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ORIGINAL ARTICLE

Dickkopf3 overexpression inhibits pancreatic cancer cell growth *in vitro*

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Abstract

AIM: To elucidate the role of dickkopf3 (Dkk3) in human pancreatic cancer cell growth.

METHODS: Dkk3 mRNA and protein expression in human pancreatic cancer cell lines were detected by real-time reverse transcription polymerase chain reaction (real-time RT-PCR), Western blotting and immunofluorescence. Methylation of the Dkk3 promoter sequence was examined by methylation-specific polymerase chain reaction (MSP) and Dkk3 mRNA expression was determined by real-time RT-PCR after 5-aza-2′-deoxycytidine (5-aza-dC) treatment. The effects of Dkk3 on cancer cell proliferation and *in vitro* sensitivity to gemcitabine were investigated by CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) after transfecting the Dkk3 expression plasmid into human pancreatic cancer cells. The expression of β-catenin, phosphorylated extracellular signal-regulated protein kinases (pERK) and extracellular signal-regulated protein kinases (ERK) was also examined by real-time RT-PCR and Western blotting after upregulating Dkk3 expression in human pancreatic cancer cells.

RESULTS: The results show that the expression levels of both Dkk3 mRNA and protein were low in all pancreatic cancer cell lines tested. The Dkk3 promoter sequence was methylated in the MIA PaCa-2 and AsPC-1 cell lines, which showed reduced Dkk3 expression. These two cell lines, which initially had a methylated Dkk3 promoter, showed increased Dkk3 mRNA expression that was dependent upon the dosage and timing of the DNA demethylating agent, 5-aza-dC, treatment ($P < 0.05$ or $P < 0.01$). When Dkk3 expression was upregulated following the transfection of a Dkk3 expression plasmid into MIA PaCa-2 cells, the ability of cells to proliferate decreased ($P < 0.01$), and the expression of β-catenin and pERK was downregulated ($P < 0.01$). Sensitivity to gemcitabine was enhanced in Dkk3 expression plasmid-transfected cells.

CONCLUSION: Our findings, for the first time, implicate Dkk3 as a tumor suppressor in human pancreatic cancer, through the downregulation of β-catenin expression *via* the ERK-mediated pathway.

Key words: Cell growth; Dickkopf3; *In vitro*; Overexpression; Pancreatic cancer

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INTRODUCTION

Pancreatic cancer is the sixth leading cause of cancer death in China[1]. The overall five-year survival rate is approximately 1%-3%, and the median survival period after diagnosis is only 4 to 5 mo. Pancreatic cancer remains one of the most aggressive human cancers, with an exceedingly poor prognosis because of its late onset of symptoms[2], rapid progression, frequent metastasis and insensitivity to chemotherapy and radiotherapy. Therefore, recognizing the factors associated with pancreatic cancer progression is critical for its treatment.

Dickkopf (Dkk) family proteins, including Dkk1/2/3/4, are secreted modulators of the canonical Wnt signaling pathway[3]. Dkk1, Dkk2 and Dkk4, antagonists of Wnt signaling[4,5], interact with Wnt coreceptors, low-density lipoprotein receptor-related protein 5/6 (LRP5/6) and Kremen[6,7]. Dkk3 interacts with kremen1 and kremen2, but not with LRP5/6[8], and has been proposed to act as a tumor suppressor. Dkk3 is downregulated in some tumors, and it inhibits tumor growth[9-25]. For example, in cervical cancer and malignant glioma, Dkk3 regulates tumor cell growth and decreases β-catenin expression[16,23]. Dkk3 can induce cancer cell apoptosis by c-Jun-NH2-kinase (JNK) activation in testicular and prostate cancer cells[9,26]. The Dkk3 promoter sequence is methylated in several tumors, such as breast cancer, hepatoma, bladder cancer and malignant astrocytic gliomas[27-32]. In lung adenocarcinomas, however, Dkk3 inhibits cancer cell apoptosis by decreasing the intracellular level of reactive oxygen species and functions as an oncogene[33]. Dkk3 knock-out mice showed no enhanced tumor formation[34]. Recently, other studies have demonstrated that Dkk3 plays distinct roles in different cells[8].

To date, no study has investigated Dkk3 expression and its roles in human pancreatic cancer cell behavior. To better understand the role of Dkk3 in pancreatic cancer progression, we investigated Dkk3 expression and promoter sequence methylation in human pancreatic cancer cells. The effects of Dkk3 on cell proliferation and sensitivity to gemcitabine were simultaneously observed after expression was increased in MIA PaCa-2 cells, following transfection with the Dkk3 expression plasmid.

