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ORIGINAL RESEARCH

Protocadherin 1 (PCDH1) Promotes Pancreatic Cancer Metastasis by Regulating Epithelial-Mesenchymal Transition (EMT) Progression Through the NF-κB Pathway

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Objective: This study investigates the role of the protocadherin-1 (PCDH1) gene in pancreatic cancer metastasis, focusing on its influence on the epithelial-mesenchymal transition (EMT) process and the NF-κB signaling pathway.

Methods: Clinical specimens from pancreatic cancer patients were analyzed through bioinformatics to correlate PCDH1 expression with disease staging, pathological grade, metastasis, and prognosis. Cellular experiments using siRNA knockdown and plasmid overexpression were conducted, with assays such as CCK-8, Transwell, and flow cytometry to evaluate cell proliferation, invasion, and apoptosis. Western blot, qRT-PCR, and immunofluorescence assays were used to assess PCDH1 expression and its effects on the EMT process and NF-κB pathway.

Results: PCDH1 was significantly overexpressed in pancreatic cancer tissues compared with normal tissues, and its expression correlated positively with pathological grade and disease progression. Knockdown of PCDH1 inhibited cell proliferation, migration, and invasion while promoting apoptosis. Conversely, overexpression of PCDH1 promoted cell proliferation, migration, and invasion. Notably, both knockdown and overexpression of PCDH1 increased apoptosis, suggesting a complex, context-dependent role in apoptotic regulation. PCDH1 suppressed E-cadherin expression and promoted N-cadherin and vimentin, driving EMT progression. Additionally, PCDH1 activated the NF-κB pathway by promoting nuclear translocation of P65, and inhibition of NF-κB with SC75741 reversed PCDH1-induced EMT, confirming that PCDH1 promotes pancreatic cancer metastasis via the NF-κB/EMT axis.

Conclusion: PCDH1 acts as an oncogene in pancreatic cancer, promoting cell invasion, migration, and EMT progression through the NF-κB signaling pathway, making it a potential therapeutic target.

Keywords: PCDH1, pancreatic cancer, invasion and metastasis, epithelial-mesenchymal transition, NF-κB pathway

Introduction

Pancreatic cancer is a digestive system tumor that is of great concern to the medical community due to its strong concealment, difficulty in early diagnosis, rapid disease progression, and poor prognosis.[1] The survival rate of pancreatic cancer is below 10% after five years, making it a leading cause of cancer-related death.[2,3] Invasion and metastasis are the primary factors leading to poor prognosis in pancreatic cancer.[4] The rapid development of molecular biology has provided new research perspectives and treatment methods. Epithelial mesenchymal transition (EMT) is a biological phenomenon that involves the transformation of cell phenotype and is a key step in tumor invasion and metastasis. During this process, epithelial cells lose their original intercellular connections and polarity, gaining the mobility of stromal cells. This promotes the migration and metastasis of tumor cells.[5] Therefore, studying the molecular mechanism of regulating EMT is of great theoretical and practical significance for treating pancreatic cancer.

Protocadherins (PCDHs) are cell adhesion molecules that play a crucial role in maintaining normal tissue structure and function, and have significant biological importance.[6] Abnormal expression and dysfunction of cell adhesion molecules are important causes of tumor invasion and metastasis during tumorigenesis and development. PCDHs are frequently identified as candidate tumor suppressor genes, although in some tumors, they may play a carcinogenic role. For instance, PCDH7

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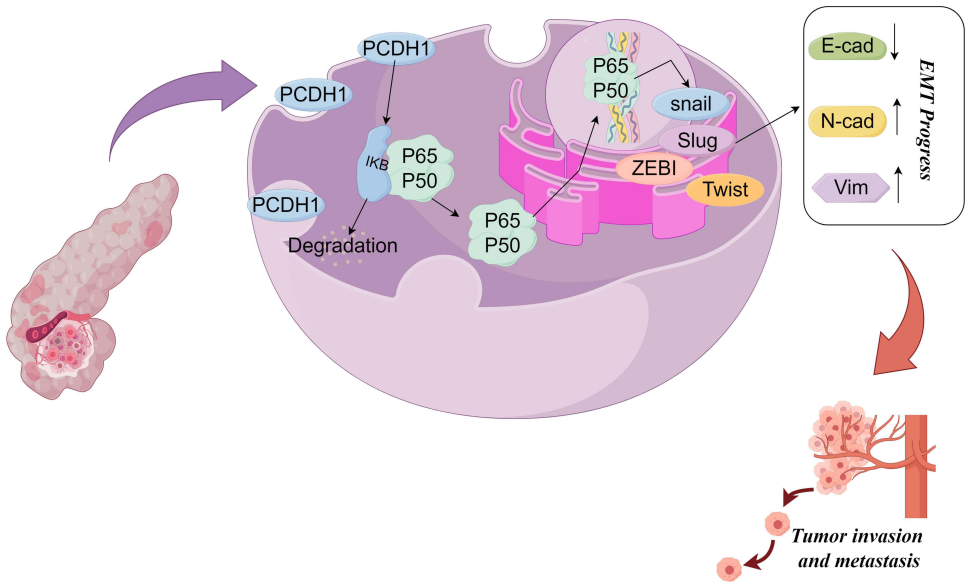
Graphical Abstract



facilitates the formation of gap junctions between cancerous astrocytes, resulting in the production of Interferon alpha (IFNα), which in turn activates the nuclear factor kappa B (NF-κB) pathway.[7] The removal of PCDH8 expression is linked to the advancement of breast cancer cells.[8] In pancreatic cancer, the overexpression of PCDH1 is associated with a poor prognosis in patients, suggesting a specific correlation between pancreatic cancer and PCDH1.[9,10]

The NF-κB signaling pathway is a crucial pathway for cells to respond to internal and external stimuli.[11] It plays a significant role in cell growth, differentiation, apoptosis, and immune response. In oncology, the NF-κB signaling pathway is closely associated with various biological processes, including tumorigenesis, progression, drug resistance, and immune escape.[12] The NF-κB signaling pathway is significant in regulating the process of EMT. This process is a key step for tumor cells to acquire invasive and metastatic abilities, and to resist chemotherapy and radiotherapy. During EMT, the activation of the NF-κB signaling pathway can induce the expression of downstream target genes, including important EMT transcription factors, extracellular matrix degrading enzymes, and factors that promote cell migration. This drives the transformation of the tumor cell phenotype.[13]

This study aims to investigate the role of the NF-κB signaling pathway and its regulatory mechanism in the EMT process of pancreatic cancer. The research background provides the necessary context for this investigation. The findings will contribute to a comprehensive understanding of the molecular mechanisms of pancreatic cancer invasion and metastasis. The interaction between PCDH1 and the NF-κB signalling pathway and its synergistic mechanism in pancreatic cancer are revealed in this study. This discovery may lead to the identification of new targets for pancreatic cancer treatment and provide a theoretical basis for clinical treatment of pancreatic cancer patients.

