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Girdin influences pancreatic cancer progression by regulating c-MYC expression

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Pancreatic ductal adenocarcinoma is a complex gastrointestinal tumor with high metastatic potential and poor prognosis. Actin-binding protein Girdin is highly expressed in a variety of tumors and promotes tumorigenesis and progression. However, the mechanisms underlying the involvement of Girdin in pancreatic cancer have not been clarified. In this study, we observed that the expression of Girdin was upregulated in pancreatic cancer cells. The siRNA-mediated gene knockdown experiments showed that reduced expression of Girdin in pancreatic cancer cells inhibited cell proliferation, migration, and invasion while promoting cell apoptosis. Functional assays revealed that c-MYC overexpression in pancreatic cancer cells could significantly increase the cell proliferation ability and rates of cell migration and invasion while decreasing the apoptosis rate. It has been shown that phosphorylation plays a role in the functional regulation of the c-MYC gene. Subsequently, we examined the expression level of c-MYC in cells with manipulated expression of Girdin and identified a positive correlation between Girdin expression and c-MYC expression. Moreover, we found that Girdin knockdown in c-MYC-overexpressing pancreatic cancer cells slowed cell growth, blocked the cell cycle progression, significantly promoted apoptosis, and markedly decreased the cell migration and invasion. This finding indicated that silencing Girdin could mitigate the effect of c-MYC on promoting proliferation and metastasis of pancreatic cancer. Overall, this study provided evidence that Girdin promoted pancreatic cancer development presumably by regulating the c-MYC overexpression.

Key words: pancreatic carcinoma, Girdin, proliferation, apoptosis, c-MYC

Pancreatic cancer remains one of the deadliest human cancers, with a 5-year survival rate of only 1–5% and a median survival of about 6 months despite treatment [1, 2]. As a major histologic subtype [3], ductal adenocarcinoma of the pancreas accounts for 90% of all cases of pancreatic cancer. While this type of pancreatic cancer is a highly complex and aggressive malignancy with early local invasion and metastasis, it is resistant to most therapies such as gemcitabine, leading to a poor prognosis of the cancer patients [4]. Therefore, it is of great significance to explore the pathogenesis of pancreatic cancer and develop new treatment methods.

Actin-binding protein Girdin is highly expressed in a variety of malignancies and is involved in tumor invasion and metastasis [5]. Girdin plays a key role in vascular remodeling during angiogenesis [6]. It has also been shown that the expression of Girdin is upregulated in hepatocellular carcinoma tissues, and Girdin knockout can inhibit the proliferation, migration, and invasion of hepatocellular carcinoma cells. Girdin was identified as a direct target of the tumor suppressor gene miR-101, and the downregulation of miR-101 can greatly restore Girdin knockout-mediated inhibition of hepatocellular carcinoma cells [7]. Girdin expression in patients with invasive breast cancer was found to be significantly associated with the tumor size, lymph node invasion, tumor stage, and estrogen and progesterone receptor expression [8]. To date, the mechanism that underlying the role of Girdin in pancreatic cancer has yet to be fully elucidated.

c-MYC is a multi-targeted transcription factor functioning in cell proliferation, mitochondrial biosynthesis, and glucose metabolism. Studies have shown that the c-MYC gene is overexpressed in various types of malignant tumors [9], and acts as a key oncogene involved in the development of pancreatic cancer [10]. c-MYC overexpression reduces the sensitivity of cancer cells to gemcitabine during pancreatic cancer chemotherapy [11, 12], leading to poor therapeutic

efficacy. Lu et al. demonstrated that mycro3, a small molecule inhibitor of c-MYC, could inhibit cell proliferation of transplanted pancreatic tumors in the mouse model [13]. Gharibi et al. found that lactate dehydrogenase A and c-MYC played a synergistic role in aerobic glycolysis, promoting pancreatic cancer progression [14]. Protein post-translational modifications, such as phosphorylation, acetylation, and ubiquitination, display significant changes during tumor development and play a key role in tumor migration and invasion. It has been shown that phosphorylation affects the function of the c-MYC gene [15]. Although the phosphorylation regulation of Girdin known as Akt-phosphorylation enhancer (APE) has been extensively studied, it remains to be determined whether Girdin can affect c-MYC expression. Thus, functional characterization of the interaction between Girdin and c-MYC may help improve targeted therapy for pancreatic cancer.

Materials and methods

Cell culture. Normal human pancreatic ductal epithelial cells (HPE6C7) and six pancreatic cancer cell lines (PANC-1, PANC-28, CAPAN-1, ASPC-1, BXPC-3, and MIAPACA-2) were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. The cells were digested with trypsin and washed with PBS. Thereafter, cell pellets for each cell line were collected by centrifugation. All cell lines were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (FBS) at 37 °C in a 5% humidified CO_2 incubator. After another digestion with 0.25% trypsin (GIBCO) and washing twice with PBS, cells were collected for subsequent analysis.

Cell transfection. The synthesis and screening of Girdin small interfering RNA (Girdin-siRNA), overexpressed Girdin, and overexpressed c-MYC gene were performed by Sailan Biotechnology Co., Ltd. (Hangzhou, China). Girdin-siRNA was transfected into BXPC-3 cells to silence the Girdin gene. Girdin overexpression plasmid was designed and constructed based on the sequence of Girdin. Capan-1 cells were transfected with Girdin overexpression plasmid. The target cell lines were digested with trypsin and washed with PBS one more time. Cell transfection was performed in a fresh and antibiotic-free medium at approximately 90% of fusion. Lipofectamine RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA) was used for transfection with Girdin-siRNAs or the overexpression plasmid according to the manufacturer's instructions. After 48 h of transfection, cells were harvested for western blot analysis and PCR assay.

