2024–2040. https://doi.org/10.1053/j.gastro.2019.01.259

Soto, E., Yanagisawa, M., Marlow, L.A., Copland, J.A., Perez, E.A., Anastasiadis, P.Z., 2008. p120 catenin induces opposing effects on tumor cell growth depending on E-cadherin expression. J Cell Biol 183, 737–749. https://doi.org/10.1083/jcb.200805113

Spring, C.M., Kelly, K.F., O'Kelly, I., Graham, M., Crawford, H.C., Daniel, J.M., 2005. The catenin p120ctn inhibits Kaiso-mediated transcriptional repression of the beta-catenin/TCF target gene matrilysin. Exp Cell Res 305, 253–265. https://doi.org/10.1016/j.yexcr.2005.01.007

Sun, D., Cao, M., Li, H., He, S., Chen, W., National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100021, China, 2020. Cancer burden and trends in China: A review and comparison with Japan and South Korea. Chinese Journal of Cancer Research 32, 129–139. https://doi.org/10.21147/j.issn.1000-9604.2020.02.01

Tang, B., Tang, F., Wang, Z., Qi, G., Liang, X., Li, B., Yuan, S., Liu, J., Yu, S., He, S., 2016. Overexpression of CTNND1 in hepatocellular carcinoma promotes carcinous characters through activation of Wnt/β-catenin signaling. J Exp Clin Cancer Res 35, 82. https://doi.org/10.1186/s13046-016-0344-9

Thoreson, M.A., Reynolds, A.B., 2002. Altered expression of the catenin p120 in human cancer: implications for tumor progression. Differentiation 70, 583–589. https://doi.org/10.1046/j.1432-0436.2002.700911.x

Torphy, R.J., Fujiwara, Y., Schulick, R.D., 2020. Pancreatic cancer treatment: better, but a long way to go. Surg Today 50, 1117–1125. https://doi.org/10.1007/s00595-020-02028-0

van Roy, F.M., McCrea, P.D., 2005. A role for Kaiso–p120ctn complexes in cancer? Nat Rev Cancer 5, 956–964. https://doi.org/10.1038/nrc1752

Wong, C.H., Lou, U.K., Fung, F.K.-C., Tong, J.H.M., Zhang, C.-H., To, K.-F., Chan, S.L., Chen, Y., 2022. CircRTN4 promotes pancreatic cancer progression through a novel CircRNA-miRNA-lncRNA pathway and stabilizing epithelial-mesenchymal transition protein. Mol Cancer 21, 10. https://doi.org/10.1186/s12943-021-01481-w

Xue, X., Zhang, J., Lan, H., Xu, Y., Wang, H., 2017. Kaiso protects human umbilical vein endothelial cells against apoptosis by differentially regulating the expression of B-cell CLL/lymphoma 2 family members. Sci Rep 7, 7116. https://doi.org/10.1038/s41598-017-07559-0