Materials and Methods

Experimental Materials

Cells: Human pancreatic epithelial cell lines HPDE6-C7 (BFN60807571) was purchased from the BFB, pancreatic cancer cell lines PANC-1 (SCSP-535), MiaPaCa-2 (SCSP-568), AsPC-1 (SCSP-5080), Capan-2 (TCHu259), BxPC-3 (TCHu 12), and CPFAC-1 (TCHu112) were purchased from the cell bank of Shanghai Academy of Sciences, China. All cells have been identified by STR profiling and confirmed to be free from mycoplasma contamination. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (item no. 11965092, Thermo Fisher, Shanghai, China) medium containing 10% fetal bovine serum and placed in a constant temperature incubator at 37°C with 5% CO_2 for cell culture.

Reagents: PCDH1 gene silencing and overexpression plasmid was purchased from Shanghai Sangong Bioengineering Service Co. SC75741 inhibitor (1 mg, purity: 99.51%, item no. HY-10496) was purchased from Med Chem Express (Shanghai, China). TRIzol reagent (100 mL, item no. 15596026CN) was purchased from Thermo Fisher. SuperScript™ III one-step RT-PCR system (100 rxns, item no. 12574026) containing Platinum™ Taq DNA Polymerase was purchased from Thermo Fisher. SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase (100 rxns, item #12574026) was purchased from Thermo Fisher. PCDH1 Antibody (50 ul, item #abs133856) was purchased from Absin (Shanghai, China), GAPDH Antibody (100 ul, item (Article No.: #2118s), P65 antibody (20 ul, Article No.: #8242T), E-cadherin antibody (20 ul, Article No.: #3195T), N-cadherin (20 ul, Article No.: #13116T) antibody and vimentin antibody (20 ul, Article No.: #5741T) were purchased from Cell Signaling Technology (Shanghai, China). StarSignal Western Protein Marker (10–200 kDa, Cat# M227-01, GenStar, Beijing, China) was used for molecular weight estimation.

Pathological slides: The pathology department of Ning Bo No.2 Hospital provided the tumor sections of patients with pancreatic cancer. All participating patients provided written informed consent prior to the collection of tumor tissues, authorizing the use of their biological samples for scientific research purposes. The study was approved for use by the Ning Bo No.2 Hospital's medical ethics committee (NO.20230719), and all procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki.

Bioinformatics Analysis

Gene expression data from normal and pancreatic cancer tissues were collected from public databases, such as The Cancer Genome Atlas (TCGA). Data analysis was obtained using GEPIA 2 website analysis (http://gepia2.cancer-pku.cn/#survival), were used to quantify the expression level of PCDH1 in pancreatic cancer tissues and compare it with normal tissues. The study employed survival analysis to evaluate the impact of PCDH1 expression levels on the survival of pancreatic cancer patients. Proteinatlas data revealed that the mRNA and protein expression levels of PCDH1 in pancreatic cancer tissues (https://www.proteinatlas.org/ENSG00000156453-PCDH1/pathology).

Immunohistochemistry

Human pancreatic cancer tissues and cancer-adjacent tissues were collected, fixed, embedded, and sectioned using a paraffin slicer. Tissue sections underwent a 10-minute incubation with 0.3% hydrogen peroxide at room temperature, followed by thorough rinsing with PBS to remove excess reagents. The PCDH1 antibody was diluted with diluent in a ratio of 1:1000 and incubated at 4°C overnight. Subsequent to this procedure, the sections were subjected to three washes with Tris-Buffered Saline Tween (TBST, item no. T1085, Solarbio, Beijing, China) to ensure the removal of any unbound antibodies. Thereafter, the sections were incubated with the secondary antibody, diluted at a ratio of 1:4000, for a duration of 30 minutes at room temperature. The visualization of tumor sections employed DAB solution for staining, lasting between 3 and 15 minutes. Following this, slides underwent brief hematoxylin counterstaining at ambient temperature, lasting 8 to 15 minutes. Images were captured by an inverted microscope (Ts2/Ts2-FL, NIKON, Tokyo, Japan) and then underwent analysis with the Image J 7.0 software (NIH, Bethesda, MD, USA). The staining intensity of PCDH1 in cancerous versus adjacent normal tissues was quantified using ImageJ and compared by Student's *t*-test.

Cell Treatment

Cell Grouping: The cells were planted in 6-well plates at a density of 1×10^6 cells/well when the cell density reached 80% or more. The cells were then randomly divided into four groups: the control group (si-NC group), PCDH1 silencing group (si-PCDH1 group), overexpression control group (OE-NC group), and PCDH1 overexpression group (OE-PCDH1 group).

Cell processing: The cells were transfected with the corresponding empty vector or plasmid in each group, following the instructions of the Lipo2000 transfection kit. The liquid was changed 6 hours after transfection, and the relevant indexes were detected after 48 hours of further culture.

qRT-PCR

Following cell treatment, total RNA was extracted from the cells using TRIzol reagent. The concentration and purity of RNA were determined using a NanoDrop spectrometer. The extracted RNA was then reverse transcribed into cDNA using SuperScript

III reverse transcriptase, which served as a template for the PCR reaction. During the real-time PCR stage, the fluorescence detection reporter used was SYBR Green I dye, which was amplified under specific thermal cycling conditions to monitor changes in the fluorescence signal during amplification. To calculate the amount of change in gene expression, GAPDH was used as an internal reference, according to the $2^{-\Delta\Delta Ct}$ formula. The forward and reverse primers targeting human PCDH1 were 5′-ACGCCACTCGGGTAGTGTA-3′ and 5′-TCACGGTCGATGGAGGTCTC-3′, respectively, and those targeting GAPDH were 5′-CAGGAGGCATTGCTGATGAT-3′ and 5′-GAAGGCTGGGGCTCATTT-3′, respectively.

Western Blot

Following the above-mentioned cell treatment, total cellular proteins were extracted using RIPA lysate. The protein concentration was determined using the BCA protein concentration assay kit. Subsequently, 40 μg of protein samples from each group underwent SDS-PAGE electrophoresis and were transferred to a polyvinylidene fluoride membrane. The primary antibody for the target protein was diluted 1:1000 with diluent. The membrane was incubated with the primary antibody overnight at 4°C, washed with TBST solution the following day, and then combined with the corresponding horseradish peroxidase-labelled secondary antibody (diluted 1:8000) for 5 hours. The expression level of the target protein was detected by developing a chemiluminescent substrate and quantitatively analyzed using Image J 7.0 software (NIH, Bethesda).