The cell model of c-MYC overexpression was constructed by using the lentivirus stable transmutation technique. Briefly, BXPC-3 cells in the exponential growth phase were seeded into a six-well plate. When the degree of cell fusion reached 45%, the medium was replaced with 1/2 volume of 5 μg/ml polybrene, and an appropriate amount of virus (MOI=10) was added. After 4 h of growth, a half volume of the fresh medium was added; 24 h later, the liquid was changed. After 48 h of virus infection, 1 μg/ml puromycin was used to screen for viruses with puromycin resistance, and a large number of samples were amplified and stored. The stable c-MYC-overexpressing cell strains were verified by using western blot analysis. Transfection with Girdin-siRNA was conducted in c-MYC-overexpressing cells to construct cell strain with Girdin knockdown and c-MYC overexpression. No-load stable cell lines were generated as the experimental controls.

Western blot analysis. Each sample was re-suspended with an appropriate volume of lysis buffer containing PMSF (ST506, Beyotime Biotechnology). The samples were placed in 1.5 ml centrifuge tubes and lysed at 4 °C for 30 min, followed by centrifugation at 13,000×g for 10 min. The resulting supernatants were stored at −80 °C. The protein concentration was determined using the BCA protein assay kit (P0010, Beyotime Biotechnology). 30 μg protein of each sample was separated by 12% SDS-PAGE and electro-transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore). After being blocked with 5% skim milk for 2 h at 25 °C, the membrane was incubated with anti-calpain-1 primary antibody (ab108400, Abcam) at 4 °C overnight. Thereafter, the membrane was washed five times with TBST for 5 min each and then subjected to incubation with the secondary antibodies for 1 h at 25 °C. Protein bands were visualized using the enhanced chemiluminescence (ECL) and the data were analyzed by the ImageJ software.

Total RNA isolation and quantitative real-time PCR (qRT-PCR). Total RNA was isolated using Invitrogen™ TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and reverse transcribed into cDNAs according to the manufacturer's instructions (Takara Bio, Inc., Otsu, Japan). GAPDH was used as the internal reference. The primers were as follows: GAPDH, 5'-GGAGCGAGATCCCTCCAAAAT-3' and 5'-GGAGCGAGATCCCTCCAAAAT-3'; Girdin, 5'-AGGAAATGGGACCAACCTTGA-3' and 5'-GTGCATTCTAAGTGAGGCATCAT-3'; and c-MYC, 5'-GGCTCCTGGCAAAAGGTCA-3' and 5'-CTGCGTAGTTGTGCTGATGT-3'. The following thermal conditions were applied for qRT-PCR: denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s. The fluorescence signal for quantitative PCR analysis was counted and calculated by the software CFX Manager 3.1 attached to the PCR instrument. All cDNA primers were designed and synthesized by Sailanbiotechnology Co., Ltd. (Hangzhou, China).

CCK-8 assay. Cells were seeded into 96-well plates at a density of 0.4×10^4 cells/ml and incubated in an incubator at 37 °C until being completely adhered to the wall. CCK-8 solution was added into each well with the cell suspension in a ratio of 1:10 and the plates were incubated at 37 °C. The OD value at 450 nm was measured respectively after incubation

for 0, 24, 48, 72, and 96 h, while the cell growth curve was drawn based on the absorbance values.

Apoptosis assay. Pancreatic cancer cells were digested with trypsin and re-suspended in a fresh culture medium. Cells were resuspended with 1× Binding Buffer and adjusted to a density of 3–5×10^5 cells/ml. Then, 5 µl Annexin V-FITC was added to the cells. After 15 min of incubation in the dark, another 5 µl Annexin V-FITC was added, and the cells were cultured for an additional 5 min. Finally, 200 µl of 1× Binding Buffer was added and the cells were transferred to the flow tube for the assay.

Cell cycle analysis. Aliquots of cells from the control and synchronized cultures were collected and suspended in 70% ethanol overnight at –20 °C. After being washed with PBS, fixed cells were suspended in 4 mM sodium citrate containing 30 U/ml RNase A, 0.1% Triton X-100, and 50 µg/ml propidium iodide (PI). Flow cytometric analysis was conducted after 30 min of incubation at 37 °C in the dark. Data were collected using a FACScan apparatus (BD Biosciences, San Jose, CA, USA) and analyzed by BD CellQuest software.

Cell migration and invasion assays. For the migration and invasion assays, cells were inoculated at a density of 5×10^4 cells/well and allowed to migrate for 24 h. 100 µl of cell suspension (in a serum-free medium) and 600 µl of 10% FBS-containing medium as chemoattractant were added respectively into the upper chamber and lower chamber of Transwell. The Transwell chambers were incubated at 37 °C in a humid environment with 5% CO_2. After 24 h of incubation, the chambers were removed from the incubator, and non-migrating cells in the upper chambers were wiped off with cotton swabs. The membranes were air-dried and treated with 500 µl of 0.1% crystal violet at 37 °C for 30 min. Subsequently, the samples were washed and the number of migratory cells was counted under a microscope at ×200 magnification.

Statistical analysis. All data was analyzed using SPSS statistical software (version 13.0, Chicago, IL, USA). Statistical significance was determined by using the analysis of variance (ANOVA). p-value <0.05 was considered statistically significant.

