Original Article

KIF23 enhances cell proliferation in pancreatic ductal adenocarcinoma and is a potent therapeutic target

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Background: In recent research, high expression of kinesin family member 23 (KIF23), one of the kinesin motor proteins involved in the regulation of cytokinesis, has been shown to be related to poor prognosis in glioma and paclitaxel-resistant gastric cancer, as a results of the enhancement of proliferation, migration, and invasion. In this study, we analyzed the role of KIF23 in the progression of pancreatic ductal adenocarcinoma.

Methods: A bioinformatic method was used to analyze the KIF23 mRNA level in pancreatic tumor tissues compared with normal pancreatic tissues and to analyze the connection between high KIF23 expression and prognosis. We examined the expression of KIF23 using immunohistochemistry and analyzed the connection between the expression of KIF23 and clinicopathological features in pancreatic ductal adenocarcinoma patients. In addition, a colony formation assay, MTT assay, and western blot assay were performed *in vitro*, along with a mouse xenograft model *in vivo*, to analyze the effect of KIF23 on proliferation. Further, the correlation between KIF23 and CDCA8 was analyzed by TCGA and immunohistochemical data.

Results: Bioinformatic results showed that KIF23 mRNA expression was higher in pancreatic tumor tissues than in normal pancreatic tissues and a poor prognosis has been linked to the high expression of KIF23. Immunohistochemistry revealed that KIF23 was highly expressed at the protein level and high expression of KIF23 correlated with adverse clinicopathological features. Our experimental results demonstrated that knockdown of KIF23 could inhibit the proliferation of pancreatic cells. Further, a positive correlation between KIF23 and CDCA8 expression existed, and KIF23 might promote pancreatic cancer proliferation by affecting CDCA8 expression.

Conclusions: Our data showed that high expression of KIF23 is associated with a poor prognosis, and KIF23 might be a potential therapeutic target for pancreatic ductal adenocarcinoma.

Keywords: Kinesin family member 23 (KIF23); proliferation; pancreatic cancer; prognosis; therapeutic target

Introduction

In recent years, significant progress has been made in terms of cancer research and treatment. However, there has not been any remarkable advance in survival rates for pancreatic cancer (1,2). The morbidity and mortality of pancreatic ductal adenocarcinoma (PDAC) have risen significantly in recent years: with 56,770 new cases (male 29,940 and female 26,830 cases) of pancreatic cancer and 45,750 deaths (male 23,800 and female 21,950 cases) expected in 2019 (3), 55,400 new cases (male 29,200 and female 26,240 cases) and 44,330 deaths (male 23,020 and female 21,310 cases) reported in 2018 (4), and 53,670 new cases (male 27,970 and female 25,700 cases) and 43,090 (male 22,300 and female 20,790 cases) deaths in 2017 (5). The five-year survival rate for pancreatic cancer is very low, and it is considered one of the worst malignant tumors due to its high surgical mortality rate and low cure rate (6). In China, the mortality rate from pancreatic cancer is almost equal to the incidence rate because of the poor prognosis (7). Although there are treatment options for pancreatic cancer, such as chemotherapy and radiotherapy, surgical resection is still the main treatment of choice. However, most patients with pancreatic cancer are diagnosed at an advanced stage and surgery is feasible in less than 20% of cases (8). Recent studies have found that the occurrence of pancreatic cancer is largely affected by gender, aging, smoking, and diabetes (9). However, the pathogenesis of pancreatic cancer is complex and our understanding remains limited. Therefore, more in-depth studies on the mechanism of pancreatic cancer may help us to identify novel therapeutic targets (10).

Kinesin family member 23 (KIF23), a human homolog of mouse KIF23, is one of the kinesin motor proteins involved in the regulation of cytokinesis (11,12). Based on cDNA microarray or by quantitative reverse transcription-polymerase chain reaction (PCR), KIF23 overexpression has been detected in lung cancer, breast cancer, gliomas, paclitaxel-resistant gastric cancer, hepatocellular carcinoma, and pancreatic cancer (13,14), and has been associated with poor prognosis for several cancers (15).

In our research, we discovered that high expression of KIF23 was associated with poor prognosis by immunohistochemical analysis of tumor sections and clinical data from patients with PDAC. We also found that knockdown of KIF23 decreased proliferation in PANC-1 and BxPC-3 PDAC cells, and suppression of KIF23 in PANC-1 cells inhibited tumor growth in nude mice.

We present the following article in accordance with the ARRIVE reporting checklist (available at http://dx.doi.org/10.21037/atm-20-1970).

Methods

Bioinformatic analysis

We used GEPIA (http://gepia.cancer-pku.cn/) (16) to collate and analyze The Cancer Genome Atlas (TCGA) data with a threshold of $P<0.05$ and LogFC >1 or <–1 for the differential genes, and we divided the patients into two groups using the median value as the basis for Kaplan-Meier survival analysis. The 95% confidence interval is marked with a dotted line. In addition, the correlation between KIF23 and CDCA8 was analyzed by GEPIA.

Sample collection

Based on the institutional review board's approval, all of the 82 adult PDAC patients with PDAC included in this study were from Tianjin Medical University Cancer Institute and Hospital from 2009 to 2017. These cases were included on the basis of a clear pathological diagnosis. The informed consent of each patient was obtained. All procedures performed in research involving human participants complied with the ethical standards of the institution and/or the National Research Council and with the 1964 Helsinki Declaration and its subsequent amendments or similar ethical standards.

Immunohistochemistry

The expression of KIF23 was detected in paraffin-embedded tumor tissue by a rabbit-anti-KIF23 antibody (ab235955, 1:200 dilution; Abcam, USA), following the procedure specified in the immunohistochemistry kit (ZSGB-BIO, pv6000, China). Briefly, we fixed tumor tissues and matched normal adjacent tissues in 10% neutral-buffered formalin and embedded them in paraffin blocks by the pathology department of our hospital. Then, the paraffin sections (4 μm) were placed into a microwave oven for 15 minutes to repair the antigens and were then cooled at room temperature. Endogenous peroxidase was blocked, and sections were incubated with an anti-KIF23 antibody overnight at 4 ℃, followed by a goat anti-rabbit secondary antibody at 37 ℃ for 1 hour. Sections were stained with 3,3-diaminobenzidin (DAB) at room temperature for

10 minutes and images were then recorded by microscopy (Olympus BX43). All samples were evaluated by two independent pathologists.

To analyze the results, KIF23 nuclear staining was scored using the 4-point scale (0, no staining; 1+, light staining at high magnification; 2+, intermediate staining; and 3+, dark staining of the linear membrane at low magnification). According to the distribution of scores of KIF23 staining intensity, samples were divided into high expression (2+ to 3+) and low expression (0 to 1+) groups.