Cell Invasion Experiment

Prior to commencing the experiment, the consumables were pre-cooled in a −20°C refrigerator. Matrigel gel was diluted with serum-free DMEM medium at a ratio of 1:10. Subsequently, 50 μL of the diluted Matrigel gel was added to the upper chamber of the Transwell and incubated at 37°C for 30 min. Following transfection for 48 h, 1×10^5 cells were seeded in the upper chamber of the treated chamber. Finally, 600 μL of medium containing 20% FBS was added to the lower chamber. After 48 hours, the supernatant from the chambers was discarded, excess cells in the upper chamber were removed, and the cells were stained with a 1% crystal violet solution. The stained cells were then photographed under a microscope (Ts2/Ts2-FL, NIKON) for analysis.

Wounding Healing Assay

Prior to the experiment, horizontal lines were evenly drawn on the back of the 6-well plate using a ruler and a marker pen. The cells were then grouped and transfected following the aforementioned method. Once the cell fusion rate reached 100%, the cells were evenly scraped using a 100 μL pipette tip. The unattached floating cells were washed away with PBS, and 2 mL of medium containing 2% FBS was added. After 48 hours of cultivation, the cells were observed and photographed under a microscope. The scratch photographs were then analyzed using Image J 7.0 software. The cell healing rate was calculated according to the following formula: Healing Rate = (Initial Scratch Area - Final Scratch Area)/Initial Scratch Area.

Cell Counting Kit-8 (CCK8) Experiment

The CCK8 experiment involved planting cells in 96-well plates at a density of 3×10^3 cells per well, as previously described. The cells were then grouped and transfected using the lipo2000 transfection kit, following the provided instructions. Absorbance values were analyzed at 1, 3, 5, and 7 days post-transfection, with 5 replicate wells in each group. At each corresponding time point, 10 μL of CCK8 solution was added to each well and incubated for 2 hours. The absorbance was measured at 450 nm.

Immunofluorescence Experiment

After grouping and treating the cells as described above, they were fixed with 4% paraformaldehyde. Then, Triton X-100 (item.no T8200, Solarbio) was used for permeabilisation, and an appropriate amount of sealing solution was added before incubating with a diluted primary antibody solution for the target proteins at a ratio of 1:500. 50 μL of the target proteins were added to each well and incubated overnight in the refrigerator at 4°C. The following day, the cells were incubated with the corresponding fluorescent-labelled secondary antibody solution (diluted 1:8000) for 5 hours. The cell nuclei

were re-stained with DAPI (item.no C0065, Solarbio) solution and analyzed under a fluorescent inverted microscope (Ts2/Ts2-FL, NIKON).

Apoptosis Assay

After grouping and treatment as described above, the cells were operated following the instructions of the Annenxin-V5 /PI Apoptosis Detection Kit. Briefly, the cells in each group were collected, washed, and then treated with 5 μL of Annenxin-V5 solution and Propidium Iodide (PI)solution, respectively. The cells were then incubated at room temperature, protected from light, for 15–30 minutes. The assay was carried out on the machine, and the data were analyzed using Flow jo10.0 software.

Data Analysis

During the data analysis process of the study, we utilized various statistical software and methods to ensure the accuracy and scientific validity of the results obtained from processing the experimental data. The $2^{-\Delta\Delta Ct}$ method was used to calculate the change in gene expression in the real-time fluorescence quantitative PCR data. This accurately reflects the change in the expression level of the target gene relative to that of the internal reference gene GAPDH. In the quantitative analysis of Western blot and immunohistochemistry, image analysis software, such as ImageJ, was used to perform grey value analysis of protein bands to quantify protein expression levels. For immunohistochemistry, the optical density (OD) of staining was measured in at least five randomly selected fields per section, and the mean OD of cancerous tissues was compared with that of adjacent normal tissues using Student's *t*-test. For data processing of cell invasion and migration experiments, cells passing through the Transwell were counted using analysis software, such as Image-Pro Plus, and compared and analyzed with the control group.

In all statistical analyses, a statistical significance level of 0.05 was set, meaning that differences were considered statistically significant at $p < 0.05$. For multiple group comparisons (eg, across four treatment groups in Figure 1), one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was applied; the asterisks (*) denote comparisons between specific groups as indicated in each figure legend, while the pound signs (#) denote comparisons specifically between the OE-PCDH1 group and the OE-PCDH1+SC75741 group. The analyses were conducted under strict supervision to ensure data integrity and analysis objectivity.

Results

Expression of PCDH in Pancreatic Cancer Tissues and Prognostic Implications

To gain a better understanding of the expression pattern of the PCDH1 gene in pancreatic cancer, this study conducted a comprehensive analysis of pancreatic cancer gene expression data from various public databases using GEPIA 2. After conducting a thorough analysis, PCDH1 expression was found to be significantly elevated in pancreatic cancer tissues compared with normal pancreatic tissues (Figure 2A). Further subgroup analysis revealed that PCDH1 expression was progressively higher with increasing pathological grade, with the highest expression observed in poorly differentiated (G3) tumors compared with well-differentiated (G1) and moderately differentiated (G2) tumors, indicating a significant correlation between PCDH1 expression and the degree of malignant differentiation. Immunohistochemical analyses confirmed high expression of the PCDH1 protein in pancreatic cancer tissues and relatively high expression levels in some highly differentiated pancreatic cancer samples (Figure 2D). According to the GEPIA 2.0 survival analysis, patients with high PCDH1 expression had significantly shorter overall survival (OS) times compared to those with low expression. Additionally, patients with high PCDH1 expression had significantly shorter Disease Free Survival (DFS) times compared to those with low expression (Figure 2B and C). These findings collectively suggest that PCDH1 overexpression is associated with worse clinicopathological features and poorer prognosis in pancreatic cancer. Statistical analysis showed a higher proportion of PCDH1-positive cells in pancreatic cancer tissues compared with adjacent normal tissues (Figure 2E). The qRT-PCR results indicate a significant up-regulation of PCDH1 mRNA expression in pancreatic cancer cell lines compared to non-tumor control tissues (Figure 2F). CPFAC-1 cells exhibited the most significant upregulation of PCDH1 mRNA expression among the various cell lines tested. Consequently, this study prioritized this