Figures

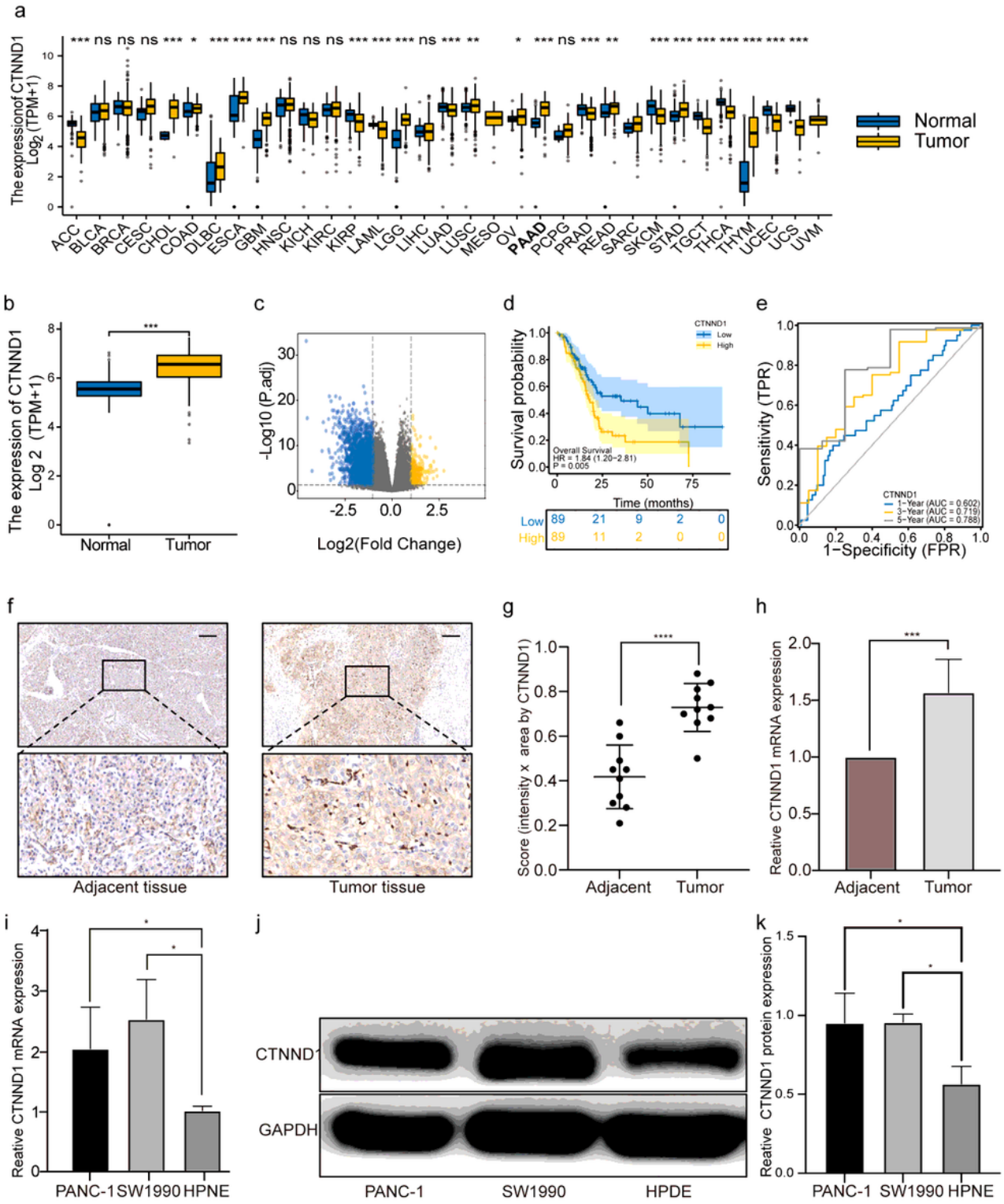

Figure 1

Bioinformatics analysis by expression of TCGA-PAAD and in pancreatic cancer tissues and cells.(a)The expression level of pancreatic cancer CTNND1 mRNA in TCGA database; (b) Boxplot of CTNND1 expression between pancreatic cancer tissue and normal pancreatic tissue in TCGA dataset; (c) TCGA data concentration of volcanic maps expressed in different genes of pancreatic cancer and normal pancreatic tissue (d) K-M survival analysis of CTNND1 in pancreatic cancer; (e) ROC curves and AUC of

1,3,5-year survival in CTNND1 patients; (f) CTNND1 in 10 PAAD and normal tissues Representative images of IHC staining Representative images of CTNND1 IHC staining of 10 pancreatic cancer and normal tissues (X400, scale bar 100 μm). (g) The score (intensity x area by CTNND1) anaylsis; (h) qRT-PCR results of CTNND1 mRNA expression in pancreatic cancer primary tumors (n=10) and adjacent normal tissues (n=10); (i) pancreatic cancer cells (PANC-1, SW1990) and normal pancreatic epithelial cells (HPNE) qRT-PCR results of CTNND1 mRNA expression; (j) Western blot results of CTNND1 protein expression in pancreatic cancer cells (PANC-1, SW1990) and normal pancreatic epithelial cells (HPNE); (k) CTNND1 protein expression in pancreas Statistical analysis of expression in cancer cells (PANC-1, SW1990) and normal pancreatic epithelial cells (HPNE); Data are shown as mean±SEM. *P<0.05, **P<0.01, ***P<0.001.