MATERIALS AND METHODS

Cell lines and cell culture

The human pancreatic cancer cell lines PANC-1, MIA PaCa-2, AsPC-1 and BxPC-3 were purchased from the American Type Culture Collection (Manassas, Virginia, United States). AsPC-1 and BxPC-3 cells were cultured in RPMI-1640 medium (Sigma-Aldrich, MO, United States) and PANC-1 and MIA PaCa-2 cells were cultured in Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, MO, United States). All media were supplemented with 10% fetal calf serum (Tianjin Haoyang Biological Manufacture Co., LTD, China), 100 μg/mL streptomycin and 100 U/mL penicillin, and the cultures were grown at 37 ℃ in a humidified atmosphere containing 5% CO_2.

Construction of and transient transfection with a plasmid expressing human Dkk3

Total RNA was extracted from PANC-1 cells using TRIzol reagent (Invitrogen, CA, United States), according to the manufacturer's protocol. The cDNAs were synthesized using the TaKaRa RNA polymerase chain reaction (PCR) Kit (TaKaRa, Japan). A full-length cDNA encoding human Dkk3 was cloned by PCR using 500 ng cDNA as a template and primers containing HindIII and BamHI restriction enzyme sites (Table 1). The PCR products were ligated into pcDNA3.1 (Invitrogen, CA, United States) to create the plasmid pcDNA3.1-Dkk3. MIA PaCa-2 cells were transfected with the pcDNA3.1 vector or pcDNA3.1-Dkk3 using FuGENE (Roche Diagnostic GmbH, Mannheim, Germany), according to the manufacturer's protocol.

Reverse transcription polymerase chain reaction

Total RNA was isolated from the cells using TRIzol reagent (Invitrogen, CA, United States) according to the manufacturer's protocol. The cDNAs were synthesized using the TaKaRa RNA PCR Kit (TaKaRa, Japan). The optimal PCR conditions were 94 ℃ for 5 min; 35 cycles at 94 ℃ for 40 s, 61 ℃ (Dkk3)/52 ℃ (β-actin) for 40 s, 72 ℃ for 40 s; and 72 ℃ for 10 min. PCR products (5 μL) were separated by electrophoresis in a 2.0% agarose gel. Primer sequences for Dkk3 and β-actin are listed in Table 1.

RNA preparation and real-time reverse transcription polymerase chain reaction

Total RNA was isolated from the cells, with or without 5-aza-2'-deoxycytidine (5-aza-dC) treatment, using TRIzol reagent (Invitrogen, CA, United States) according to the manufacturer's protocol. First-strand cDNA was synthesized from 500 ng of total RNA using the TaKaRa RNA PCR Kit (TaKaRa, Japan). PCR was conducted on a 7500 Real Time PCR System (Applied Biosystems, United Kingdom) in combination with the SYBR green PCR master mix (Applied Biosystems, United Kingdom). Melting curve analyses following amplification were performed to ensure product specificity. The relative expression levels of Dkk3 mRNA and β-catenin mRNA were normalized to mRNA level of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the same cDNA sample. ΔCt was calculated by subtracting the Ct of GAPDH mRNA from the Ct of the mRNA of interest. ΔΔCt was then calculated by subtracting the ΔCt of the control from the ΔCt of the sample. The fold change in mRNA was calculated according to the equation $2^{-\Delta\Delta Ct}$. Primer sequences for Dkk3, β-catenin and GAPDH are listed in Table 1.

Table 1 Oligonucleotide primers used in the study

	Sequence (5′ to 3′)	T_A (℃)	Cycles
PCR			
Dkk3 (full-length)	Forward: CCCAAGCTTATGCAGCGGCTTGGGGC Reverse: CGCGGATCCCTAAATCTCTTCCCCTCCCAGCAGT	53	35
Real-time RT-PCR			
Dkk3	Forward: ACAGCCACAGCCTGGTGTA Reverse: CCTCCATGAAGCTGCCAAC	60	40
β-catenin	Forward: AAAATGGCAGTGCGTTTAG Reverse: TTTGAAGGCAGTCTGTCGTA	60	40
GAPDH	Forward: GCACCGTCAAGGCTGAGAAC Reverse: GCCTTCTCCATGGTGGTGAA	60	40
RT-PCR			
Dkk3	Forward: AAGGCAGAAGGAGCCACGAGTGC Reverse: GGCCATTTTGGTGCAGTGACCCCA	61	35
β-actin	Forward: AAATCGTGCGTGACATTAA Reverse: CTCGTCATACTCCTGCTTG	52	35
MSP			
Dkk3 unmethylated	Forward: TTAGGGGTGGGTGGTGGGGT[32] Reverse: CTACATCTCCACTCTACACCCA[32]	59	34
Dkk3 methylated	Forward: GGGCGGGCGGCGGGGC[32] Reverse: ACATCTCCGCTCTACGCCCG[32]	59	34

T_A: Annealing temperature; Real-time RT-PCR: Real-time reverse transcription polymerase chain reaction; Dkk3: Dickkopf3; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; MSP: Methylation-specific polymerase chain reaction.