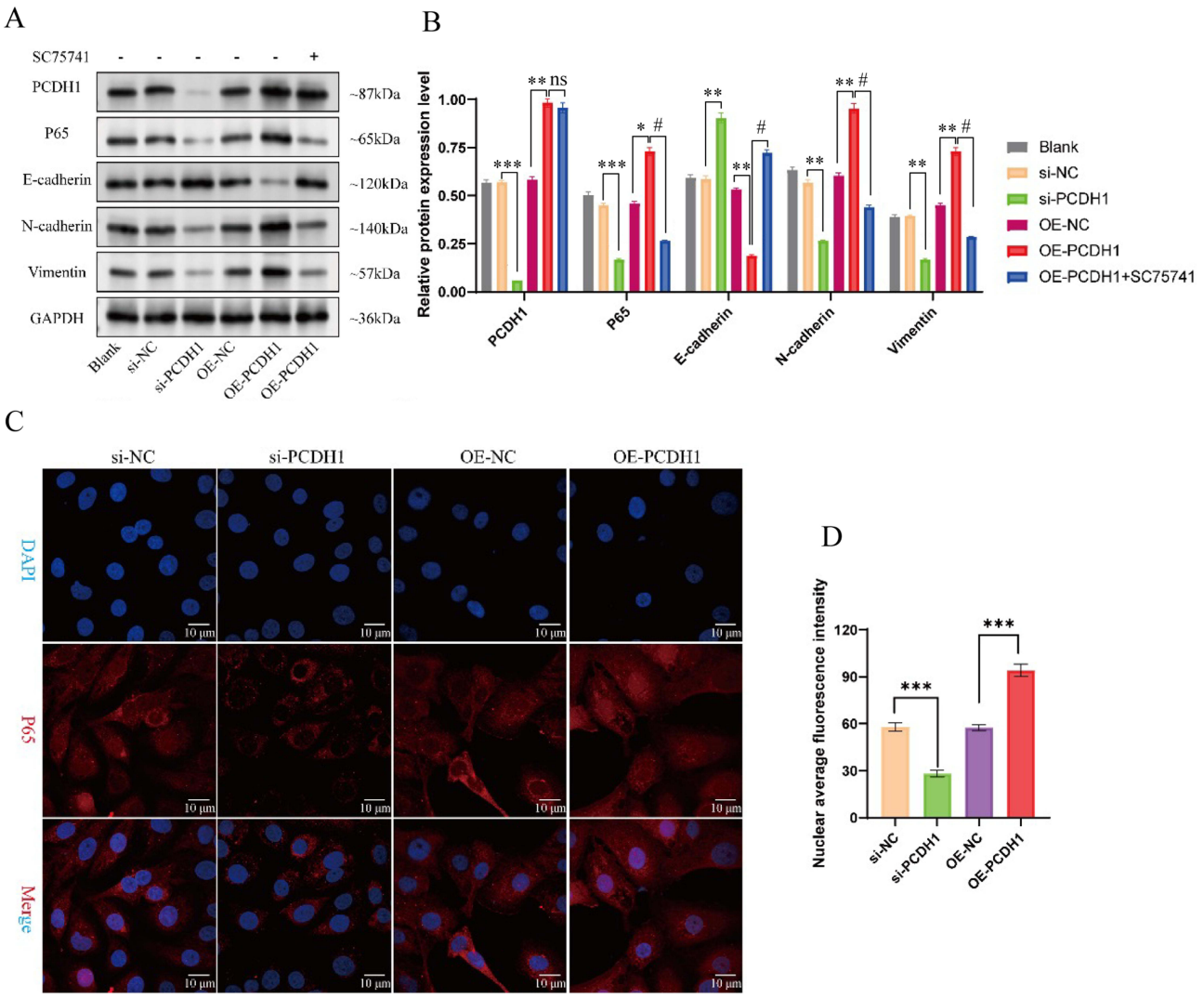


Figure 1 PCDH1 regulates NF-κB signaling pathway promotes invasion and metastasis of pancreatic cancer. (**A**) Western Blot was used to detect the expression levels of PCDH1 and EMT-related proteins in each group; (**B**) Statistical analysis of protein levels in each group using one-way ANOVA (n=3). Asterisks (*) indicate comparisons between si-NC vs. si-PCDH1 and OE-NC vs. OE-PCDH1 groups; pound signs (#) indicate comparisons between OE-PCDH1 vs. OE-PCDH1+SC75741 group. (**C**) Fluorescent analysis of P65 into the nucleus; (**D**) Statistical results of P65 fluorescence intensity in the nucleus using the Student's *t*-test (n=3); $^{**}p<0.01$, $^{***}p<0.001$. $^{\#}p<0.05$; ns, no significant difference.

cell line for subsequent experimental investigations. Furthermore, HE staining showed that compared with the paraneoplastic tissues, the cells of the cancerous tissues had an abnormal morphology and structure, with increased cell volume, and the nuclear chromatin was deeply stained and unevenly distributed (Figure 2G). Immunohistochemical analyses confirmed the high expression of PCDH1 protein in pancreatic cancer tissues compared with the paraneoplastic tissues (Figure 2H). Quantitative analysis by ImageJ demonstrated that the mean optical density of PCDH1 staining in cancerous tissues was significantly higher than in adjacent normal tissues (Student's *t*-test, $p < 0.01$). These results confirm the oncogenic expression pattern of PCDH1 in pancreatic cancer and its correlation with pathological grade and clinical prognosis.

Silence or Overexpression PCDH1 Validation

Following transfection with siRNA, knockdown or overexpression, the results of immunofluorescence demonstrated that the green fluorescence signal was diminished in the si-PCDH1 group in comparison to the si-NC control group, indicating that transfection of siRNA significantly inhibited the expression of PCDH1. In contrast, the green fluorescence