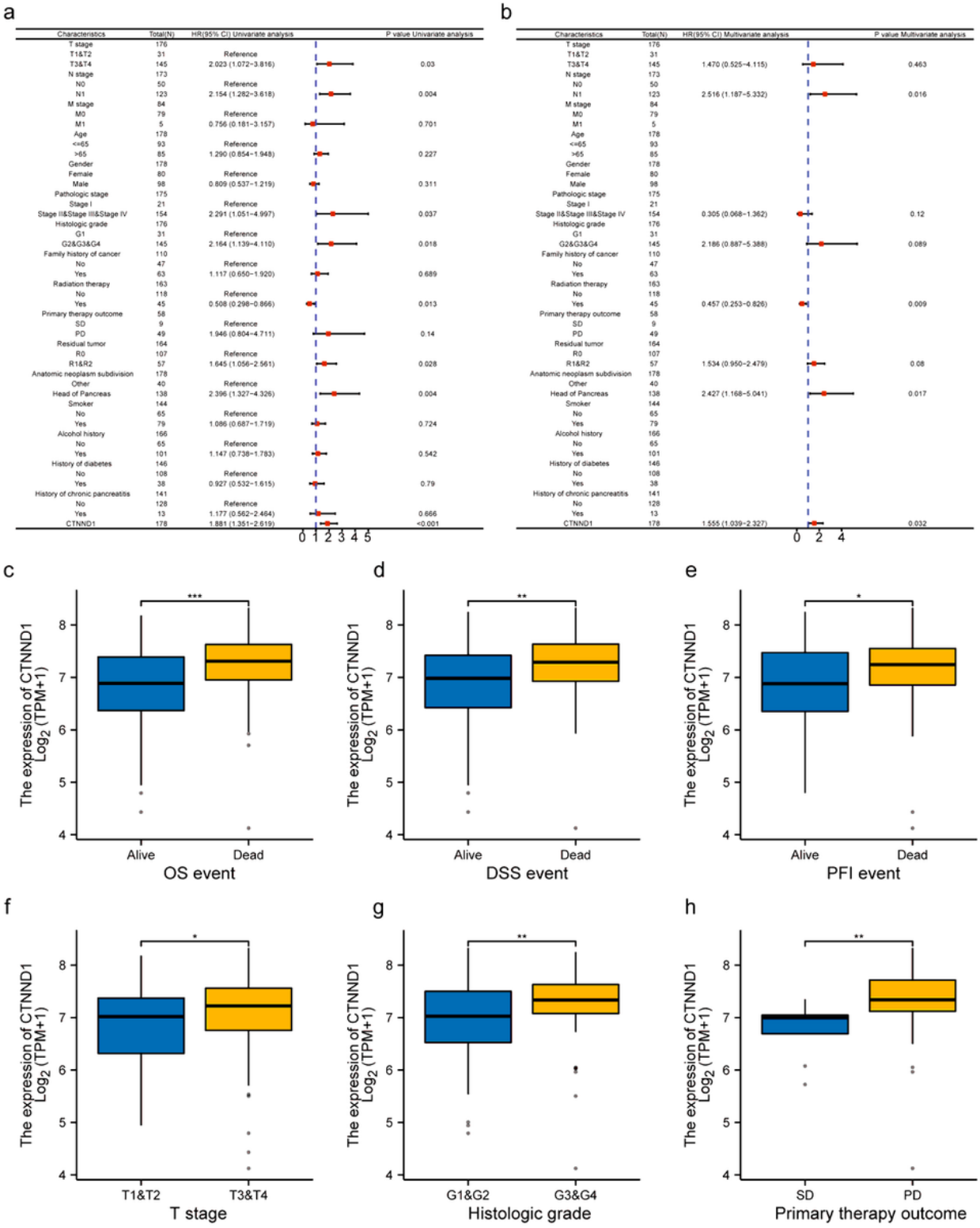

Figure 2

Analysis of clinical data in TCGA database (a) single factor Cox regression analysis of clinical data in TCGA cohort; (b) Clinical multivariate Cox regression analysis in TCGA cohort;□c□ OS event; (d)DSS event; (e) PFI event; (f)T stage; (g) Histologic grade; (h) primary therapy outcome; Data are shown as mean±SEM. *P<0.05, **P<0.01, ***P<0.001.