Bisulfite modification and methylation-specific polymerase chain reaction

Genomic DNA was isolated from pancreatic cancer cell lines using the TIANamp Genomic DNA kit (Tiangen Biotech Co., LTD, Beijing, China). One microgram of genomic DNA was bisulfite-modified using the CpGenome™ DNA Modification Kit (Chemicon, MA, United States) according to the manufacturer's protocol. Methylation-specific polymerase chain reaction (MSP) was performed at 95 ℃ for 5 min, followed by 34 cycles at 94 ℃ for 30 s, 59 ℃ for 30 s and 72 ℃ for 30 s. The final extension was at 72 ℃ for 10 min. Each PCR reaction was performed using 0.5 units of HotStarTaq Plus DNA Polymerase (Qiagen GmbH, Hilden, Germany). The primers are listed in Table 1. The specificity of the MSP primers in detecting the Dkk3 methylation status was demonstrated using unmethylated and methylated DNA as a template (EpiTect Control DNA Set; Qiagen GmbH, Hilden, Germany).

5-aza-dC treatment

Cells were seeded at a density of 4×10^4 cells/well in a six-well plate. After overnight incubation, the cells were treated with 10 μmol/L and 20 μmol/L of the DNA demethylating agent 5-aza-dC (Sigma-Aldrich, Steinheim, Germany) for 48 h or 72 h. Control cells were incubated with dimethyl sulfoxide and fresh medium.

Immunofluorescence and confocal microscopy

Cells grown on coverslips were washed and fixed with 4% paraformaldehyde, followed by washing with 0.2% Triton X-100. Coverslips were incubated with nonimmune animal serum to reduce nonspecific binding. The coverslips were subsequently incubated at 4 ℃ overnight with an anti-Dkk3 rabbit polyclonal antibody (1:100, Santa Cruz, CA, United States). Rhodamine-conjugated AffiniPure goat anti-rabbit IgG was used as the secondary antibody (1:200, Zhongshan Goldenbridge Biotechnology Co., LTD, Beijing, China). Counterstaining was performed using 1 μg/mL 4',6-diamidino-2-phenylindole. Expression and localization of Dkk3 were observed under a confocal microscope (Leica, Mannheim, Germany).

Western blotting

The cells in culture were washed twice with ice-cold PBS, and proteins were extracted with M-PER mammalian protein extraction reagent (Pierce Biotechnology, Rockford, United States). Samples were centrifuged at 14 000 × *g* for 10 min. Aliquots of cell lysates containing 40 μg protein were separated on a 12% SDS-polyacrylamide gel and transferred to PVDF membranes (Millipore, MA, United States). The membranes were blocked with 10% skim milk and incubated with Dkk3 antibody (1:1500, Santa Cruz, CA, United States), β-catenin antibody (1:1500, BD Transduction Laboratories, San Diego, United States), phosphorylated extracellular signal-regulated protein kinase antibody (pERK antibody, 1:2000, Cell Signaling, MA, United States), extracellular signal-regulated protein kinase antibody (ERK antibody, 1:2000, Cell Signaling, MA, United States) and β-actin antibody (1:2000, Santa Cruz, CA, United States) at 4 ℃ overnight, followed by their corresponding secondary antibodies (1:2000, Zhongshan Goldenbridge Biotechnology Co., LTD, Beijing, China) at room temperature for 2 h. The membrane-bound proteins were detected using the Pierce ECL Western blotting substrate (Pierce Biotechnology, Rockford, United States).