Results

Knocking down and overexpressing Girdin gene in the pancreatic cancer cell lines. Given that BXPC-3 cells display a relatively high expression of Girdin and c-MYC among all pancreatic cancer cell lines, we chose to perform a Girdin knockdown experiment in BXPC-3 cells. In the experiment, three different Girdin small interfering RNAs (siRNA1, siRNA2, and siRNA3) were transfected into BXPC-3 cells, while transfection with non-functional siRNA (siNC) was included as a control. As shown in Figure 1A, the expression of Girdin was significantly reduced in the three Girdin-siRNA groups compared with the control

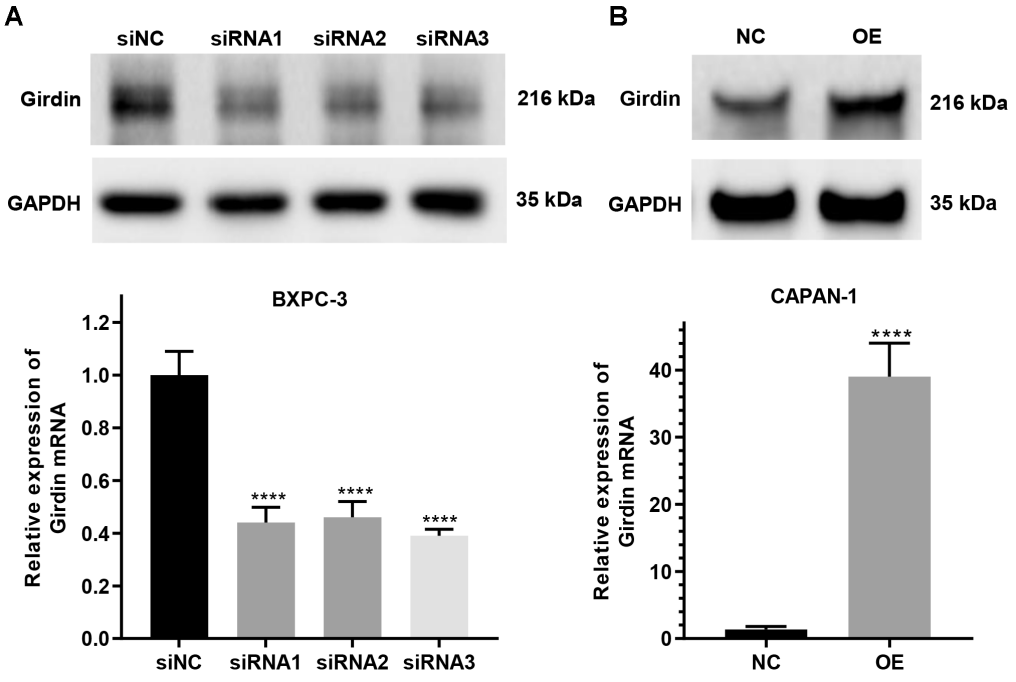

Figure 1. Manipulated expression of Girdin in pancreatic cancer cells. A) BXPC-3 cells were transfected with siNC, siRNA1, siRNA2, and siRNA3, respectively. The expression level of Girdin in transfected cells was detected by western blot analysis and RT-PCR, respectively. Human GAPDH was included as an internal reference. B) CAPAN-1 cells were transfected with Girdin-overexpressing plasmid (OE) and pcDNA (NC). The expression level of Girdin in transfected cells was detected by western blot analysis and RT-PCR, respectively. Human GAPDH was included as an internal reference. ****p<0.0001, vs. siNC or NC group.

group, indicating a successful knockdown of Girdin in BXPC-3 cells. Thus, cells transfected with the siRNA1 were selected for subsequent experiments in this study. In the meantime, based on the low level of the Girdin protein in CAPAN-1 cells, we undertook the Girdin overexpression experiment in this pancreatic cancer cell line. From Figure 1B, the expression of Girdin in the OE group was significantly upregulated by 38 times compared with the NC group, indicating the success of cell transfection with the overexpression plasmids. In the experiment, untreated CAPAN-1 cells and cells transfected with the plain vector (pcDNA) were used as the blank group (CK) and negative control group (NC), respectively.

Role of Girdin in regulating proliferation, apoptosis, cell cycle progression, migration, and invasion of pancreatic cancer cells. We performed a CCK-8 assay to measure the proliferation of pancreatic cancer cells with reduced expression or overexpression of Girdin. As illustrated in Figure 2A, after 96 h of transfection, the siGirdin group exhibited a significantly lower rate of cell proliferation than the siNC and CK groups ($p<0.01$), while the cell proliferation was increased in the OE as compared to the NC ($p<0.01$). These observations indicated that Girdin knockdown inhibited the proliferation of BXPC-3 cells, whereas the proliferation of CAPAN-1 cells was promoted by Girdin overexpression.

We next analyzed apoptosis by flow cytometry. As shown in Figure 2B, while apoptosis was markedly increased in BXPC-3 cells transfected with Girdin-siRNA (siGirdin) compared with the siNC ($p<0.0001$), CAPAN-1 cells overexpressing Girdin (OE) displayed a significantly lower rate of apoptosis than the NC group ($p<0.01$).