Antibodies

The antibodies used in this study were: rabbit anti-KIF23 antibody (Abcam, ab235955; 1:200 dilution for immunohistochemistry and 1:1000 dilution for western blotting), rabbit anti-β-actin antibody (1:1000 dilution for western blotting; ab8227, Abcam plc., Cambridge, UK), rabbit anti-Ki67 antibody (1:200 dilution for immunohistochemistry and 1:1000 dilution for western blotting; ab16667, Abcam), rabbit anti-proliferating cell nuclear antigen (PCNA) antibody (1:500 dilution for western blotting; ab18197, Abcam), and rabbit anti-Borealin/CDCA8 (ab70910, Abcam; 1:50 dilution for immunohistochemistry).

RNA isolation and qPCR

Total RNA was isolated from cancer cells using TRIzol reagent (Invitrogen, 15596026, USA) and complementary DNA (cDNA) synthesis was performed using the PrimeScript RT Reagent Kit (Clontech, RR055B, USA). The following primers were used in the reactions: KIF23 (forward primer: 5'-CAGATTTCCAACGGCCAGCA-3' and reverse primer: 5'-TGGCTTTTTGCGCTTGG-3'), and GAPDH (forward primer: 5'-CGACCACTTT GTCAAGCTCA-3' and reverse primer: 5'-GGTTGAGC ACAGGGTACTTTATT-3′).

Western blot analysis

Total protein was collected from PANC-1 and BxPC-3 cells using RIPA and RMSF reagents (100:1) on ice and was then quantified by a BCA assay. Proteins were separated on 10% PAGE gels, then transferred to PVDF membrane (Millipore, IPSN07852, USA). The membrane was washed three times using PBST and blocked with 10% nonfat milk for 1 hour,

then incubated with a primary antibody at 4 ℃ overnight. After a further three washes, PVDF was incubated with a secondary antibody for 1 hour at room temperature and then washed again. Finally, the ECL substrate reagent kit (GE Healthcare, RPN 2109, USA) was used to visualize the protein bands on the imaging capture system (Alpha Innovation).

Cell culture

The PDAC cells of PANC-1 and BxPC-3 were cultured in 1640 medium (Gibco, 61870044, USA) with 10% fetal bovine serum (Gibco, 10099-141, USA) at 37 ℃ in a 5% CO_2 incubator.

The shRNA and stable clones

Lenti-KD RNAi (GICL3071096830, Genechem, China; AAACGAACCTTAAAGACCCAGTT) against KIF23 was transfected into PANC-1 and BxPC-3 PDAC cells using Lipofectamine e3000 reagent (Invitrogen, L30000315, CA, USA). The KIF23 shRNA control sequence plasmids were transfected as a negative control. Six-well plates were seeded with 1×10^5 cells per well based on the manufacturer's protocol. Wells were divided into three groups: the KIF23 depletion group, which was transfected with shRNA targeting KIF23; a negative control (NC) group, which was transfected with the control sequence; and a Mock group, which was treated without any reagent (data not shown). Silencing efficiency was measured by both quantitative PCR and immunoblotting 48 hours after transfection. The depleted cells were used to evaluate the correlation between KIF23 expression and cellular processes. Cells transfected with shRNA targeting KIF23 were maintained in medium containing 2 μg/mL puromycin for 2 weeks to select stable clones. Then, stable KIF23-depleted cell lines were screened through lentivirus infection and used for the both *in vitro* and *in vivo* assays.

Colony formation assays

PANC-1 and BxPC-3 cells were seeded into six-well plates (500 cells/well) and incubated at 37 ℃ in 5% CO_2 for 2 weeks. Cells were fixed with 100% methanol and stained with 0.1% crystal violet. Images were collected during microscopy, and quantitation was performed using Image-Pro Plus 5.0 software.

Page 4 of 15

Gao et al. KIF23 promotes pancreatic ductal adenocarcinoma proliferation

MTT assay

An MTT assay was used to evaluate cell proliferation and survival. Cells were resuspended in DMEM medium and 200 µL of cell suspension was seeded into 96-well plates at 2,000 cells/well and incubated at 37 ℃ in 5% CO_2. After three days, the medium was removed and 20 µL of MTT solution was added into each well and further incubated at 37 ℃ for 4 hours. Then, dimethyl sulfoxide (DMSO) was added (150 µL/well) to solubilize the formazan crystals, and the plate was incubated at room temperature for 10 minutes on a shaking table. The absorbance was then measured at 570 nm by spectrophotometry.

Mouse xenograft model

Nude BALB/c mice (female, 6–8 weeks, 18–22 g, four mice in each group) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). PANC-1 PDAC cells ($5×10^6$, 100 µL) were injected subcutaneously into the armpit of the nude mice. The size of tumors was measured every 3 days, and the tumor volume was calculated using the formula $V = 0.5 \times L \times W^2$, where V is the volume, L is the length, and W is the width. After 29 days, the tumors were harvested, weighed, and photographed. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Animal research was performed according to guidelines approved by the Tianjin Medical University Cancer Institute and the Hospital Animal Ethics Committee (approval No.: IRM-DWLL-2019113).

Semi-quantitative analysis of the immunohistochemical data from in vivo tumors

The expression of KIF23 or Ki67 was detected in paraffin-embedded tumors using a rabbit-anti-KIF23 or rabbit-anti-Ki67 antibody, following the procedure detailed in the immunohistochemistry kit (ZSGB-BIO, pv6000, China). Briefly, the pathology department of our hospital excised the tumor tissues *in vivo* and fixed them in 10% neutral-buffered formalin and then embedded them in paraffin blocks. Then, the paraffin-embedded sections (4 µm) were placed into a microwave oven for 15 minutes to repair the antigens and were then cooled at room temperature. Endogenous peroxidase was blocked and sections were incubated with an anti-KIF23 (or Ki67) antibody overnight at 4 ℃ and then a goat anti-rabbit antibody at 37 ℃ for

1 hour. Sections were stained with DAB at room temperature for 10 minutes. Images were obtained under microscopy (Olympus BX43). Then, positive cells were counted in five randomly selected fields under a light microscope at a 200× magnification, and the data were expressed as the mean value ± standard deviation (SD).

Statistical methods

Statistical analyses were conducted using SPSS edition 22.0. The relationship between KIF23 expression and clinical features was assessed using a chi-square test. The Kaplan-Meier method were employed to analyze disease-free survival and overall survival in pancreatic cancer patients. A *t*-test was performed for comparison of the data between the groups. Data were expressed as the mean value ± SD. Two-sided P values of <0.05 were considered significant.