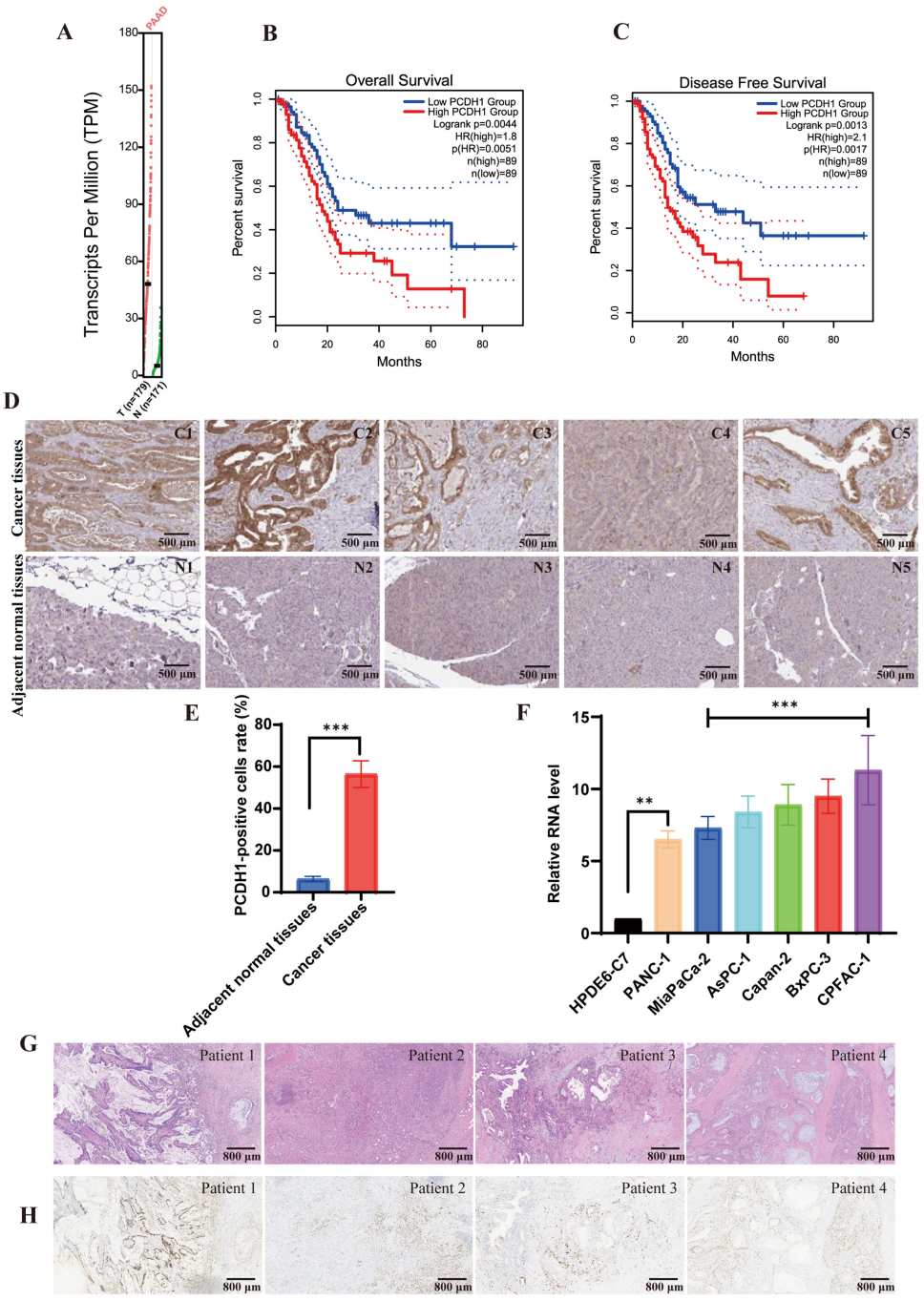


Figure 2 Expression of PCDH1 in pancreatic cancer and its influence on prognosis. (**A**) Bioinformatics analysis of PCDH1 expression in normal tissues and pancreatic cancer tissues; (**B**) Bioinformatics analysis of the effect of PCDH1 expression level on the overall survival rate of patients; (**C**) Bioinformatics analysis of the effect of PCDH1 expression level on the progression-free survival of patients; (**D**) Immunohistochemistry was used to detect the expression of PCDH1 in pancreatic cancer tissues and normal tissues at different differentiation stages. (**E**) Statistical results of the proportion of PCDH1-positive cells in pancreatic cancer tissues and adjacent normal tissues. (**F**) RT-qPCR was used to detect the expression of PCDH1 mRNA in normal pancreatic epithelial cells and pancreatic cancer cell lines, one-way analysis of variance was used for statistics (n=3). (**G**) HE staining was used to detect the pathology of cancerous and paraneoplastic tissue. (**H**) Immunohistochemistry was used to detect the expression of PCDH1 in cancerous and paraneoplastic tissue. $^{**}p < 0.01$, $^{***}p < 0.001$.

signal was enhanced in the OE-PCDH1 group compared to the OE-NC control group, indicating that the plasmid could significantly induce PCDH1 expression (Figure 3A and B). The PCDH1 protein expression in each group was detected by Western Blot. The results demonstrated that PCDH1 protein expression was significantly decreased in the si-PCDH1 group in comparison to the si-NC group, and significantly increased in the OE-PCDH1 group in comparison to the OE-

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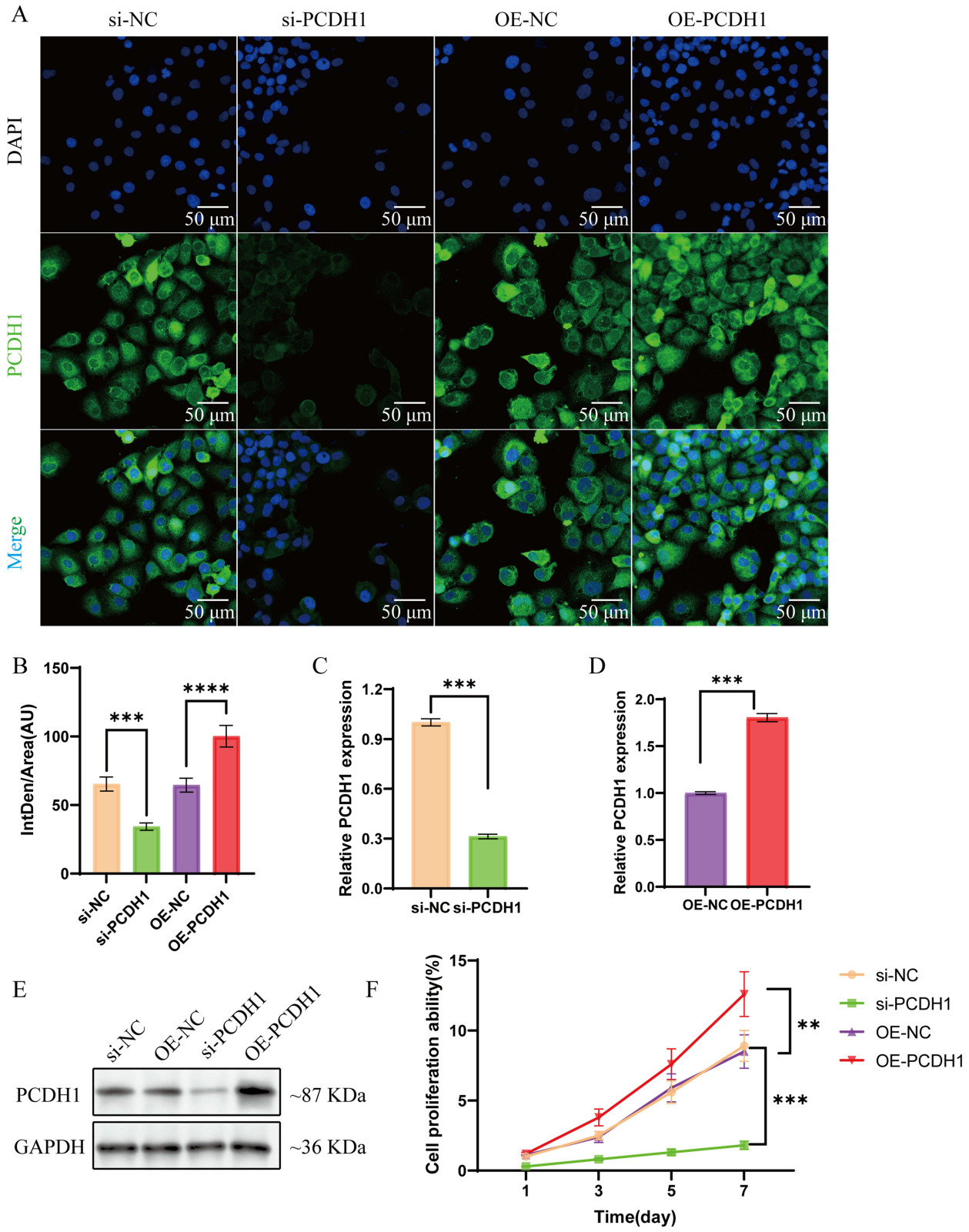