Furthermore, we examined if Girdin knockdown or overexpression affects the cell cycle distribution in pancreatic cancer cells. Flow cytometry showed that the proportion of cells in the G1 phase was significantly increased in siGirdin compared with the siNC ($p<0.01$), while the proportion of S phase cells in siGirdin was significantly lower than that in siNC ($p<0.01$, Figure 2C). In the meantime, we observed that while the proportion of G1 phase cells in OE was remarkably lower than that in either CK or NC ($p<0.01$), a slight elevation in the proportion of S phase cells was evident in OE ($p>0.05$, Figure 2C). These data demonstrated that Girdin knockdown in pancreatic cancer cells could trigger a cell cycle arrest in the G1 phase, whereas Girdin overexpression accelerated the cell cycle progression.

To assess the effects of manipulated expression of Girdin on migration and invasion of pancreatic cancer cells, the Transwell assays were performed on both BXPC-3 and CAPAN-1 cells. As shown in Figure 3A, the cell migration and invasion were markedly decreased in the siGirdin compared with the siNC ($p<0.01$). On the contrary, a significant increase in the migration and invasion of CAPAN-1 cells was detected in the OE as compared to the NC ($p<0.01$, Figure 3B). Together, these observations indicated that while reduced expression of Girdin inhibited the migration and invasion of pancreatic cancer cells, Girdin overexpression promoted the cell migration and invasion.

The expression pattern of Girdin and c-MYC among the pancreatic cancer cell lines. We carried out western blot analysis and qRT-PCR assay to examine the protein and mRNA expressions of Girdin and c-MYC in human pancreatic duct epithelial cells HPE6C7 as well as the in six pancreatic cancer cell lines. As shown in Figure 4A, among all cells examined, the highest and the lowest expressions of Girdin protein were detected in ASPC-1 cells and CAPAN-1 cells, respectively. Notably, the expression pattern of c-MYC protein was in accordance with that of Girdin protein among those different types of pancreatic cell lines.

Correlation of Girdin expression with c-MYC expression in pancreatic cancer cells. We further examined if the manipulated expression of Girdin affects the c-MYC expression in pancreatic cancer cells. As revealed by western blot analysis and qRT-PCR, the expression of c-MYC was decreased in the siGirdin as compared to the siNC, while the OE had a significantly higher level of c-MYC protein than the NC (Figure 4B). These data suggested that Girdin knockdown led to reduced expression of c-MYC, whereas Girdin overexpression increased the c-MYC expression.

Girdin knockdown inhibited the role of c-MYC in promoting pancreatic cancer proliferation and metastasis. To further investigate the relationship between Girdin and c-MYC, we first constructed a c-MYC-overexpressing BXPC-3 cell line by the lentiviral stable transformation technique. Then, the transfection of c-MYC-overexpressing cells with Girdin small interfering RNA (siRNA1) was performed to knock down Girdin in the c-MYC-overexpressing cells. For subsequent experiments, the following groups of cells were used: 1) no-load stable cell strain (pcDNA+siNC) (cells as a control group); 2) no-load Girdin knockdown stable cell strain (pcDNA+siGirdin) (cells with reduced expression of Girdin only); 3) c-MYC-overexpressing stable cell strain (c-MYC+siNC) (cells with c-MYC overexpression only); and 4) co-transfected stable cell strain (c-MYC+siGirdin) (cells with reduced expression of Girdin and c-MYC overexpression).

CCK-8 assay was conducted to comparatively analyze the rate of cell survival among the different groups of cells. As depicted in Figure 5A, the cell growth of the c-MYC+siNC group and that of the pcDNA+siNC group at 96 h were 7.08±0.12 and 3.66±0.05, respectively. Moreover, we observed that the proportions of the G1 phase and S phase cells in the pcDNA+siNC group were 58.13±2.16% and 40.85±1.8% respectively, while the proportions of the G1 phase and S phase cells in the c-MYC+siNC group were 41.35 ± 0.16% and 55.00±0.68%, respectively (Figure 5B). The above data suggested that the c-MYC overexpression shortened the G1 phase and prolonged the S phase ($p<0.05$), thus promoting cell proliferation. Flow cytometry showed that apoptosis was decreased in the c-MYC+siNC group compared with the pcDNA+siNC group (Figure 5C). Besides, the Transwell