Results

High expression of KIF23 was related to poor prognosis

A bioinformatic method was used to analyze the expression of KIF23 at the mRNA level in pancreatic tumor tissues and normal pancreatic tissues. As shown in *Figure 1*, KIF23 mRNA expression was higher in pancreatic cancer tissues than in normal tissues (*Figure 1A*, P<0.05). As shown in *Figure 1B* and 1C, the median KIF23 mRNA expression level was used as the cut-off point to divide all pancreatic cancer patients into two groups: high expression (n=89, from the TCGA dataset) and low expression (n=89, from the TCGA dataset). Patients in the high expression group showed worse overall survival and progression-free survival rates. We also examined the expression of KIF23 in peripheral blood and tumors. We found that KIF23 has guiding significance for the prognosis of pancreatic cancer, and KIF23 was also expressed in tumors (Figure S1). These data suggested that KIF23 might be a poor prognostic factor in pancreatic cancer patients. To verify these results, we conducted immunohistochemical studies to investigate the expression of KIF23 protein in PDAC patients from our institute. As shown in *Figure 2A*, KIF23 positive staining was localized in the nucleus. Weak or no reactivity was observed in the low expression samples, whereas strong staining was observed in the high expression samples. Compared to *Figure 2A*, the KIF23 negative staining was observed in normal tissue adjacent to the cancer tissue in *Figure 2B*. We also
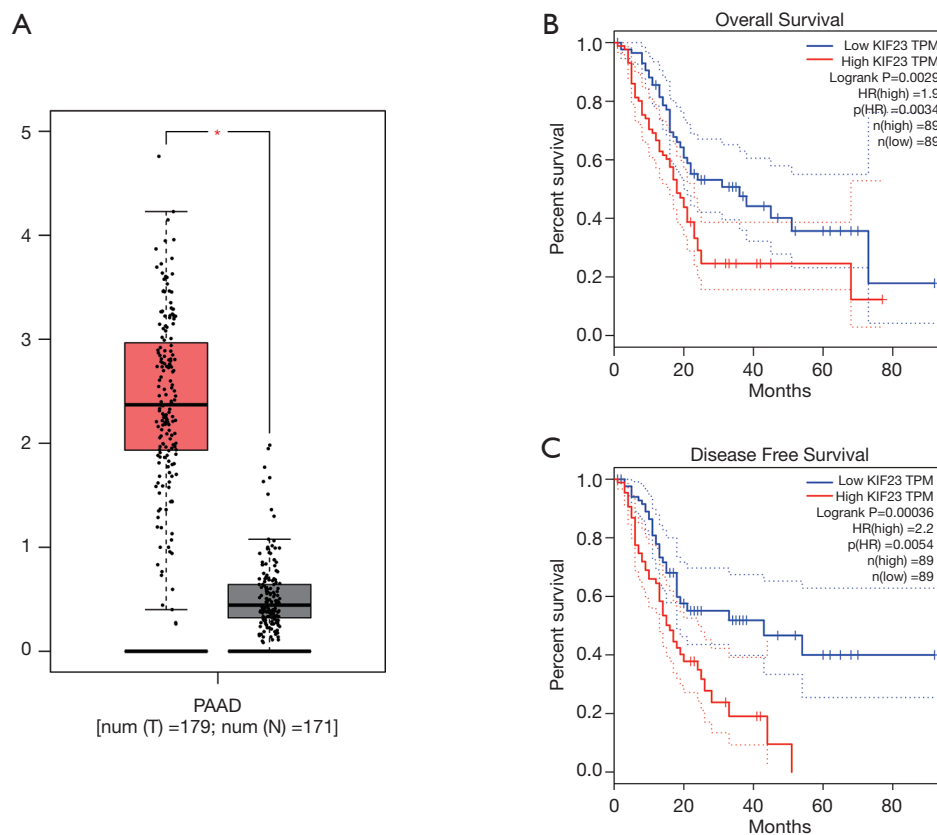
Figure 1 KIF23 mRNA expression in pancreatic tumor tissue and normal pancreatic tissue and its association with poor prognosis. (A) KIF23 mRNA expression was high in 179 tumor tissues and low in 171 normal tissues (*P<0.05); (B,C) pancreatic cancer patients with high KIF23 expression showed poor overall survival (OS) and poor progression-free survival (PFS).

analyzed the connection between KIF23 expression and the clinical features of pancreatic cancer patients. As shown in *Table 1*, high expression of KIF23 was related to tumor size (P=0.04). However, high expression of KIF23 was not related to age (P=0.088), gender (P=0.278), or the degree of differentiation (P=0.113).

KIF23 knockdown in PANC-1 and BxPC-3 PDAC cells by short hairpin RNA (shRNA)

First, to observe the function of KIF23 in PDAC, PANC-1 and BxPC-3 cells were transfected with specific shRNA to knockdown KIF23. Total RNA was isolated from the cells and the expression of the gene encoding KIF23 was analyzed by PCR. As shown in *Figure 3A*, when PANC-1 and BxPC-3 cells were transfected with shRNA, the low expression of KIF23 was detected by PCR. The control cells were transfected with empty plasmid. Second, we used

western blotting to analyze the KIF23 protein expression levels. The results were consistent with those for the RNA levels (*Figure 3B*). The PANC-1 and BxPC-3 cells transfected with shRNA were selected with puromycin until the cells formed stable clones.