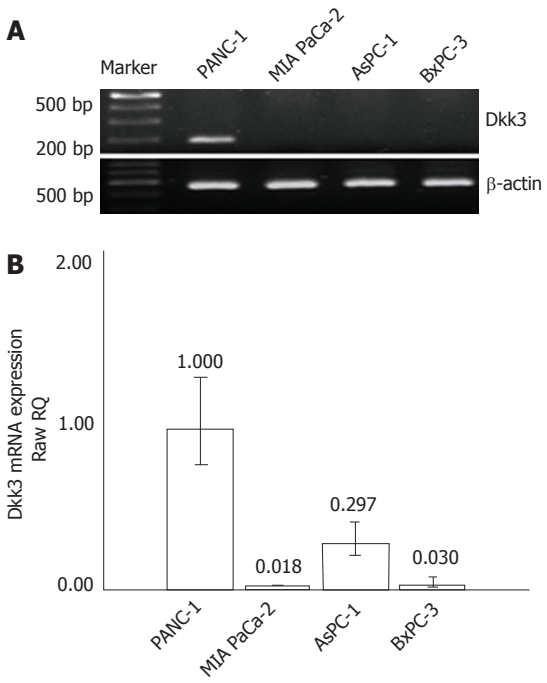

Figure 1 Dickkopf3 expression in human pancreatic cancer cell lines (PANC-1, MIA PaCa-2, AsPC-1 and BxPC-3). A, B: Dickkopf3 (Dkk3) mRNA expression was detected by reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR. Dkk3 mRNA expression was low in all cell lines examined. Dkk3: Dickkopf3; RQ: Relative quantitation.

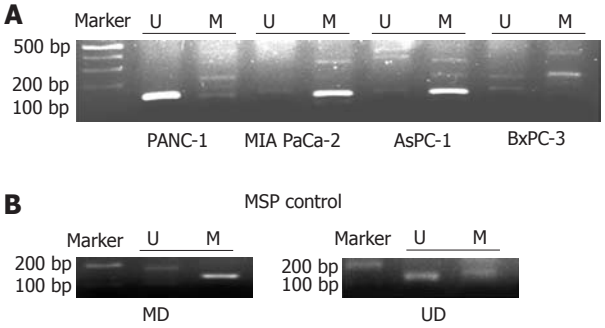

Figure 2 Dickkopf3 promoter methylation analysis in human pancreatic cancer cell lines. A: Methylation-specific PCR (MSP) was performed with bisulfite-treated DNA from pancreatic cancer cells. The Dickkopf3 (Dkk3) promoter was significantly methylated in MIA PaCa-2 and AsPC-1 cells; B: MSP controls demonstrate the specificity of the Dkk3 primers used. Methylated bisulfite-converted DNA exclusively yields amplification products with primers specific to methylated Dkk3 promoter sequences; unmethylated bisulfite-converted DNA yields exclusively amplification products with primers recognizing unmethylated Dkk3 promoter sequences. MD: Methylated bisulfite-converted DNA; UD: Unmethylated bisulfite-converted DNA; U: PCR products amplified with primers recognizing unmethylated Dkk3 promoter sequences; M: Amplification generated with methylation-specific primers.

Determination of dose-response curve

For determination of the dose-response curve, MIA PaCa-2 cells were transfected with pcDNA3.1-Dkk3 or pcDNA3.1. Six hours after transfection, cells were seeded in 96-cell plates in triplicate at a density of 3000 cells/well and were allowed to adhere. Gemcitabine (LILLY, France) was added to the medium 24 h after transfection. Cell proliferation was determined 72 h after gemcitabine addition using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS, Promega, WI, United States), according to the manufacturer's protocol. The spectrophotometric absorbance of each sample was measured at 490 nm using the TECAN spectra (Thermo, Austria). Percent proliferation relative to the controls was calculated based on the MTS read-out; the IC_{50} value was defined as the concentration of drug that produced a 50% reduction in absorbance relative to the control.

Cell growth assay

For the cell growth assay, MIA PaCa-2 cells were transfected with pcDNA3.1-Dkk3 or pcDNA3.1. At 6 h after transfection, cells were seeded in 96-well plates in triplicate at a density of 1000 cells/well and were allowed to adhere overnight. At 24 h, 48 h and 72 h, cell proliferation was determined using MTS (Promega, WI, United States) according to the manufacturer's protocol. The spectrophotometric absorbance of each sample was measured at 490 nm using the TECAN spectra (Thermo, Austria).

Statistical analysis

Statistical analysis was performed using SPSS 16.0 software. Unless otherwise indicated, the level of significance for differences between data sets was assessed using *t* test and one-way analysis of variance. Data are expressed as the mean ± SD. $P < 0.05$ was considered statistically significant.

RESULTS

Dkk3 is downregulated in pancreatic cancer cell lines

Dkk3 expression was assessed in four human pancreatic cancer cell lines (PANC-1, MIA PaCa-2, AsPC-1, BxPC-3). A low level of Dkk3 mRNA was observed in all cell lines, although Dkk3 expression in PANC-1 cells was slightly higher than in the other three cell lines (Figure 1). Dkk3 protein expression was too low to detect by Western blotting or immunofluorescence (data not shown).