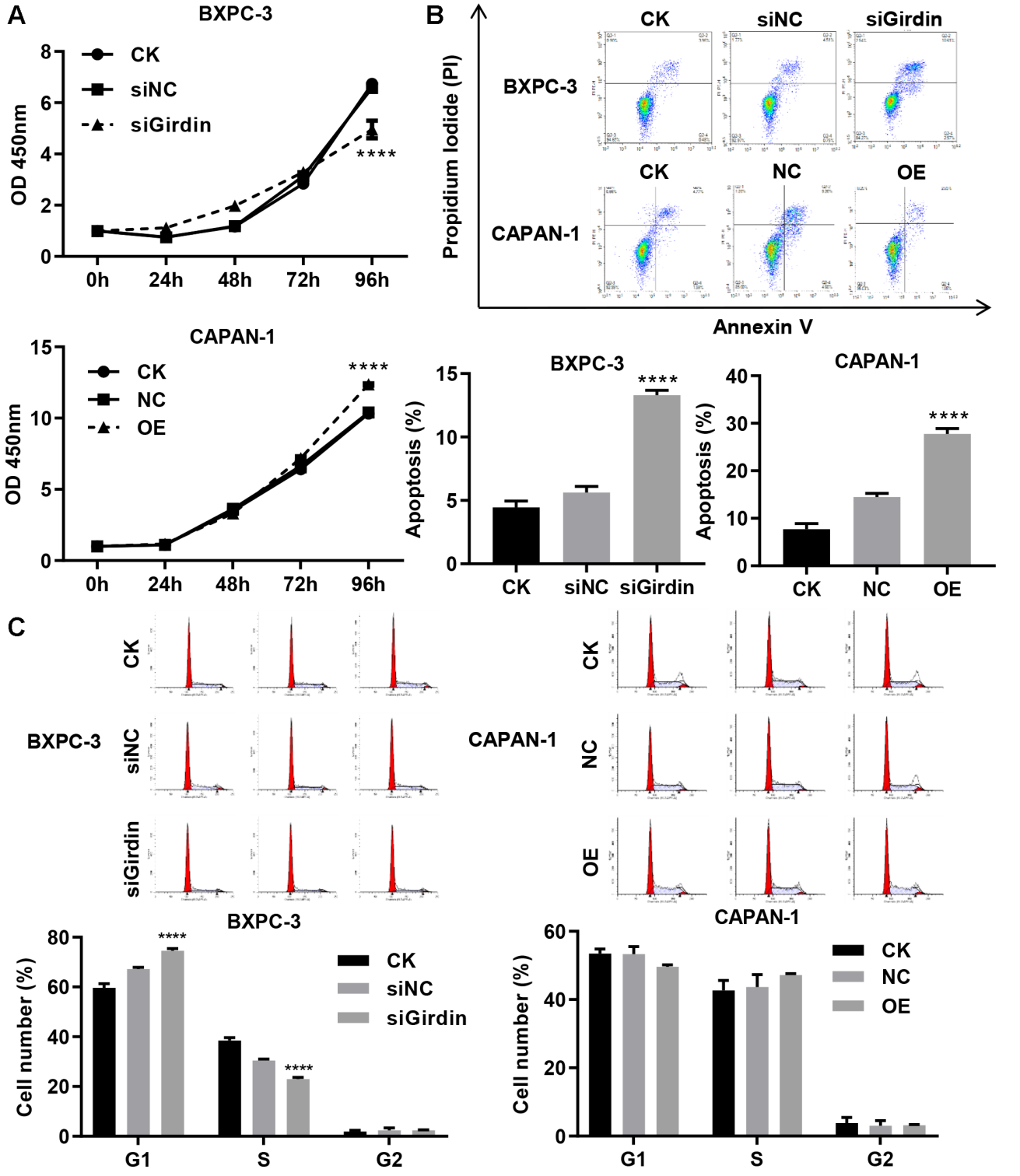

Figure 2. Girdin regulates proliferation and apoptosis in pancreatic cancer. The following groups of transfected cells were used in the experiment: siGirdin (BXPC-3 cells transfected with Girdin-siRNA1), siNC (BXPC-3 cells transfected with non-functional siRNA), OE (CAPAN-1 cells transfected with Girdin overexpression plasmid, NC (CAPAN-1 cells transfected with pcDNA), and CK (untreated BXPC-3 cells or CAPAN-1 cells). A) The cell proliferation rates were measured by CCK-8 assay. B) PI staining analysis was performed to assess apoptosis in the indicated groups of cells. Note that while a significantly higher level of apoptosis was detected in the siGirdin group compared with the siNC group, the OE group displayed a significantly lower level of apoptosis than the NC group. C) The cell cycle distribution in the indicated groups was analyzed using flow cytometry. ****p<0.0001, vs. siNC or NC group.