Knockdown of KIF23 inhibited the proliferation of PANC-1 and BxPC-3 cells

To study the influence of KIF23 on PDAC cells, a colony formation assay was conducted and KIF23 was knocked down by shRNA in PANC-1 and BxPC-3 PDAC cells. Compared with the control cells, the proliferation of PDAC cells was inhibited following knockdown of KIF23 (*Figure 4A*). Moreover, the results of the MTT assay were similar to those obtained with the colony formation assay (*Figure 4B*). Ki67 and PCNA are two markers of cell proliferation. When transfected with KIF23 shRNA, the
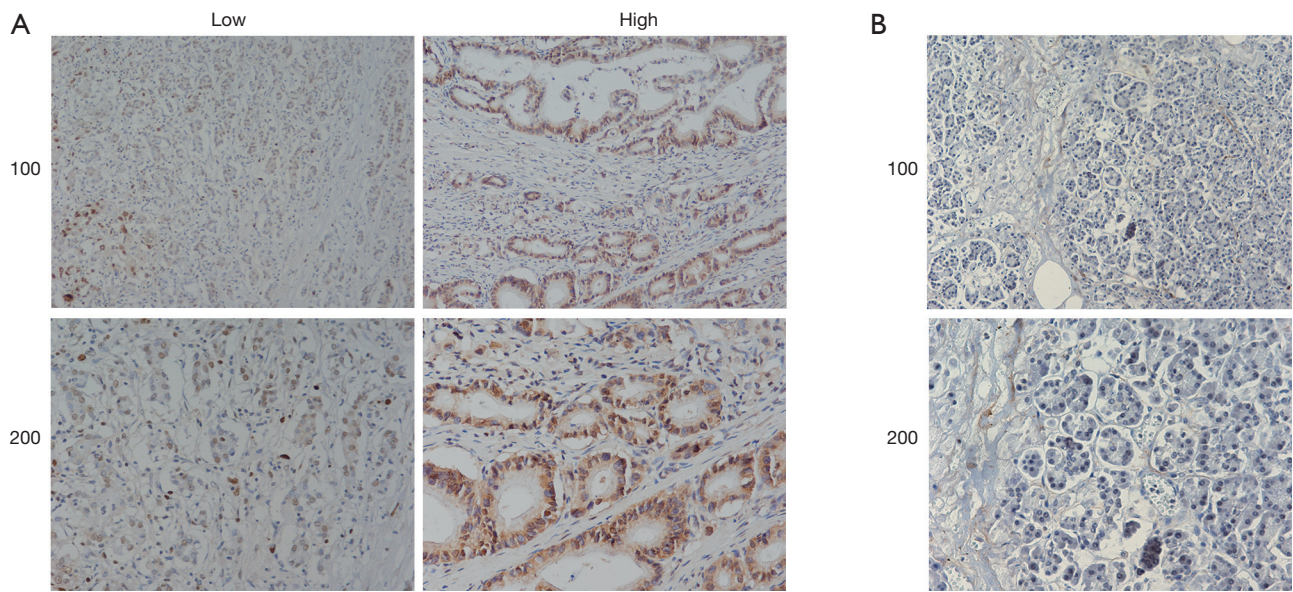
Figure 2 KIF23 expression at the protein level as analyzed by immunohistochemistry. (A) KIF23 positive staining was localized in the nucleus. Weak or no reactivity was observed in the low expression group and strong staining was observed in the high expression group. (B) KIF23 negative staining in normal tissue adjacent to the cancer tissue.

Table 1 The relationship between KIF23 and clinicopathological characteristics in 82 patients with pancreatic ductal adenocarcinoma

| Feature | All (n=82) | KIF23 expression | | χ^2 | P |
		Low (n=42)	High (n=40)		
Age (year)				2.905	0.088
<55	54	24	30		
≥55	28	18	10		
Gender				1.179	0.278
Male	46	26	20		
Female	36	16	20		
Differentiated degree				2.513	0.113
Low	36	22	14		
High	46	20	26		
Tumor size				4.228	0.040*
<5 cm	34	22	12		
≥5 cm	48	20	28		

*, $P<0.05$.

expression levels of Ki67 and PCNA in PANC-1 cells and in BxPc-3 cells were lower (*Figure 4C,D*). These data indicated that KIF23 affects the proliferation of PANC-1 and BxPc-3 cells.

Knockdown of KIF23 inhibited tumor growth in vivo

To study the effect of KIF23 *in vivo*, PANC-1 cells transfected with KIF23 shRNA were injected into nude
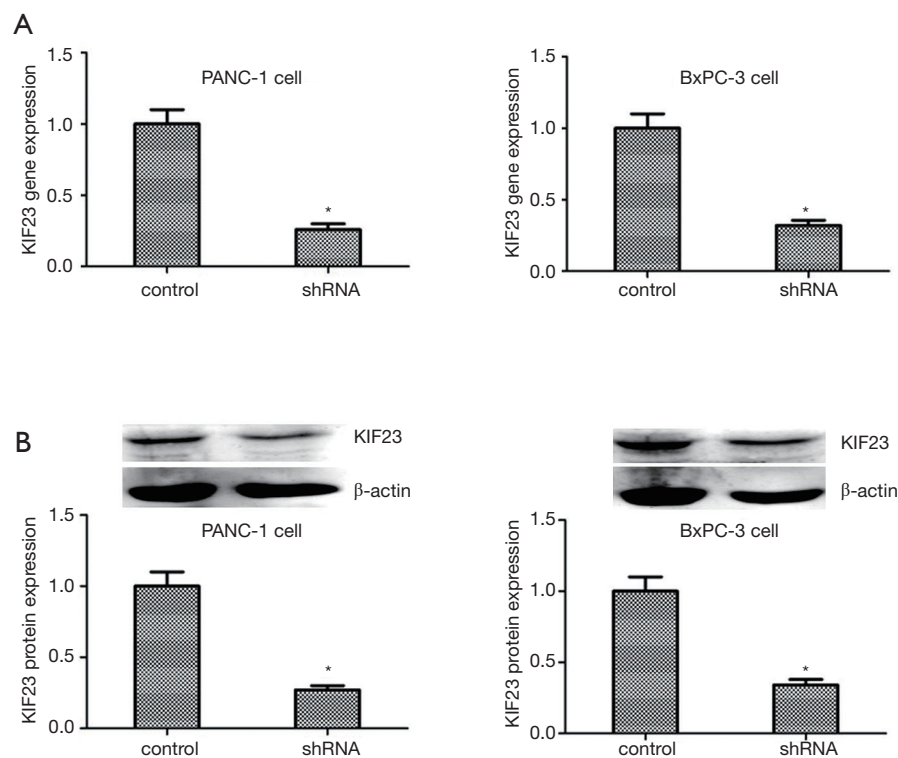
Figure 3 Knockdown of KIF23 by shRNA. **(A)** When PANC-1 and BxPC-3 cells were transfected with shRNA, low expression of KIF23 was detected by PCR. (B) Following transfection, low expression of KIF23 protein was detected by western blotting. *P<0.05.

mice subcutaneously. Tumors were measured every 3 days and excised after 29 days. As shown in *Figure 5A*, tumor growth in the shKIF23 group was significantly inhibited. Then, we used immunohistochemistry to detect the expression of KIF23 in tumors. The expression level of KIF23 protein in the control group was obviously higher than that in tumors (*Figure 5B*). These data showed that KIF23 was successfully knocked down in the shKIF23 group. The expression level of Ki67 in the control group was also higher than in tumors (*Figure 5C*). These results revealed that KIF23 is a positive regulator of PDAC progression.