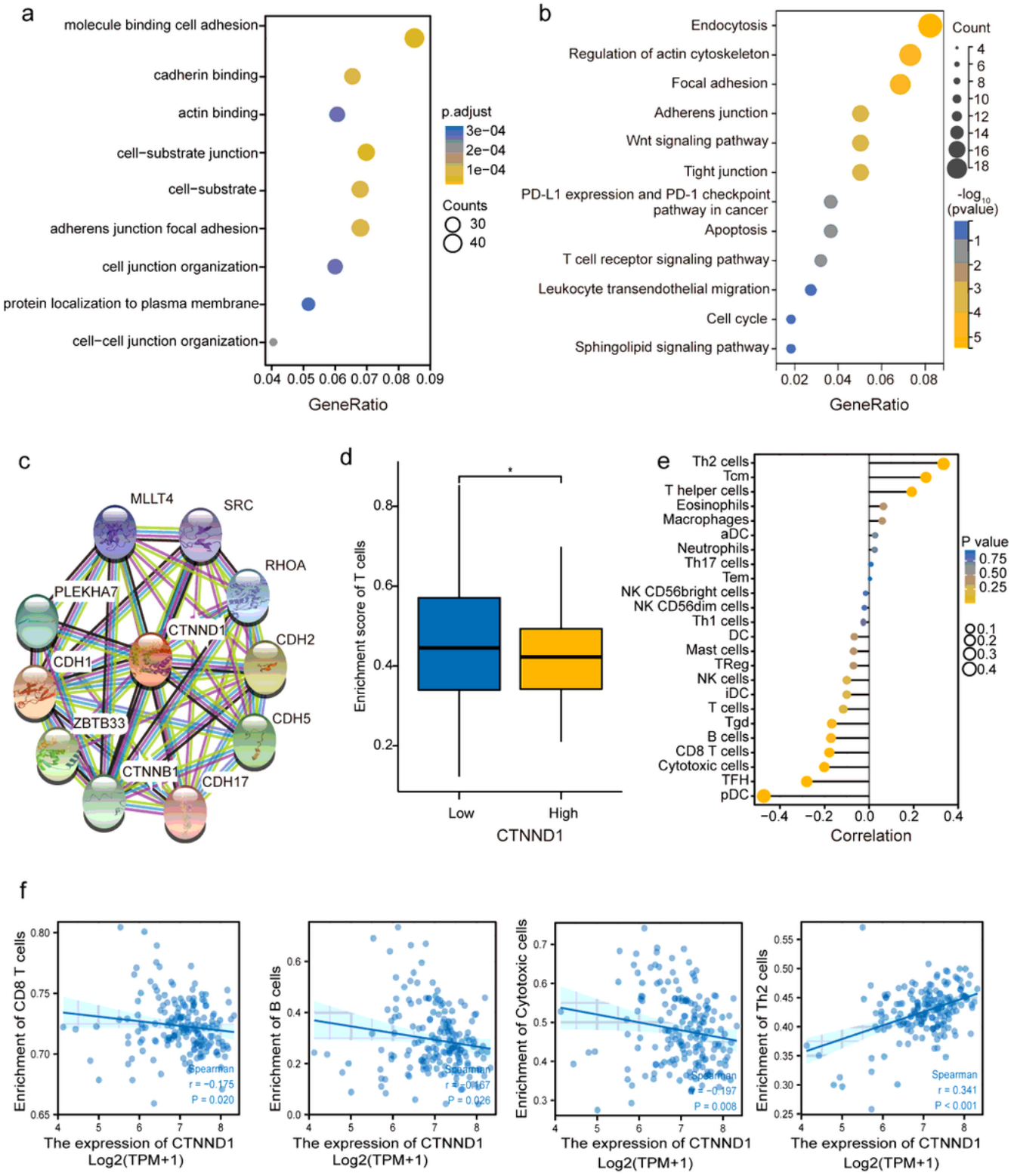

Figure 3

Relationships between CTNND1 and GO, KEGG, PPI, Enrichment score of T cells and Tumor immune cells in pancreatic cancer; (a) GO analysis; (b) KEGG; (c) PPI network;□d□Relationships between CTNND1 and Enrichment score of T cells; (e,f) Relationships between CTNND1 and Tumor immune cells. Data are shown as mean±SEM. *P<0.05, **P<0.01, ***P<0.001.