Figure 3 Silence or overexpression PCDH1 validation. (**A**) Immunofluorescence assay was used to detect the expression of PCDH1 in each group after transfection; (**B**) Statistical results of immunofluorescence analysis of PCDH1 expression levels; (**C**) Western Blot analysis was used to detect the expression of PCDH1 protein levels in each group; (**D**) Statistical results of protein expression in si-NC and si-PCDH1, OE-NC and OE-PCDH1 groups using the Student's *t*-test (n=3); (**E**) CCK-8 assay was used to detect the proliferation of cells in each group; (F) CCK-8 assay was used to detect the proliferation of cells in each group $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

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NC group (Figure 3C and D). Knockdown of PCDH1 significantly inhibited the proliferation of CPFAC-1 cells, while overexpression of PCDH1 significantly promoted cell proliferation (Figure 3E). These results consistently indicate that PCDH1 acts as a pro-proliferative oncogene in pancreatic cancer cells.

Regulation of CPFAC-1 Cell Migration, Invasion, and Apoptosis by PCDH1

The wound healing assay showed that cell scratch healing was significantly slowed in the si-PCDH1 group compared to the si-NC group, and cell scratch healing was significantly accelerated in the OE-PCDH1 group compared to the OE-NC group (Figure 4A and B); Transwell migration assays demonstrated that compared to the si-NC group, cell migration was significantly decreased in the si-PCDH1 group, while in the OE-PCDH1 group, the cell migration rate was significantly

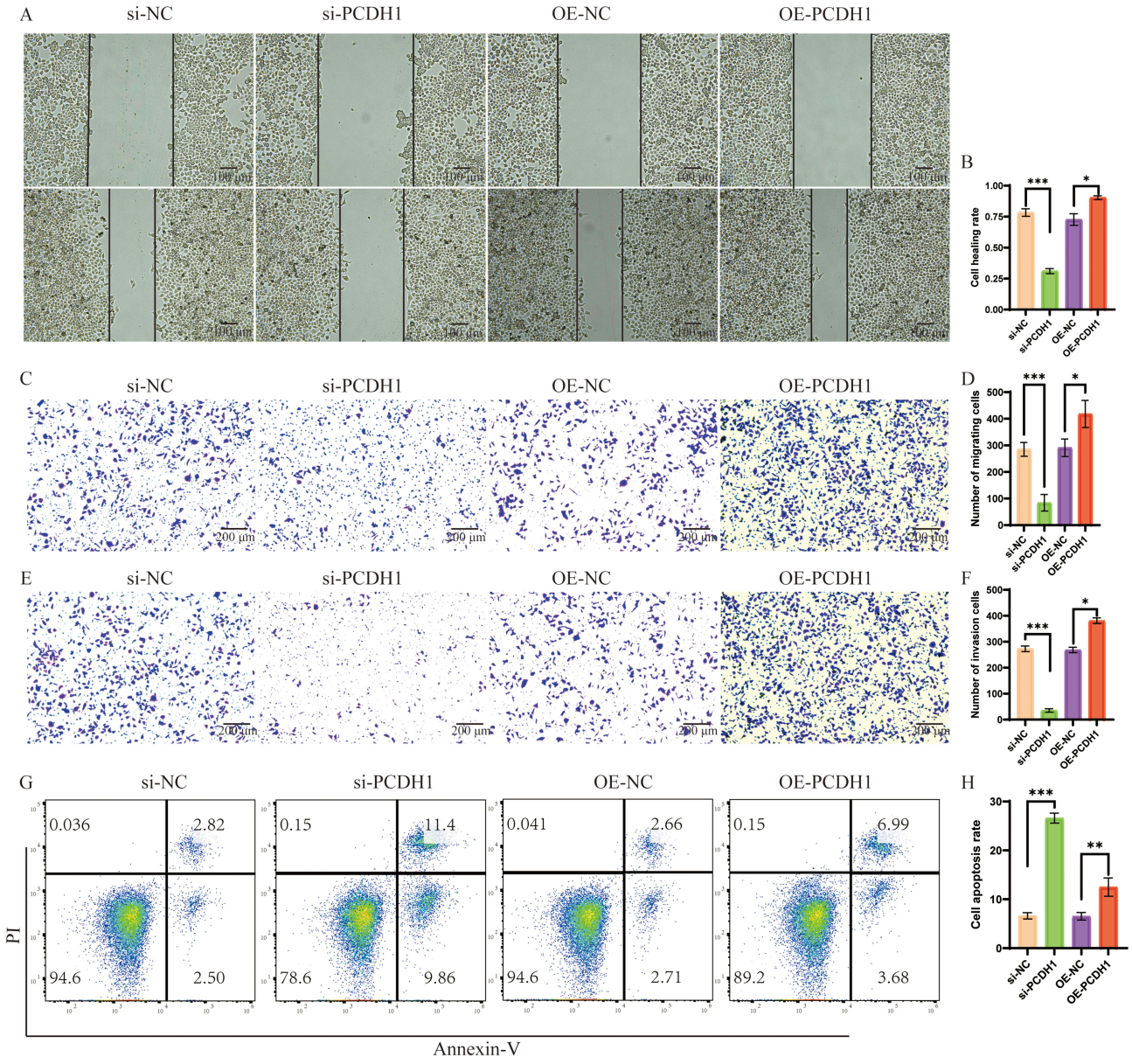

Figure 4 Effects of PCDH1 on invasion and apoptosis of CPFAC-1. (**A**) Scratch test was used to detect the migration ability of cells in each group; (**B**) Statistical analysis results of cell scratch healing rate in each group using the Student's *t*-test (n=3); (**C**) Transwell assay was used to detect the cell migrating ability of each group. (**D**) Statistical analysis results of cell migrating rate in each group using the Student's *t*-test (n=3); (**E**) Transwell assay was used to detect the cell invasion ability of each group. (**F**) Statistical analysis of cell invasion rate in each group using Student's *t*-test (n=3); (**G**) Flow cytometry was used to detect apoptosis in each group. (**H**) Statistical analysis results of apoptosis rate in each group using the Student's *t*-test (n=3). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Solid lines above bar pairs indicate statistically significant comparisons between the connected groups.