Methylation of the Dkk3 promoter in pancreatic cancer cell lines

Through the use of MSP, we found that the Dkk3 promoter sequence was significantly methylated in MIA PaCa-2 and AsPC-1 cells, which were the cell lines with reduced Dkk3 expression. Conversely, the Dkk3 promoter sequence was unmethylated in the PANC-1 cells, which had slightly higher Dkk3 expression (Figure 2).

Demethylation of the Dkk3 promoter

Because methylation of the Dkk3 promoter sequence was detected in MIA PaCa-2 and AsPC-1 cells, we chose to treat these two cell lines with 10 μmol/L and 20 μmol/L, respectively, of the DNA methyltransferase inhibitor 5-aza-dC. After treatment with 5-aza-dC, for 48 h or 72 h, the cells were harvested to determine Dkk3 mRNA expression by real-time reverse transcription PCR. The results showed that these two cell lines with methylated Dkk3 promoters showed increased Dkk3 mRNA expression,

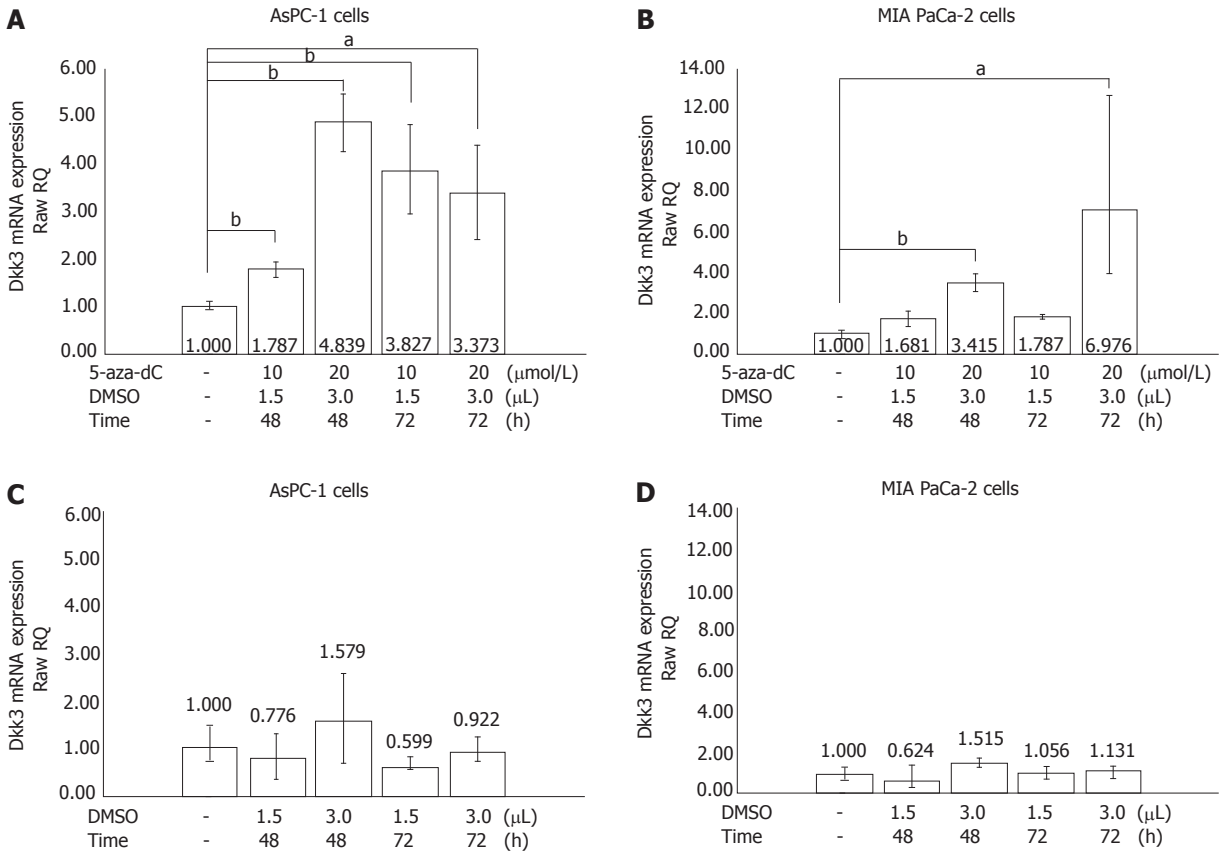

Figure 3 Dickkopf3 mRNA expression after demethylation *in vitro*. A, B: AsPC-1 and MIA PaCa-2 cells were treated with 10 μmol/L and 20 μmol/L of the DNA demethylating agent, 5-aza-dC, for 48 h or 72 h, respectively. The results show that these two cell lines, in which the Dickkopf3 (Dkk3) promoter was initially heavily methylated, had increased Dkk3 mRNA expression that was dependent on the dosage and timing of 5-aza-dC treatment ($^aP < 0.05$ *vs* untreated MIA PaCa-2 cells or untreated AsPC-1 cells, $^bP < 0.01$ *vs* untreated MIA PaCa-2 cells or untreated AsPC-1 cells); C, D: Control cells were incubated with dimethyl sulfoxide and fresh medium. RQ: Relative quantitation; **Dkk3: Dickkopf3; DMSO:** Dimethyl sulfoxide.

which was dependent on the dosage and timing of 5-aza-dC treatment ($P < 0.05$ or $P < 0.01$) (Figure 3).

Overexpression of Dkk3 suppresses pancreatic cancer cell growth and β-catenin expression