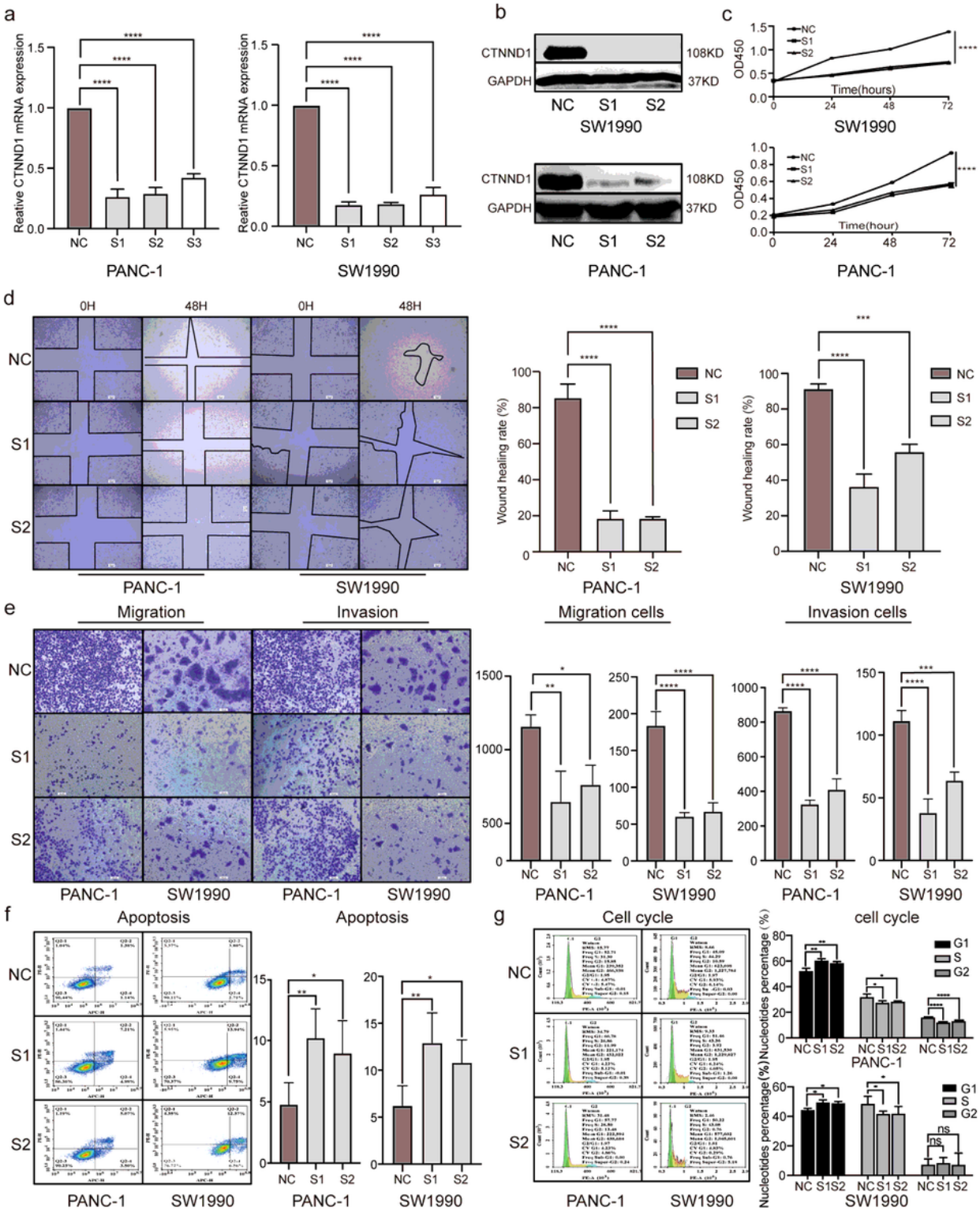

Figure 4

Silencing expression of CTNND1 attenuates proliferation，migration，cell apoptosis and cell cycle arrestof pancreatic cancer cells in vitro. (a,b) qRT-PCR and Western blot analyses affirmed the interfering efficiency of CTNND1 in pancreatic cancer cells (PANC-1 and SW1990);(c) The CCK-8 method was used to evaluate the proliferation and viability of CTNND1 silenced pancreatic cancer cells，(d) Wound-healing assays show that CTNND1 silencing reduces migration of CTNND1-silenced pancreatic cancer cells and

the cell number was statistically analyzed;（e）The transwell migration assay calculated the migration and invasion ability in CTNND1 silencing pancreatic cancer cells and the cell number was statistically analyzed;（f）Apoptosis analysis showed that CTNND1-silencing induced pancreatic cacner cell apoptosis. (g) Cell cycle analysis CTNND1-silencing induced G1 phase arrest. Data are shown as mean±SEM. *P<0.05, **P<0.01, ***P<0.001.