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increased compared with the OE-NC group (Figure 4C and D). Similarly, silencing PCDH1 expression significantly decreased the invasive capacity of CPFAC-1 cells, while overexpression of PCDH1 significantly increased the invasive ability of CPFAC-1 cells (Figure 4E and F). These results demonstrate that PCDH1 promotes the migration and invasion of pancreatic cancer cells in a dose-dependent manner.

Flow cytometry demonstrated that the apoptotic rate in the si-PCDH1 group was significantly elevated compared to the si-NC group, while the apoptotic rate in the OE-PCDH1 group was also significantly elevated compared to the OE-NC group (Figure 4G and H). This paradoxical finding—that both PCDH1 knockdown and overexpression increased apoptosis—suggests that PCDH1 may regulate apoptosis in a complex, non-linear fashion. It is possible that disruption of PCDH1 expression in either direction perturbs cellular homeostasis and triggers apoptotic signaling through distinct mechanisms, as has been observed for other oncoproteins that exhibit biphasic apoptotic responses. This finding is discussed further in the Discussion section.

PCDH1 Regulates the NF-κB Signalling Pathway to Promote Pancreatic Cancer Invasion and Metastasis

Having established that PCDH1 has a significant effect on the migration and invasion ability of pancreatic cancer cells, we further investigated its key mechanism of action. It has been reported that PCDH1 promotes the nuclear entry of P65, which activates the NF-κB signaling pathway in pancreatic cells and tissues, and that silencing PCDH1 affects the binding of P65 and KPNB1 and reduces the nuclear entry of P65.[14] In the present study, silencing PCDH1 expression significantly decreased nuclear P65 protein levels, whereas overexpression of PCDH1 significantly increased nuclear P65 levels. Importantly, treatment with SC75741, an NF-κB pathway inhibitor, significantly reduced the elevated nuclear P65 levels induced by PCDH1 overexpression (Figure 1A and B). P65 entry into the nucleus was verified by immunofluorescence, and it was also found that intranuclear P65 fluorescence intensity was significantly decreased under PCDH1 knockdown, whereas overexpression of PCDH1 significantly increased intranuclear P65 fluorescence intensity (Figure 1C and D). PCDH1 regulates the NF-κB signaling pathway and further regulates the EMT process. Regarding EMT regulation, when PCDH1 was knocked down, the expression level of the EMT-related marker E-cadherin was increased, while the expression level of vimentin and N-cadherin were decreased, which was associated with the reversal of the EMT process. On the other hand, when PCDH1 was overexpressed, the expression level of E-cadherin decreased while that of vimentin and N-cadherin increased, and the cells belonged to the state of EMT progression, which promotes the proliferation, migration and invasive ability of tumor cells (Figure 1A and B). Critically, treatment with SC75741 in the OE-PCDH1 group significantly reversed these EMT changes—restoring E-cadherin and reducing N-cadherin and vimentin—demonstrating that PCDH1-driven EMT is mediated through NF-κB pathway activation. These results provide mechanistic evidence that the PCDH1/NF-κB/EMT axis is a key driver of pancreatic cancer invasion and metastasis.

Discussion

Pancreatic cancer is a highly malignant gastrointestinal tumor that involves abnormalities in several genes and signaling pathways during its development.[15,16] Recent studies have shown that PCDHs exhibit abnormal expression patterns in various cancers. For instance, ectopic expression of PCDH10 in colorectal cancer (CRC) cells induced cell cycle retardation and increased apoptosis through regulation of the p53/p21/Rb axis and Bcl-2 expression. Overexpression of PCDH10 reversed the EMT process with morphological changes and EMT marker alterations.[17] Besides, Zhang and his team discovered that the Pcdh10 gene has the ability to produce a circular RNA known as circPcdh10 within pancreatic cancer (PC) tissue, suggesting a poorer outcome for patients.[18,19] PDCH-B9 expression levels were abnormally elevated in human esophageal squamous carcinoma cells, and exhibited tumor-promoting effects.[20] Therefore, PCDHs are considered to be possibly universal cancer-related genes, and there may be some heterogeneity in their mechanisms of action in different tumors. The PCDH1 gene has attracted much attention due to its important role in cancer. This study focuses on the expression of PCDH1 in pancreatic cancer and its prognostic impact. A series of

biological experiments were undertaken to explore the regulatory function of PCDH1 on pancreatic cancer cell proliferation, invasion, and apoptosis, along with the underlying mechanisms involved.

The expression level of PCDH1 in pancreatic cancer tissues was found to be significantly higher than that in normal tissues. This confirms the oncogenic role of PCDH1 in pancreatic cancer. Notably, PCDH1 expression was found to correlate with pathological grade, with higher expression in more poorly differentiated tumors, suggesting that PCDH1 upregulation may accompany or drive malignant dedifferentiation. This is consistent with its role in promoting EMT, a process closely associated with loss of differentiation. The expression up-regulation was correlated with the degree of differentiation of pancreatic cancer, patient prognosis, and other clinical features. This provides strong evidence for the importance of PCDH1 in pancreatic cancer. Additionally, this study verified the oncogenic function of PCDH1 in pancreatic cancer cells through biological experiments. The study demonstrated that knockdown of PCDH1 inhibited proliferation, migration, and invasion, while overexpression promoted these malignant phenotypes, consistently confirming the pro-cancer role of PCDH1 and identifying it as a potential therapeutic target.