To study the roles of Dkk3 in the progression of pancreatic cancer, MIA PaCa-2 cells were transfected with pcDNA3.1-Dkk3 or pcDNA3.1. After transfection, Dkk3 mRNA and protein levels significantly increased in the pcDNA3.1-Dkk3-transfected cells ($P < 0.01$), while no significant changes were observed in the pcDNA3.1-transfected cells (Figure 4A and B). At 48 h and 72 h after transfection, the β-catenin mRNA and protein expression levels were significantly decreased in the pcDNA3.1-Dkk3-transfected cells ($P < 0.01$) (Figure 4B and C). The protein expression of pERK was also decreased, but there was no significant change in total ERK expression (Figure 4B). The results of the MTS assay showed that in the pcDNA3.1-Dkk3-transfected cells, proliferation capacity was lower than in the pcDNA3.1-transfected cells ($P < 0.01$) (Figure 4D).

Sensitivity of Dkk3-overexpressing pancreatic cancer cells to gemcitabine

A dose-response curve was constructed, and the IC_{50} values were compared to determine the influence of Dkk3 overexpression in pancreatic cancer cells on the effect of gemcitabine on cell growth. MIA PaCa-2 cells were transfected with pcDNA3.1-Dkk3 or pcDNA3.1. Seventy-two hours after gemcitabine addition, the IC_{50} values for gemcitabine were 0.621 μmol/L for pcDNA3.1-Dkk3-transfected cells and 1.877 μmol/L for pcDNA3.1-transfected cells (Figure 4E). These results show that the IC_{50} value of the Dkk3-overexpressing cells was significantly lower than that of the control cells.

DISCUSSION

Dkk3 is expressed in many normal human tissues[35]. It was previously reported that Dkk3 expression is generally low in some tumors, such as sporadic epithelial ovarian cancer, cervical cancer, mammary tumors, malignant melanoma, hepatoma and kidney, pancreas, gastric and lung cancers[12,15,16,18,19,31,32]. Additional studies also revealed the association between Dkk3 expression and cancer metastasis or prognosis in gastric cancer, renal cancer and head and neck squamous cell carcinoma[36-38]. However, Dkk3 expression and its roles in pancreatic cancer remain unknown. In this study, we detected Dkk3 expression in human pancreatic cancer cells. We found that both Dkk3