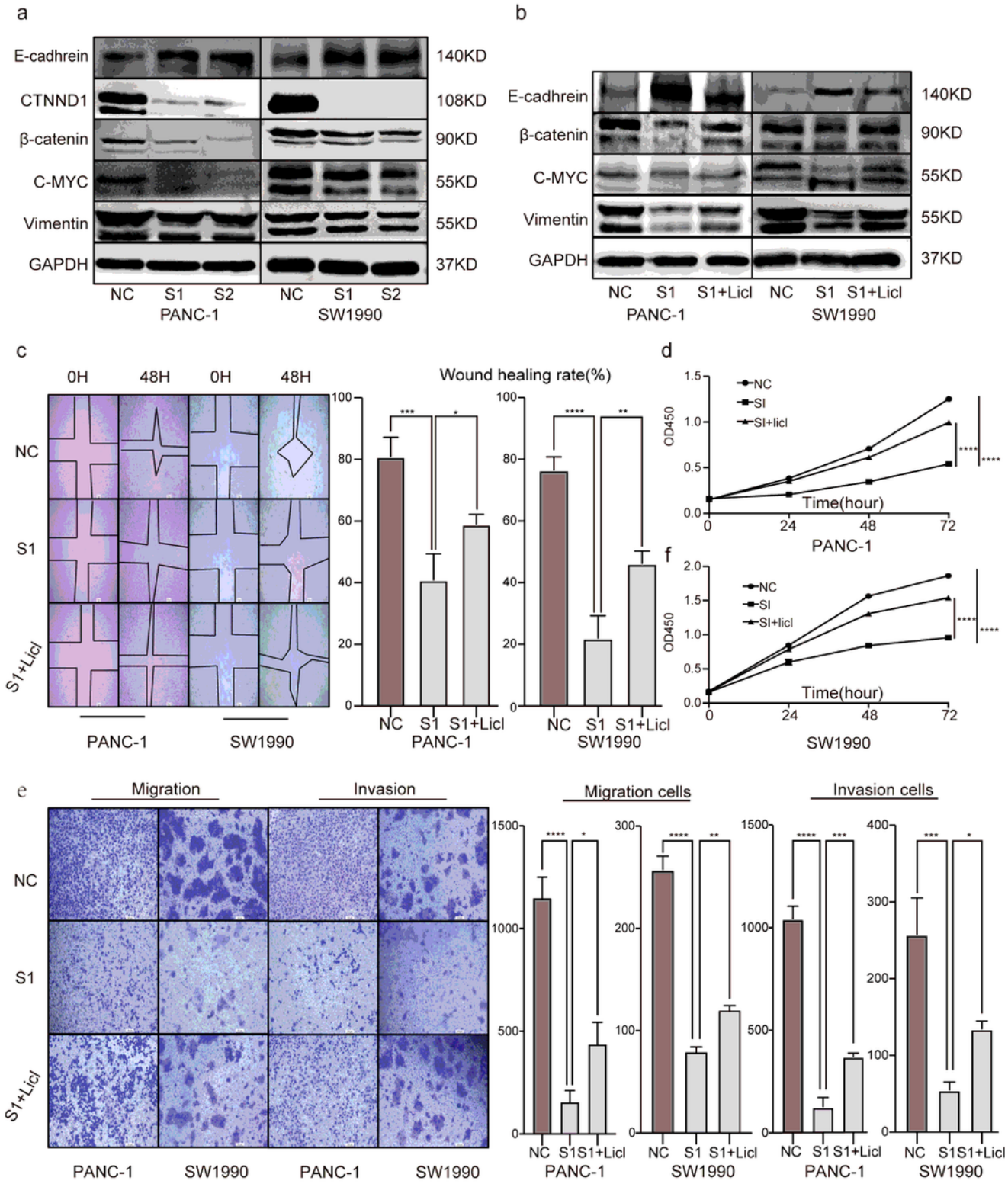

Figure 5

CTNND1 drives the EMT of pancratic cancer through Wnt/β-catenin signalling; (a) WB analysis showed that CTNND1 knockdown downregulated the expression of EMT markers(E-cadherin,vimentin) and wnt/β-catenin pathway markers(β-catenin,C-myc) in PANC-1 and SW1990 cellsl; (b) LiCl restored the expression of Wnt/β-catenin target genes and EMT markers in CTNND1-deficient cells; (c) Wound healing assays showed that LiCl restored the migration of CTNND1-deficient cells; (d) LiCl restored the proliferation and viability of CTNND1 silenced pancreatic cancer cells;(e) LiCl restored the migration and invasion of CTNND1 silenced pancreatic cancer cells. Data are shown as mean±SEM. *P<0.05, **P<0.01, ***P<0.001.