The apoptosis data in this study revealed an intriguing paradox: both knockdown and overexpression of PCDH1 elevated apoptosis rates, though via potentially distinct mechanisms. PCDH1 knockdown may trigger apoptosis by disrupting cell-cell adhesion and activating stress-response pathways. In the case of PCDH1 overexpression, the concurrent activation of NF-κB may paradoxically induce apoptosis in a subset of cells through mechanisms that remain to be fully characterized. Similar biphasic apoptotic responses have been observed for other oncoproteins when expressed at supraphysiological levels. Despite this apoptotic paradox, the net effect of PCDH1 overexpression in this study was pro-tumorigenic, as evidenced by enhanced proliferation, migration, and invasion. Future studies incorporating dose-response analyses and transcriptomic profiling following PCDH1 modulation would help clarify the mechanisms underlying this complex apoptotic regulation. Furthermore, the potential of PCDH1 and its protocadherin family members as therapeutic targets and biomarkers warrants further investigation in larger clinical cohorts, given their consistent association with malignant progression across multiple cancer types.[21,22]

Furthermore, the study revealed that the overexpression of PCDH1 enhances the invasive ability of pancreatic cancer cells. As discussed above, PCDH1 modulation in either direction exerted pro-apoptotic effects, consistent with the flow cytometry data (Figure 4G and H). This finding implies that PCDH1 may impact tumor development by regulating the invasive process of pancreatic cancer cells. PCDH1 has been previously reported as a biomarker for metastatic treatment of pancreatic cancer.[21,22] Additionally, it promotes the development of pancreatic ductal adenocarcinoma by interacting with KPNB1, which activates the NF-κB signaling pathway.[10] NF-κB is a transcription factor predominantly located in the cytoplasm. Upon stimulation by external signals, it translocates to the nucleus where it regulates gene expression. Research has demonstrated that NFKBIZ modulates hepatocellular carcinoma (HCC) tumorigenesis and metastasis by intervening in the NF-κB signaling cascade. Furthermore, the study identified the TRIM16/NFKBIZ/NF-κB pathway as a potential mechanism underlying HCC's resistance to sorafenib.[23] Wang et al's investigation into pancreatic cancer corroborated the role of NF-κB as a crucial target for Nogo-B in regulating tumor proliferation and invasion. Notably, the absence of Nogo-B suppressed PC progression, a phenomenon orchestrated by the intricate interplay between the NF-κB/GLUT1 and SREBP1 signaling pathways.[24] The results showed that PCDH1 could regulate the expression of P65, which is involved in a variety of cellular biological processes such as immunity, inflammation, apoptosis and proliferation.[25–27] Pan-cancer analyses have further revealed complex regulatory networks involving epigenetic modifications and immune landscape characteristics that intersect with NF-κB-driven oncogenic programs,[28–30] supporting the importance of multi-dimensional cancer analysis in identifying therapeutic targets.[31] The NF-κB signaling pathway was analyzed in this paper to verify the specific mechanism of PDCH1 in regulating pancreatic cancer metastasis. PCDH1 regulates pancreatic cancer metastasis through its effect on the expression level of EMT-related markers via the NF-κB signaling pathway. This finding is consistent with previous studies.[10]

EMT can cause epithelial cells to lose their original polarity and adhesion properties, resulting in enhanced migration and invasion capabilities.[32–34] Multiple signaling pathways and transcription factors are activated or inhibited during this process.[35,36] This study confirms that PCDH1 can regulate the EMT process by modulating E-cadherin, N-cadherin, and vimentin expression through NF-κB activation, and that pharmacological inhibition of NF-κB with SC75741 reverses PCDH1-driven EMT, providing direct mechanistic evidence for this regulatory axis. Therefore, targeting the PCDH1/NF-

κB/EMT axis can be an important approach for treating pancreatic cancer invasion and metastasis. This study provides a new idea for developing cancer therapeutic approaches that target the EMT process.

Although this study underscores the oncogenic role of PCDH1 in pancreatic cancer, thereby establishing a theoretical foundation for therapeutic targeting, several limitations persist. Firstly, this study is based on in vitro cellular experiments; validation in in vivo animal models and larger clinical cohorts is required to fully establish the generalizability of the findings to human disease. Secondly, the complex mechanisms underlying PCDH1-mediated oncogenesis remain only partially elucidated, with potential unexplored interactions warranting further investigation. Thirdly, while NF-κB inhibitor rescue experiments demonstrate that PCDH1-driven EMT is NF-κB-dependent, more direct mechanistic approaches such as luciferase reporter assays and ChIP-qPCR would further strengthen the mechanistic conclusions and are planned for future studies. In addition, direct rescue experiments using SC75741 in CCK-8 proliferation and Transwell migration/invasion assays would provide more definitive evidence that PCDH1-driven proliferation and invasion are also NF-κB-mediated, and are planned for future work. Furthermore, transcriptome sequencing following PCDH1 knockdown would provide a more comprehensive view of the downstream regulatory network governed by PCDH1 and is planned as a direction for future investigation. The survival analysis in this study was performed using GEPIA2-based Kaplan–Meier Log rank testing; a multivariate Cox regression analysis incorporating clinical covariates such as age, pathological stage, and differentiation grade would provide more robust prognostic insight, and is planned for future studies using prospectively collected clinical cohorts with complete covariate data. Furthermore, the study did not address the potential for therapeutic resistance, emphasizing the importance of elucidating resistance mechanisms and developing strategies to counteract them. In conclusion, while this study represents a significant advancement, addressing these limitations through ongoing research is essential to translating these findings into more precise and effective treatments for patients with pancreatic cancer.

Conclusion

In summary, this study demonstrates that PCDH1 acts as an oncogene in pancreatic cancer, promoting cell invasion, migration, and EMT progression—evidenced by decreased E-cadherin and increased N-cadherin and vimentin—through activation of the NF-κB signaling pathway. Pharmacological inhibition of NF-κB reversed PCDH1-induced EMT, confirming the functional importance of this axis. These findings provide a theoretical basis for developing therapeutic strategies targeting PCDH1 and its related signaling pathways. Further studies could focus on the interaction of PCDH1 with other signaling pathways, in vivo validation, and the potential clinical application of PCDH1 as a prognostic biomarker and therapeutic target in pancreatic cancer treatment. Continuous research aims to provide more precise and effective treatments for pancreatic cancer patients, improving their quality of life and prolonging their survival.

Data Sharing Statement

The datasets generated during and/or analyzed during the current study are available in the [GEPIA 2] repository, [http://gepia2.cancer-pku.cn/#survival] and [proteinatlas] repository, [https://www.proteinatlas.org/ENSG00000156453-PCDH1/pathology], and other data are contained within the article.

Ethics Approval

The study was approved by the Ning Bo No.2 Hospital's medical ethics committee (NO.20230719) on July 19, 2023. All study participants or their legal representatives provided written informed consent prior to the commencement of the study, including consent for tissue sample collection, data analysis, and publication of de-identified research results. No personal identifying information of patients was disclosed in the study. The study was conducted in compliance with the Declaration of Helsinki and relevant national ethical guidelines for biomedical research involving human subjects.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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