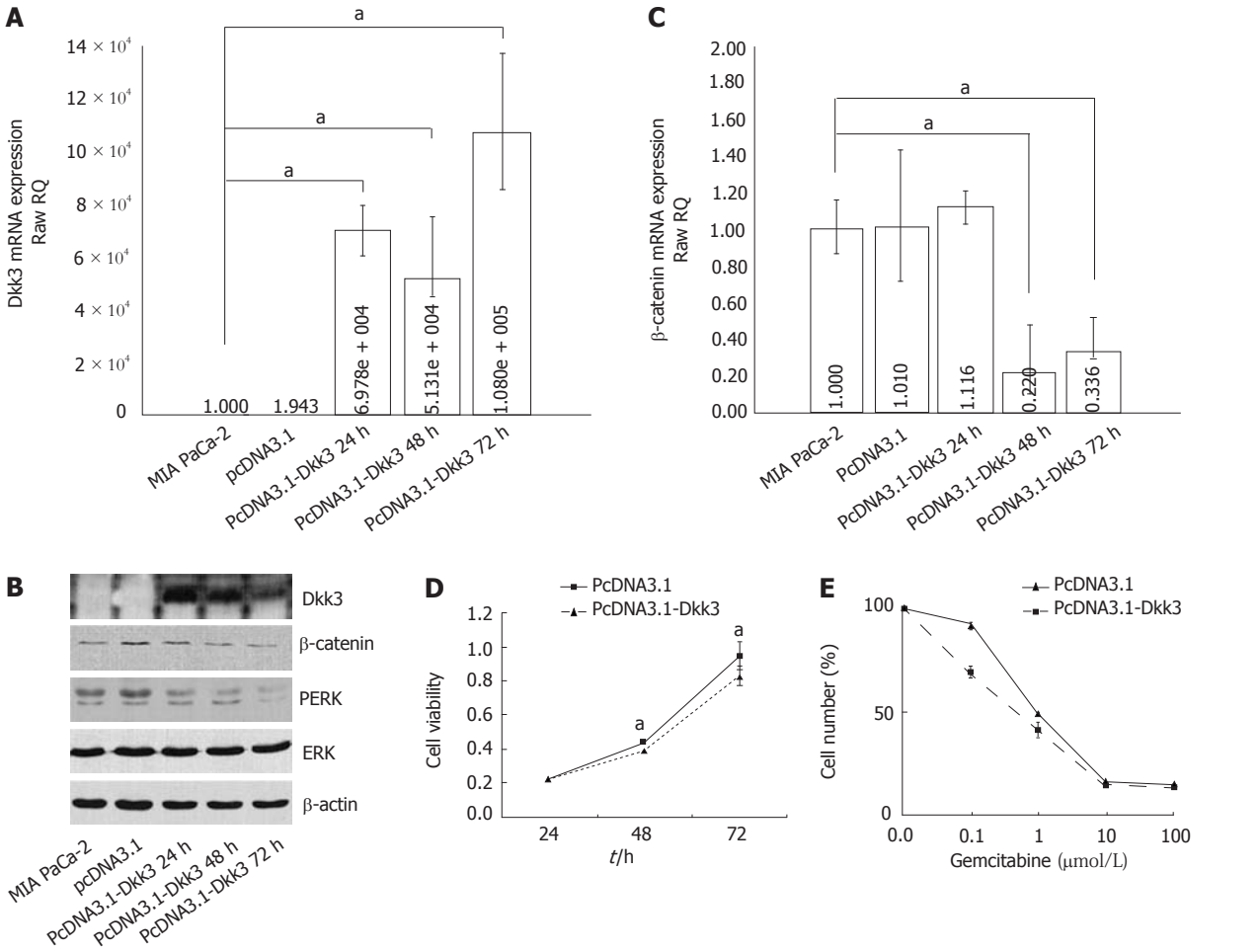

Figure 4 The effects of dickkopf3 overexpression on pancreatic cancer cells. MIA PaCa-2 cells were transfected with pcDNA3.1-dickkopf3 (Dkk3) or pcDNA3.1 vector. A, B: After transfection, Dkk3 mRNA and protein expression were examined by real-time reverse transcription polymerase chain reaction (real-time RT-PCR) and Western blotting. The results show that in the pcDNA3.1-Dkk3-transfected MIA PaCa-2 cells, Dkk3 expression was significantly upregulated ($P < 0.01$, $^aP < 0.01$ *vs* MIA PaCa-2 cells). B, C: β-catenin expression was examined by real-time RT-PCR and western blotting. β-catenin expression was downregulated 48 h and 72 h after transfecting pcDNA3.1-Dkk3 into MIA PaCa-2 cells ($P < 0.01$, $^aP < 0.01$ *vs* MIA PaCa-2 cells). B: The expression of extracellular signal-regulated protein kinases (ERK) and phosphorylated extracellular signal-regulated protein kinases (pERK) was examined by western blotting. The expression of pERK was simultaneously downregulated, without a significant change in total ERK expression. D: MTS assay results showed that the proliferative ability of pcDNA3.1-Dkk3-transfected cells was lower than that of pcDNA3.1-transfected cells ($P < 0.01$, $^aP < 0.01$). E: Dose-response analysis of pcDNA3.1- or pcDNA3.1-Dkk3-transfected MIA PaCa-2 cells with gemcitabine treatment. Seventy-two hours after gemcitabine addition, the IC50 values for gemcitabine were 0.621 μmol/L for pcDNA3.1-Dkk3-transfected cells and 1.877 μmol/L for pcDNA3.1-transfected cells. PERK: Phosphorylated extracellular signal-regulated protein kinases; ERK: Extracellular signal-regulated protein kinases; RQ: Relative quantitation; **Dkk3: Dickkopf3; DMSO:** Dimethyl sulfoxide.

protein and mRNA expression levels were low in all cell lines examined. Our results are partly in agreement with those of Takahashi N *et al*[39].