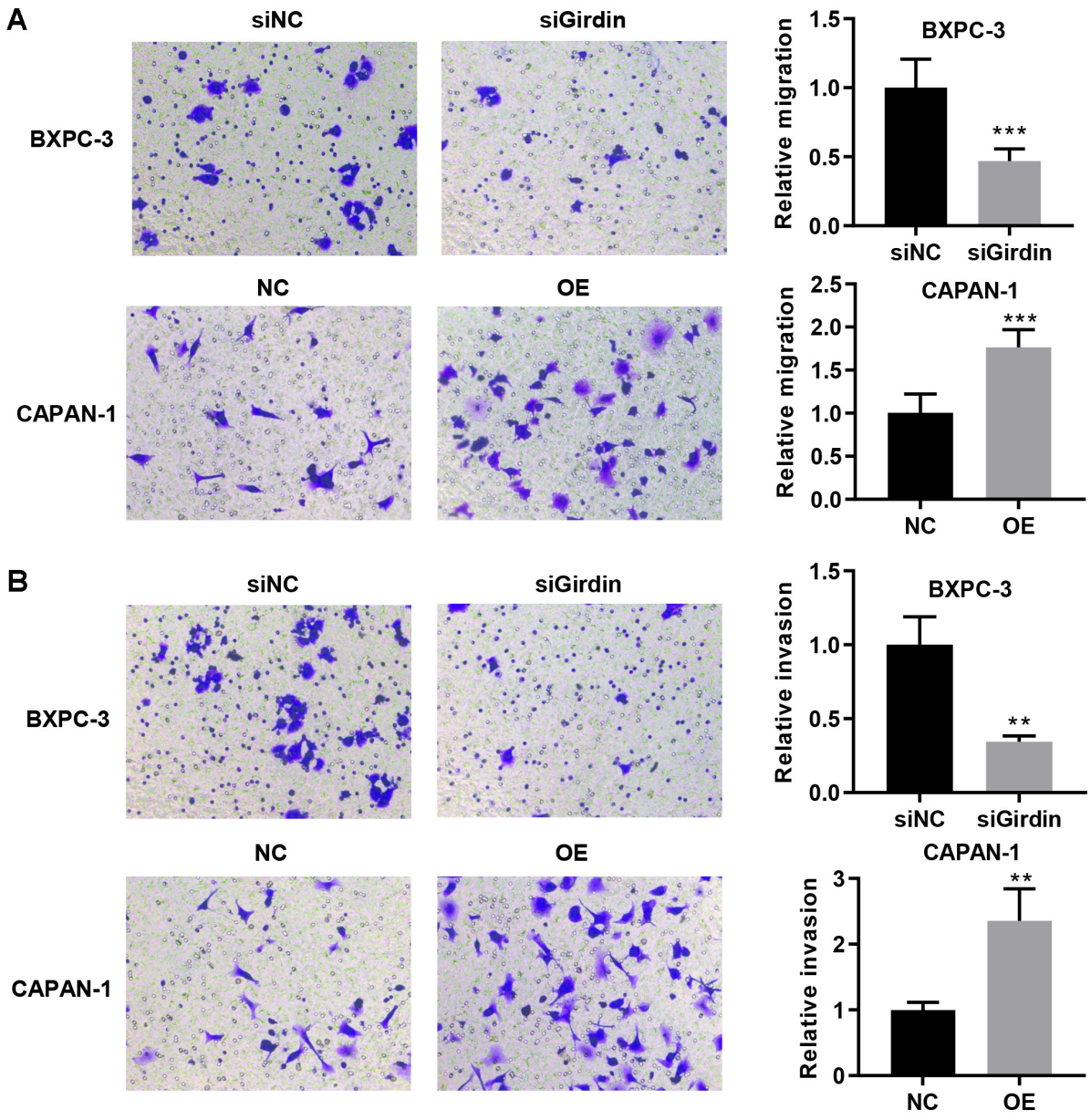

Figure 3. Girdin regulates migration and invasion in pancreatic cancer. A) The Transwell assays were conducted to assess the cell migration in the indicated groups. B) The Transwell assays were conducted to assess the cell invasion in the indicated groups. **p<0.01, ***p<0.001, vs. siNC or NC group.

assays revealed that compared with the no-load stable cell strains (pcDNA+siNC and pcDNA+siGirdin), the cell migration and invasion were increased in c-MYC stable cell strain (c-MYC+siNC and c-MYC+siGirdin) (Figure 5D). Based on the above observations, we reasoned that the c-MYC overexpression in pancreatic cancer cells promoted cell proliferation, migration, and invasion while inhibiting cell apoptosis.

We next performed similar comparative studies between c-MYC+siNC and c-MYC+siGirdin groups. As shown in Figures 5A–5D, slower cell growth, cell cycle arrest, markedly increased apoptosis rate, and a significant decrease in cell migration and invasion were detected in the c-MYC+siGirdin group compared with the c-MYC+siNC group. This finding suggested that reduced expression of Girdin could inhibit the role of c-MYC in promoting pancreatic cancer progression.

Discussion

Girdin is an actin-binding protein containing 1,870 amino acid residues, which was first reported in 2005. It has been shown that while Girdin is ubiquitously expressed in human tissues and cells, it is involved in remodeling actin cytoskel-