### KIF23 promotes pancreatic cancer proliferation by affecting CDCA8

Based on our finding that KIF23 can regulate cell proliferation of pancreatic cancer both *in vitro* and *in vivo*, we next investigated the potential mechanism responsible for the involvement of KIF23 in tumor growth. A bioinformatic method was used to analyze the relationship between KIF23 and CDCA8 (http://gepia.cancer-pku.cn/

detail.php?gene=KIF23), and a strong positive correlation was detected (R=0.68, P=0.000<0.05, *Figure 6A*). Then, using GeneCards software, positive gene interactions between KIF23 and CDCA8 were revealed (https://www.genecards.org/cgi-bin/carddisp.pl?gene=KIF23, *Figure 6B*). After confirming the expression of CDCA8 (https://www.proteinatlas.org/ENSG00000134690-CDCA8/pathology), we concluded that the expression of CDCA8 in the peripheral blood during pancreatic cancer has guiding significance for prognosis (Figure S1), and that this protein is expressed in tumors. At the same time, we found that KIF23 has similar situation in tumors (Figure S2). After checking GEPIA (http://gepia.cancer-pku.cn/), the mRNA expression of CDCA8 was confirmed to be significantly higher in tumor tissue than in normal pancreatic tissue. Taking the median as the cutoff value, the patients were divided into high and low expression groups. The results showed that the OS (overall survival) and RFS (relapse free survival) rates of the high expression group were poorer (P<0.05, *Figure 7*). Then, we performed immunohistochemistry to analyze the protein expression of CDCA8, and strong staining

Page 8 of 15

**Gao et al. KIF23 promotes pancreatic ductal adenocarcinoma proliferation**
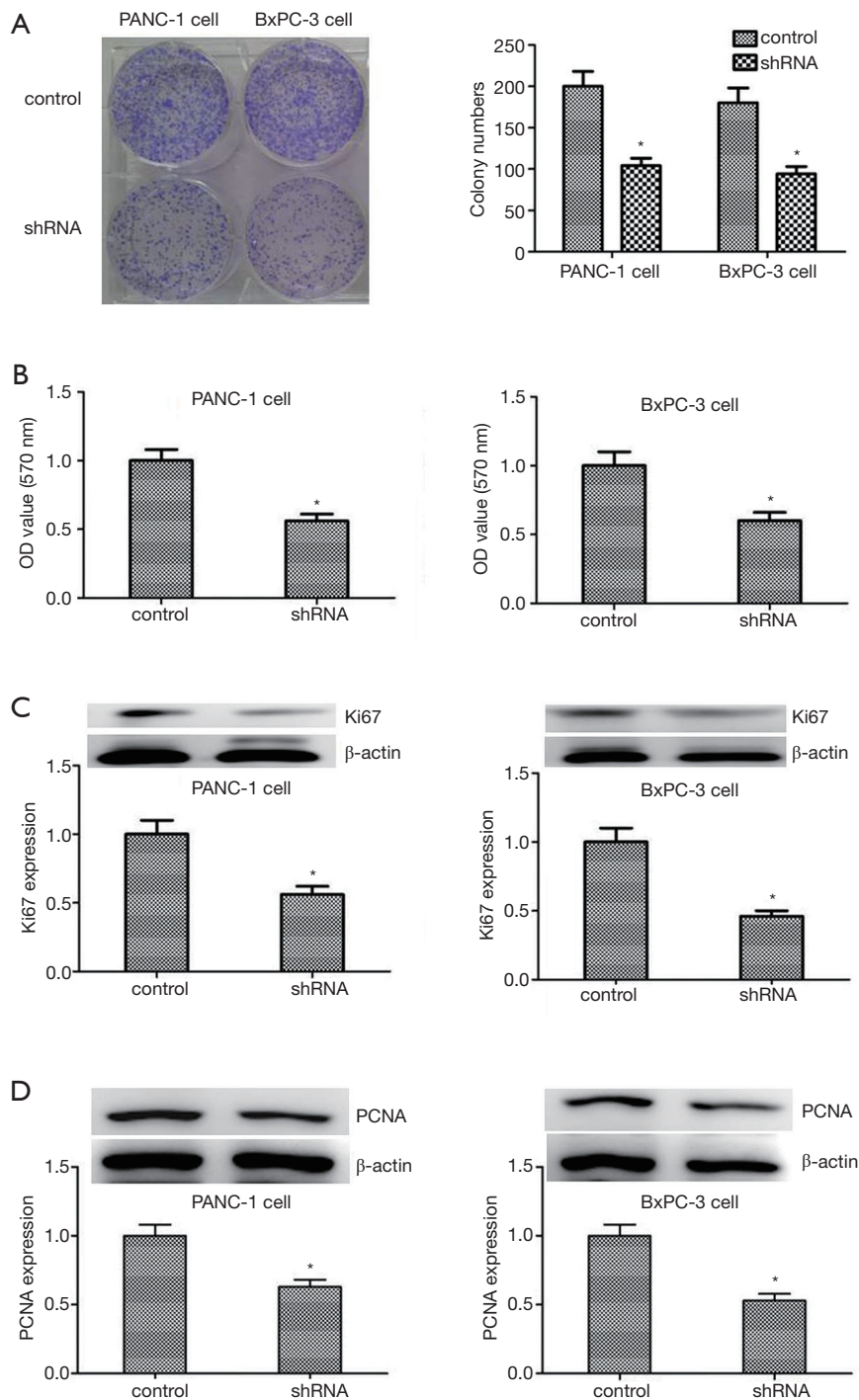
Figure 4 Knockdown of KIF23 inhibits proliferation of PANC-1 and BxPC-3 cell lines. (A,B) Colony formation assays and MTT assays were performed to analyze proliferation following transfection of PANC-1 and BxPC-3 cells with shKIF23; (C,D) The expression of Ki67 and PCNA were detected by western blotting. *P<0.05.
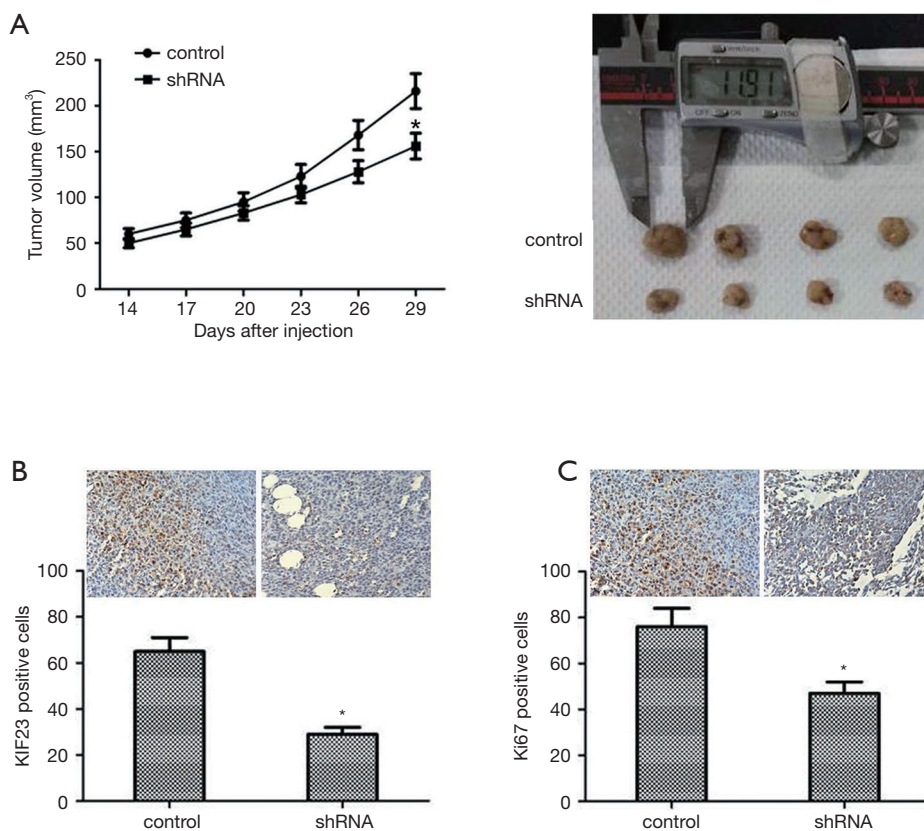
Figure 5 Analysis of the function of KIF23 *in vivo* using a mouse xenograph model. (A) Tumor growth curve in shRNA-transfected PANC-1 xenografts was significantly inhibited; (B) The expression of KIF23 in tumors was detected by immunohistochemistry. (C) The expression of Ki67 in tumors was detected by immunohistochemistry. *P<0.05. Scale bar =100 μm.
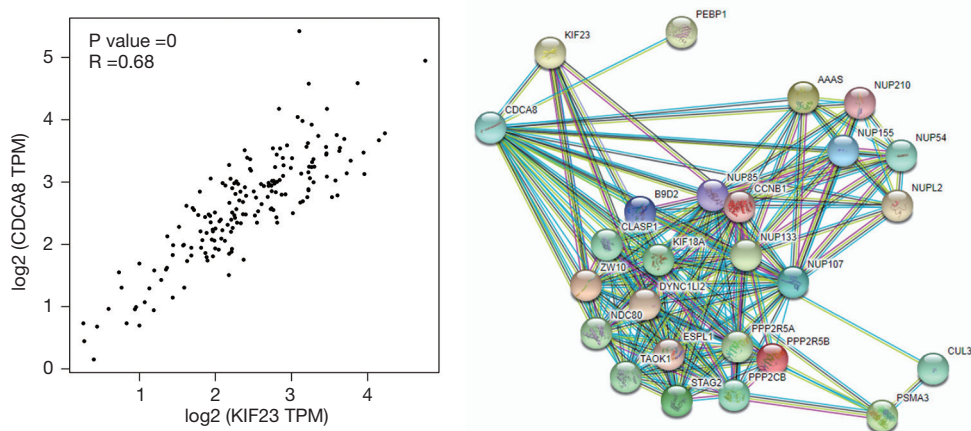
Figure 6 Correlation analysis between KIF23 and CDCA8 using bioinformatic techniques. (A) Correlation analysis of the expression of KIF23 and CDCA8 by GEPIA. (B) Mechanistic diagram of the gene interactions between KIF23 and CDCA8 as determined using Genecards.