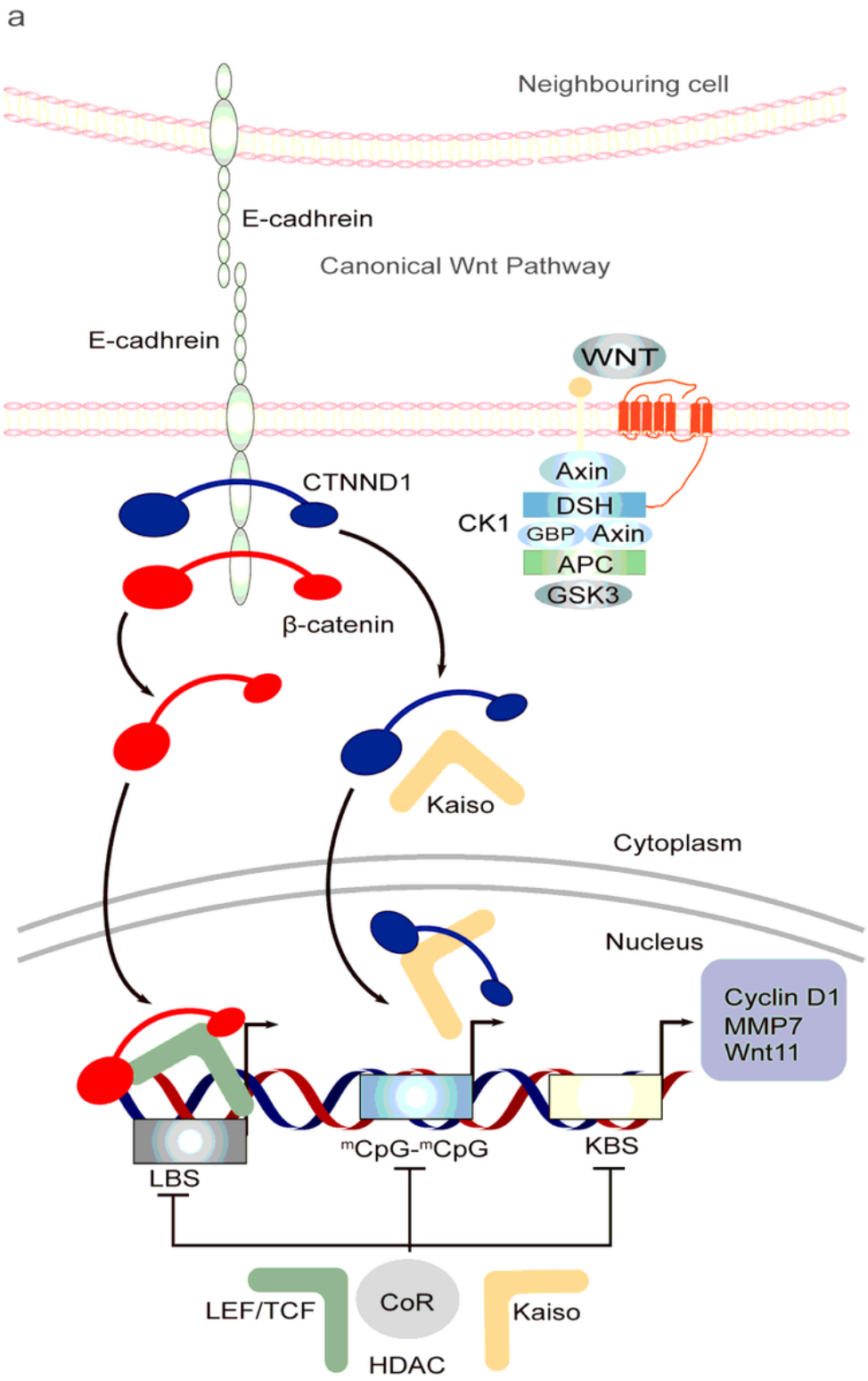

Figure 6

Schematic representation of the proposed mechanism of CTNND1 mediating EMT, proliferation, migration and invasion of pancreatic cancer through the Wnt/β-Catenin pathway.

Supplementary Files

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