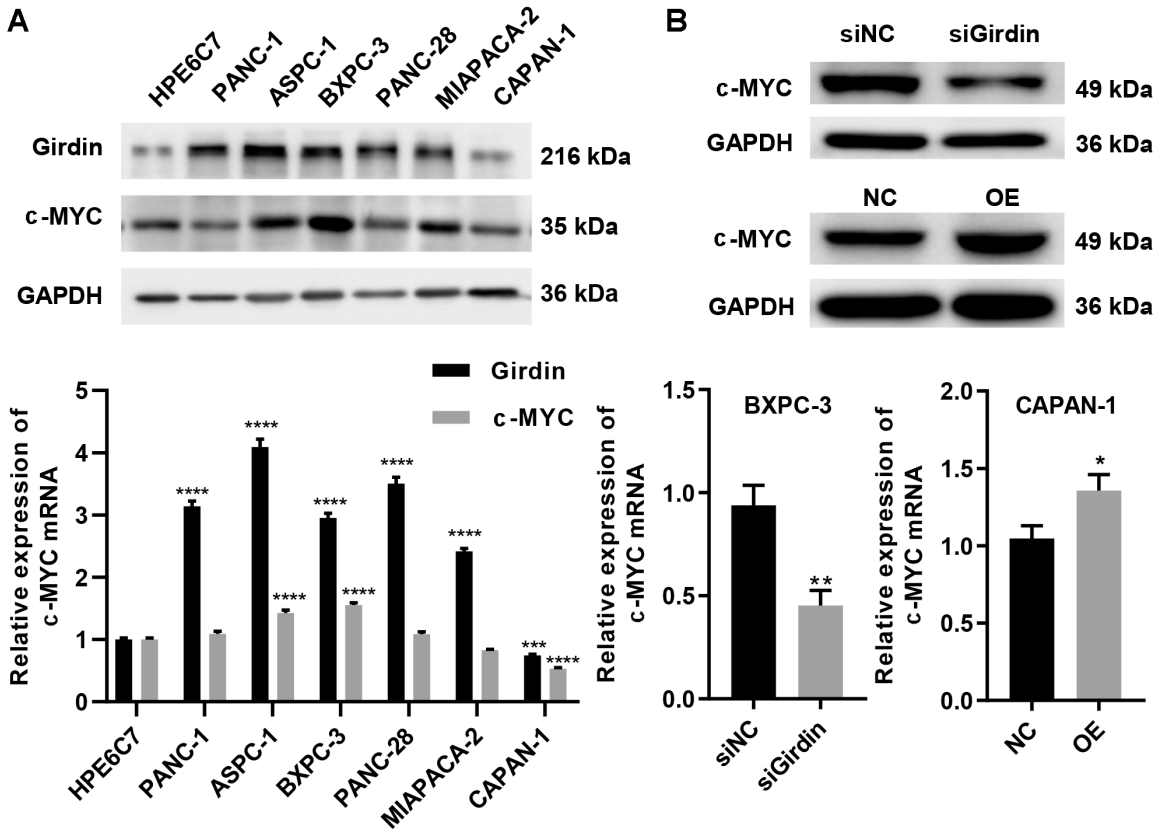

Figure 4. Correlation of Girdin expression with c-MYC expression in pancreatic cancer cells. A) The expression levels of Girdin and c-MYC in HPE6C7 normal pancreatic cells and pancreatic cancer cell lines were measured by western blot analysis and RT-PCR, respectively. ***p<0.001, ****p<0.0001, vs. HPE6C7 group. B) BXPC-3 cells were transfected with siRNA1 (siGirdin) or non-functional siRNA (siNC), while CAPAN-1 cells were transfected with Girdin overexpression plasmid (OE) or pcDNA (NC). The expression level of Girdin in the indicated groups was assayed by western blot analysis and RT-PCR, respectively. *p<0.05, **p<0.01, vs. siNC or NC group.

eton and regulating cell movement as well as the migration of neural stem cells, endothelial cells, and smooth muscle cells [16]. Multiple studies have demonstrated that the expression of Girdin is upregulated in a variety of tumors such as breast cancer, colon cancer, esophageal cancer, hepatocellular carcinoma, and glioblastoma, suggesting the involvement of Girdin in tumor cell proliferation and migration via intracellular signal transduction [16–22]. For example, it was found that the level of Girdin in tumor specimens of non-small cell lung cancer (NSCLC) is significantly higher than that in the adjacent normal lung tissue, and there is a significant correlation between the Girdin overexpression and tumor vascular invasion [23]. In hepatocellular carcinoma, tumor tissues displayed a higher expression level of Girdin than the corresponding normal tissues, and the methylation level of the Girdin gene was positively correlated with the gene expression level. Moreover, abundant immune infiltration was identified in hepatocellular carcinoma tissues with high expression of Girdin [24].

In this study, we investigated the expression of Girdin in pancreatic cancer cells as well as the effects of Girdin on proliferation, apoptosis, migration, and invasion of pancreatic cancer cells. Evidently, we showed that silencing Girdin gene inhibited the proliferation, migration, and invasion of pancreatic cancer cells and promoted apoptosis, while Girdin overexpression led to promoted cell proliferation, decreased apoptosis, and increased cell migration and invasion in the pancreatic cancer cell lines, which was consistent with another study [25]. Regarding the mechanism by which Girdin regulated the occurrence and development of pancreatic cancer, Wang et al. believed that Girdin might regulate pancreatic cancer development through the phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) signaling pathway [25]. Yang et al. suggested that Girdin regulated cortactin phosphorylation and acetylation thereby affecting pancreatic cancer progression [26]. Girdin might also promote the progression of pancreatic cancer via various ways such as intracellular signal transduction, regulation of cytoskeleton connection, and so on. Therefore, the specific underlying mechanism of Girdin in regulating pancreatic cancer still needs to be further investigated.

c-MYC is a transcription factor closely related to cell proliferation, migration, and apoptosis. It plays an important role in regulating diverse signaling pathways such as WNT,