Page 10 of 15

Gao et al. KIF23 promotes pancreatic ductal adenocarcinoma proliferation
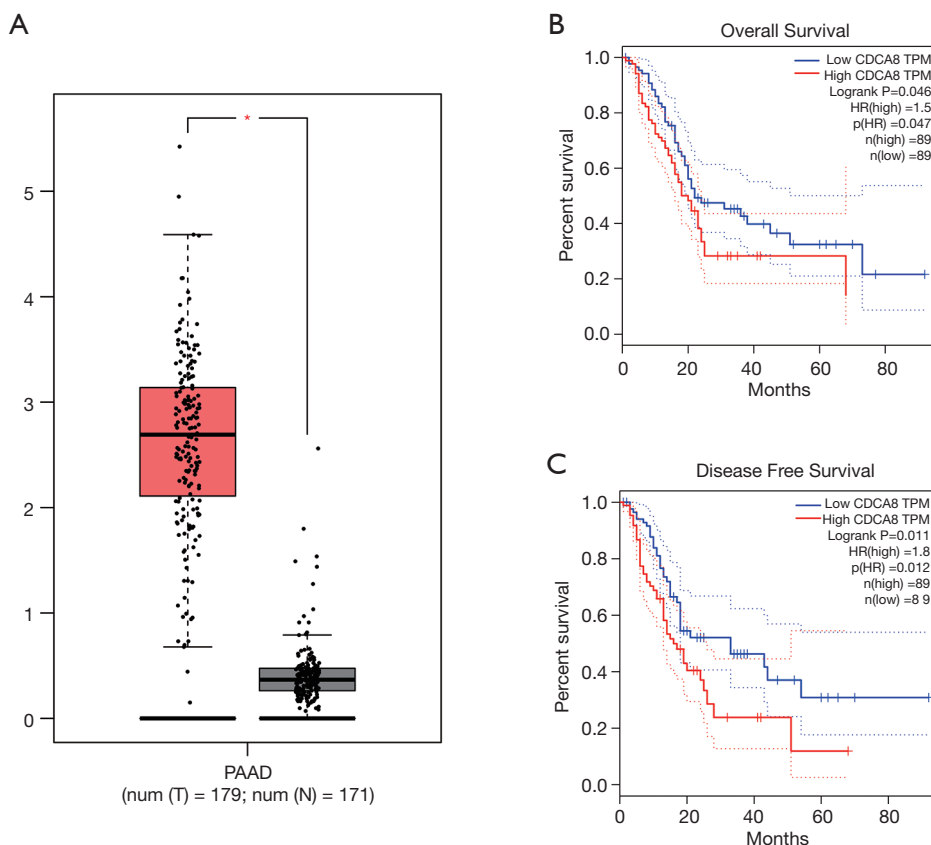
Figure 7 mRNA expression of CDCA8 in pancreatic tumor tissue compared with normal pancreatic tissue and the correlation with poor prognosis. (A) CDCA8 mRNA expression was high in 179 tumor tissues and low in 171 normal tissues (*P<0.05). (B,C) Pancreatic cancer patients with high KIF23 expression showed poor overall survival (OS) and poor progression-free survival (PFS).