Methylation of the Dkk3 promoter has been observed in hepatocellular carcinoma, breast cancer, malignant astrocytic glioma, acute myeloid and lymphoblastic leukemia and gastrointestinal and bladder cancers[27-30,40-44]. Our MSP results showed that the Dkk3 promoter was methylated in MIA PaCa-2 and AsPC-1 cells, in which Dkk3 expression was low. After treatment with the DNA methyltransferase inhibitor 5-aza-dC, MIA PaCa-2 and AsPC-1 cells, which initially bore heavily methylated Dkk3 promoters, showed increased Dkk3 mRNA expression. In the present study, we demonstrated for the first time that decreased Dkk3 gene expression was associated with promoter methylation in two human pancreatic cancer cell lines (MIA PaCa-2 and AsPC-1). The inhibition of DNA methyltransferase activity by 5-aza-dC led to a reversion of methylation and upregulated expression of the previously downregulated gene.

Additional studies have recently demonstrated that Dkk3 has distinct roles in regulating the malignant behavior of cancer cells, depending on which cells are examined. For example, Dkk3 can reduce malignancy in mouse prostate cancer RM9 cells *in vitro* and *in vivo*[25]. Dkk3 can induce apoptosis or cell death in human bladder cancer, prostate cancer, breast cancer and lung cancer cells[9,20,24,45]. Dkk3 can inhibit tumor growth and metastasis in an orthotopic prostate cancer model[10]. While Jung *et al*[33] found that Dkk3 acts as an antiapoptotic molecule in lung adenocarcinoma, our results show that Dkk3 overexpression inhibited pancreatic cancer cell growth. The results revealed that in the pcDNA3.1-Dkk3-transfected MIA PaCa-2 cells, β-catenin mRNA and protein expres-

sion levels were both downregulated. Phosphorylation of ERK was decreased. These data demonstrate that Dkk3 suppressed MIA PaCa-2 cell growth by inhibiting β-catenin expression. Our results were consistent with the findings of Yue *et al*[45] in lung cancer. We hypothesize that Dkk3 acts as a Wnt signal transduction inhibitor in human pancreatic cancer cells.

Gemcitabine is the most commonly used chemotherapy drug for pancreatic cancer. Notably, our results show that gemcitabine's IC_{50} value for pcDNA3.1-Dkk3-transfected cells was significantly lower than that for the control cells. Dkk3 overexpression enhanced the sensitivity of pancreatic cancer cells to gemcitabine.

In summary, our results suggest that Dkk3 acts as a tumor suppressor in human pancreatic cancer cells by downregulating β-catenin expression *via* the ERK-mediated pathway. Dkk3 may be a valid adjunctive target of gemcitabine for the treatment of human pancreatic cancer.

COMMENTS

Background

Pancreatic cancer is the sixth leading cause of cancer death in China. The overall five-year survival rate is approximately 1%-3%. Pancreatic cancer remains one of the most aggressive human cancers. Recognizing the factors associated with pancreatic cancer progression is critical for its treatment.

Research frontiers

Dickkopf family proteins are secreted modulators of the canonical Wnt signaling pathway. Dickkopf 3 (Dkk3) is a member of the dickkopf family proteins. Dkk3 is downregulated in some tumors, and its overexpression inhibits tumor growth. The Dkk3 promoter sequence is methylated in several tumors. However, in lung adenocarcinomas, Dkk3 functions as an oncogene. Recently, other studies have demonstrated that Dkk3 plays distinct roles in different cells.

Innovations and breakthroughs

To date, no study has investigated Dkk3 expression and its roles in human pancreatic cancer cell behavior. In this study, the authors investigated Dkk3 expression and promoter sequence methylation in human pancreatic cancer cells. The effects of Dkk3 on cell proliferation and sensitivity to gemcitabine were simultaneously observed after expression was increased in MIA PaCa-2 cells, following transfection with the Dkk3 expression plasmid. According to the experimental results, the authors for the first time, confirmed that Dkk3 acts as a tumor suppressor in human pancreatic cancer cells by downregulating β-catenin expression *via* the ERK-mediated pathway. Dkk3 overexpression enhanced the sensitivity of pancreatic cancer cells to gemcitabine.

Applications

This study indicates that Dkk3 may be a valid adjunctive target of gemcitabine for the treatment of human pancreatic cancer.

Peer review

This is a paper that reports that Dkk3 is a tumor suppressor gene in pancreatic cancer. The findings are interesting, and overall writing is good.

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