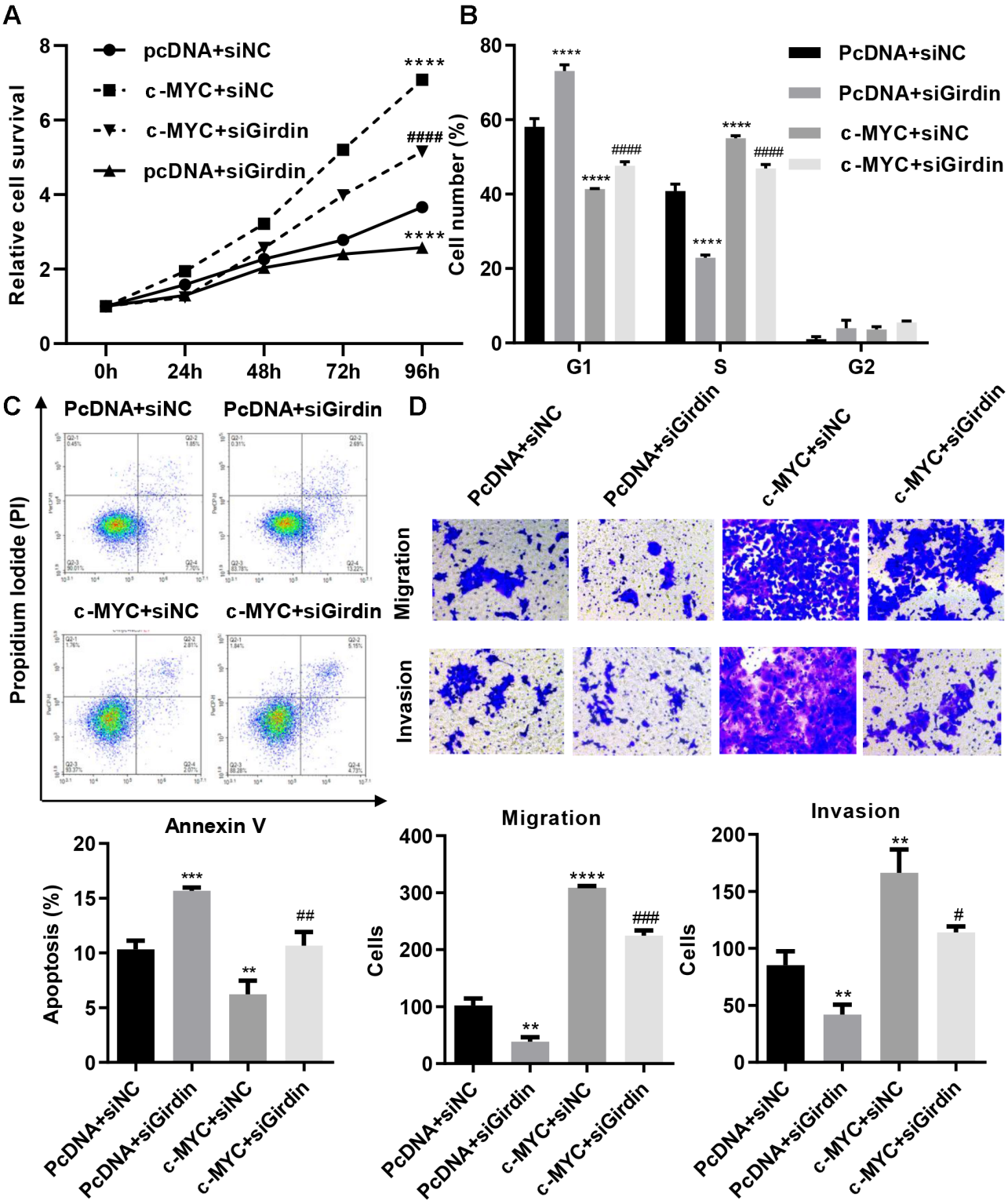

Figure 5. Girdin knockdown inhibited the role of c-MYC in promoting pancreatic cancer metastasis. A) The cell proliferation rates in the indicated groups of cells were measured by the CCK-8 assay. B) The cell cycle distributions in the indicated groups were analyzed using flow cytometry. C) PI staining analysis was performed to assess apoptosis in the indicated groups of cells. D) The transwell assays were conducted to measure the cell migration and invasion in the indicated groups. **p<0.01, ***p<0.001, ****p<0.0001, vs. pcDNA+siNC group; #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001, vs. c-MYC+siNC group.

TGF-β, and receptor tyrosine kinase related pathways [27], thus affecting various biological functions, including cell cycle regulation, apoptosis, protein synthesis, and cell senescence [28, 29]. Schleger et al. performed fluorescence in situ hybridization and immunohistochemistry to analyze the expression of c-MYC in different types of pancreatic cancer. In the study, they found that c-MYC was mainly involved in the progression of early pancreatic cancer, rather than the local spread of invasive tumors [30]. It has also been shown that aberrant expression of β-catenin at pancreatic cancer cell boundary leads to extracellular matrix degradation and uncontrolled cell proliferation and differentiation by upregulating the expression of c-MYC [31].

In the present study, we showed that the c-MYC overexpression could increase the proliferation ability, migration, and invasion rate of pancreatic cancer cells, while decreasing the rate of cell apoptosis, indicating a role of c-MYC in promoting pancreatic cancer progression. While both Girdin and c-MYC play important roles in the development of pancreatic cancer, the relationship between these two factors needs to be further clarified. In this study, we examined the expression of Girdin and c-MYC in human normal pancreatic cells and six pancreatic cancer cell lines. Compared with the normal pancreatic cells, the expression of Girdin and c-MYC was decreased in CAPAN-1 cancer cells but increased in the other five pancreatic cancer cell lines. This observation suggested that the expression pattern of Girdin and c-MYC in pancreatic cancer cells remains consistent. Furthermore, we analyzed the expression level of c-MYC in Girdin-overexpressing or Girdin knockdown cells. The analysis revealed that reduced expression of Girdin led to a decrease in the expression of c-MYC, whereas Girdin overexpression upregulated c-MYC expression. This finding suggests a regulatory relationship between c-MYC and Girdin in the cancer cells. Thus, we further functionally characterized the relationship between the two factors by using a number of cell models with the manipulated expression of c-MYC and Girdin. In the experiments, we observed that Girdin knockdown inhibited the promoting effect of c-MYC on the development of pancreatic cancer, suggestive of a regulatory role of Girdin in the biological function of c-MYC. Collectively, this study presented evidence that c-MYC is likely to be an important regulatory target of Girdin, and Girdin overexpression in pancreatic cancer tissues may promote pancreatic cancer progression through regulating c-MYC.

The regulatory role of Girdin phosphorylation has been extensively studied. Platelet phagocytosis was found to retard endothelial cell senescence through Girdin and its phosphorylation [32]. Furthermore, phosphorylation of Girdin could modulate the cardiac repair process following acute myocardial infarction [33]. Several studies have shown that certain serine and threonine residues near the middle region of c-MYC may be phosphorylated, and thus affect c-MYC function. Thus, c-MYC might be a phosphorylated transcription factor [15], and we further hypothesized that Girdin regulated c-MYC presumably via phosphorylation, but its regulatory mechanism remains to be further clarified by experiments.

In conclusion, we showed that Girdin might promote the development of pancreatic cancer by regulating the expression and function of c-MYC. This study identified a potential target for pancreatic cancer therapy.

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