was observed for the high expression group compared with weak staining for the low expression group (*Figure 8*). Moreover, we analyzed the relationship between the expression of CDCA8 and the clinicopathological characteristics of pancreatic cancer, and found that high expression was related to tumor size (P=0.026<0.05), but aging (P=0.259), gender (P=0.186) and differentiation (P=0.165) showed no correlation (*Table 2*). These results were similar to those of KIF23. Moreover, immunohistochemical analysis of the tumor tissues revealed that low CDCA8 expression correlated with low expression of KIF23, and vice versa (*Figure 9*). Correlation analysis on our data confirmed the significant positive correlation between the expression of CDCA8 and KIF23 (P=0.003<0.05) (*Table 3*). According to these results, we propose that KIF23 promotes pancreatic cancer by affecting the CDCA8 signaling pathway; however, further verification of this mechanism is necessary.

Discussion

Pancreatic cancer, known as "the king of cancer", is hard to diagnose and treat and is therefore associated with among the worst prognoses of all malignant tumors (16-22). In recent years, the morbidity and mortality rates for pancreatic cancer have markedly increased, and the 5-year survival rate is just less than 1% (18-27). Currently, the treatment methods for pancreatic cancer mainly include chemotherapy, radiotherapy, surgical treatment, and targeted treatment, but most have little effect (21-25). Although current pancreatic cancer therapeutic targets have achieved certain therapeutic effects, novel effective therapeutic targets are urgently needed and thereby, a deeper understanding of the pathogenesis of pancreatic cancer is required (21-35).

To the best of our knowledge, this is the first report of KIF23 expression in pancreatic cancer. We report that the expression of KIF23 differs among PDAC patients, and
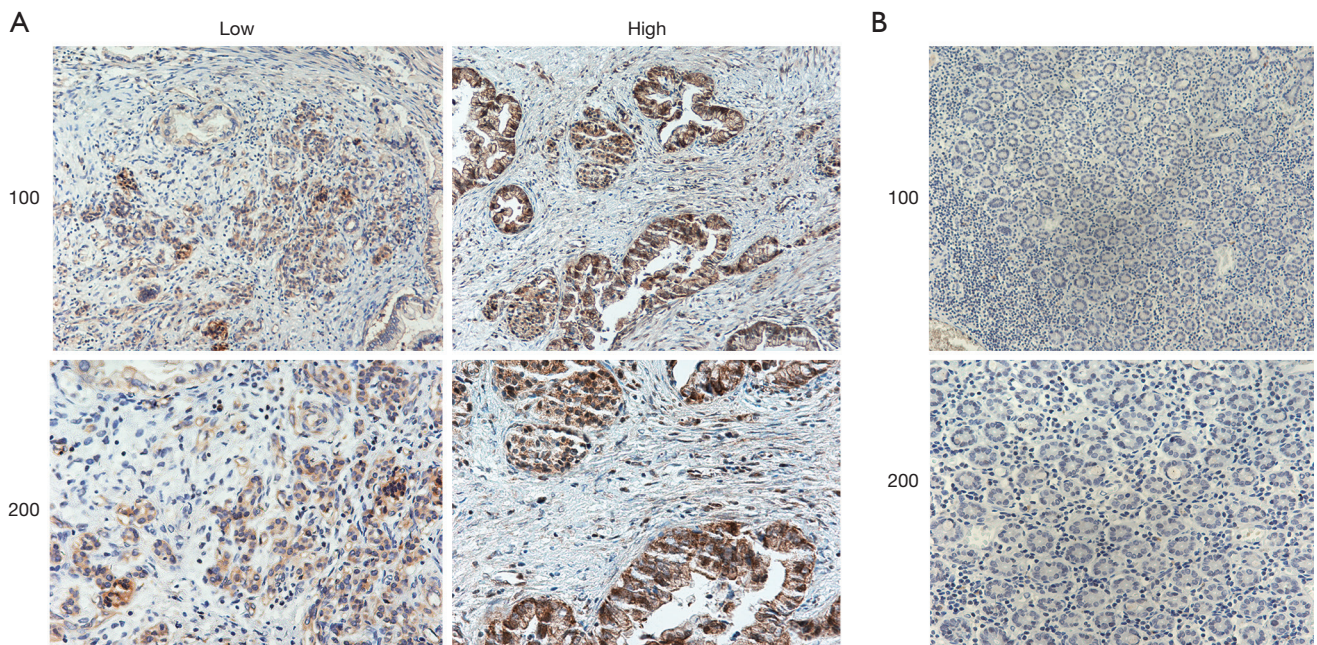
Figure 8 CDCA8 expression at the protein level as determined by immunohistochemistry. **(A)** The low expression group showed weak or no reactivity, whereas the high expression group showed strong staining. **(B)** Negative staining for CDCA8 was observed in normal tissue adjacent to cancer tissue.

Table 2 Relationships of CDCA8 and clinicopathological characteristics in 82 patients with pancreatic ductal adenocarcinoma

Feature	All (n=82)	CDCA8 expression		χ^2	P
		Low (n=34)	High (n=48)		
Age (year)				1.277	0.259
<55	54	20	34		
≥55	28	14	14		
Gender				1.748	0.186
Male	46	22	24		
Female	36	12	24		
Differentiated degree				1.927	0.165
Low	36	18	18		
High	46	16	30		
Tumor size				4.975	0.026*
<5 cm	34	19	15		
≥5 cm	48	15	33		

*$P<0.05$.
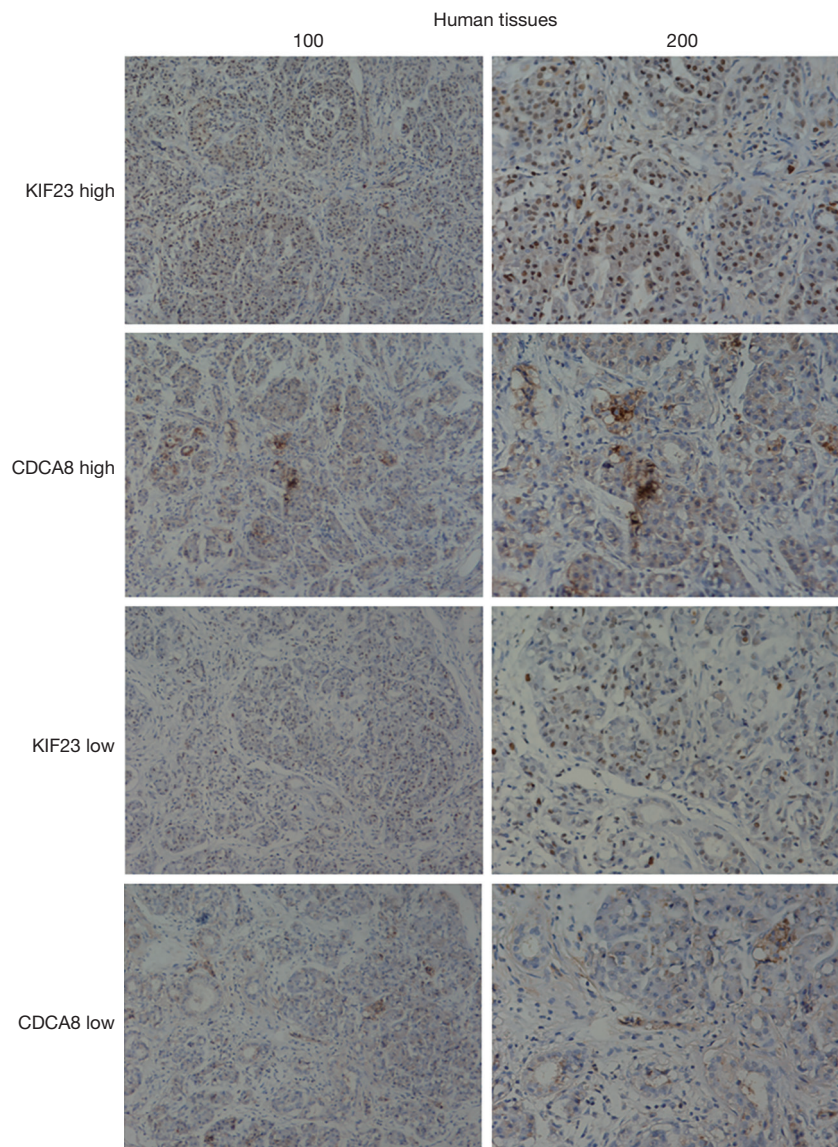
Figure 9 KIF23 expression correlated with CDCA8 expression in the tumor tissues of pancreatic cancer. Representative images of immunohistochemical staining of CD164 and CXCR4 expression in tumor tissues.

Table 3 Relationships of KIF23 and CDCA8 in 82 patients with PDAC

All (n=82)	KIF23		χ^2	P	Pearson	Spearman
	Low	High				
CDCA8			8.721	0.003	0.363	0.363
Low	24	10				
High	18	30				

PDAC, pancreatic ductal adenocarcinom.

high expression of KIF23 is related to a poor prognosis. Our results suggest that KIF23 may be a new prognosis factor in PDAC.

Nislow first identified a human KIF23 homolog in 1992 (13), which was a plus-end-directed motor enzyme that translocates antiparallel microtubules *in vitro*. Following this, other reports confirmed that KIF23 as a key regulator of cytokinesis (12,36-40). By screening a HeLa cell cDNA library, KIF23 (also known as mitotic kinesin-

like protein-1, MKLP1) was separated and confirmed as a plus-end-directed motor enzyme that translocates antiparallel microtubules *in vitro* (41). This enzyme contains the kinesin superfamily motor domain at its N-terminus, and has been found to be localized in both the cytoplasm and nucleus (42). The kinesin motor protein KIF23 was reported to be a underlying marker for several tumors (13). KIF23 is essential for the completion of cytokinesis in midbody formation and for recruitment to the spindle midzone/midbody by a chromosomal passenger protein (40). Liu and Erikson reported that a KIF23 mutant lacking nuclear localization signals, led to cell cycle arrest, further demonstrating that KIF23 is essential for cytokinesis (43). In addition, KIF23 acts as the motor enzyme for microtubules, which are known to play an important role in cellular shape and processes such as motility, mitosis, intracellular vesicle transport, organization, and positioning of membranous organelles (44-47). KIF23 overexpression is a feature of some tumors, such as glioma (48), breast (49), and paclitaxel-resistant gastric cancer (50). Our study also showed that KIF23 was overexpressed in PDAC patients. Importantly, high levels of KIF23 mRNA correlated with poor survival in patients with glioma and ER-positive breast cancer (48,50). In our study, KIF23 was demonstrated to play a vital role in PDAC cell proliferation. Our study analyzed the function of KIF23 in PDAC. PANC-1 and BxPC-3 cells were transfected with specific shRNA to KIF23 and the resulting phenotypes were observed. The results of an MTT assay and the expression level of PCNA revealed that knockdown of KIF23 with shRNA can inhibit the proliferation of PDAC cells, which was consistent with other reports in glioma (48), breast (49), and paclitaxel-resistant gastric cancer (50). Compared with the cells transfected with control shRNA, the stable clone of KIF23-knockdown cells grew slower *in vivo*. Based on these data, KIF23 may be an oncogene in PDAC.

There are some limitations in our study. It is retrospective and includes only a small number of patient samples from a single center; however, few previous studies have investigated the detailed mechanism by which KIF23 regulates tumor growth in PDAC. We reported a positive correlation between KIF23 and CDCA8 based on TCGA data (GEPIA and Genecards) and immunohistochemistry (our clinical data) further verification may be required in future studies. Furthermore, our conclusion that KIF23 may promote pancreatic cancer proliferation by affecting the expression of CDCA8 in the pathway, may also require further verification in the future.

In conclusion, our study indicated that KIF23 might play a critical role in the progression of PDAC and that it operates through enhanced cell proliferation, which was consistent with the findings of other previous studies. Further, a positive correlation between KIF23 and CDCA8 was demonstrated, and KIF23 might promote pancreatic cancer proliferation by affecting CDCA8 expression. These findings may prompt further studies of KIF23 as a therapeutic target for the treatment of PDAC. However, these findings are preliminary and further studies are needed to confirm these results.

Acknowledgments

Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in research involving human participants complied with the ethical standards of the institution and/or the National Research Council and with the 1964 Helsinki Declaration and its subsequent amendments or similar ethical standards. All applicable international,

Page 14 of 15

Gao et al. KIF23 promotes pancreatic ductal adenocarcinoma proliferation

national, and/or institutional guidelines for the care and use of animals were followed. Animal research was performed according to guidelines approved by the Tianjin Medical University Cancer Institute and the Hospital Animal Ethics Committee (approval No.: IRM-DWLL-2019113). Our protocol was approved by the committee and all operations were performed under sodium pentobarbital anesthesia, with every effort made to minimize pain.

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