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ORIGINAL ARTICLE

Rab14 overexpression regulates gemcitabine sensitivity through regulation of Bcl-2 and mitochondrial function in pancreatic cancer

Jinnian Ge[1] · Chunlin Ge[1]

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Abstract

Rab family protein Rab14 has been implicated in the development of human cancers. To date, its expression pattern, biological function, and potential mechanism in pancreatic cancer have not been explored. In this study, we analyzed Rab14 expression in 103 cases of pancreatic cancer tissues using immunohistochemistry (IHC) and found that Rab14 was overexpressed in 41/103 cases (39.8%). Rab14 overexpression correlated with the advanced stage. Moreover, elevated Rab14 levels indicated poor prognosis of patients with pancreatic cancers. We used BxPC-3 and Capan-2 respectively for plasmid and siRNA transfection. MTT and colony formation assays showed that Rab14 transfection increased cell proliferation and colony formation in BxPC-3 cells. Rab14 siRNA knockdown inhibits proliferation and colony formation ability in Capan-2 cell line. Cell cycle analysis showed that Rab14 facilitated cell cycle progression. Matrigel invasion assay showed that Rab14 promoted BxPC-3 cell invasion while its depletion inhibited Capan-2 cell invasion. In addition, MTT and AnnexinV/PI analysis demonstrated that overexpression of Rab14 reduced gemcitabine sensitivity which conversely was increased by Rab14 knockdown. We also demonstrated that Rab14 upregulated mitochondrial membrane potential (MMP) while its depletion downregulated MMP during gemcitabine treatment. In addition, western blotting revealed that Rab14 overexpression upregulated cyclin D1, cyclin A, cyclin E, p-Rb, and Bcl-2 and downregulated p21. Rab14 also downregulated caspase3, PARP cleavage, and cytochrome c release. In conclusion, our data indicated that Rab14 was overexpressed in pancreatic cancer and promotes growth and gemcitabine resistance, possibly through regulation of mitochondrial function and Bcl-2.

Keywords Rab14 · pancreatic cancer · Bcl-2 · proliferation · gemcitabine resistance

Introduction

Pancreatic cancer is the seventh most common cause of cancer-related death worldwide [1, 2]. It displays highly aggressive biological behavior, and the prognosis is poor for most patients. The mechanisms underlying pancreatic cancer progression and chemoresistance are quite complex, involving multiple genetic and epigenetic changes.

Numerous studies have explored innovative therapeutic strategies targeting pancreatic cancer. Gemcitabine has served as a standard first-line drug for chemotherapy. However, the 5-year survival rates for patients with pancreatic cancer after surgical resection are only about 20%, even with gemcitabine supplementation [3]. Identification of novel biomarkers which is related to cancer progression and chemosensitivity will be important for the development of novel therapies.

Rab14 is a member of RAS oncogene superfamily of small G proteins which regulate membrane vesicle transport and signal transduction [4]. Rab14 has been implicated in several cancers. Rab14 was reported as a miR-451 target gene which promotes lung cancer cell survival [5]. Rab14 is overexpressed in human ovarian cancers and activates the Wnt signaling [6]. Rab14 also acts as an oncogene and induces gastric cancer

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✉ Chunlin Ge
pvd691@163.com

[1] Department of General Surgery, The First Affiliated Hospital of China Medical University, Shenyang 110001, Liaoning Province, China

proliferation through the AKT signaling [7]. Silencing Rab14 suppressed proliferation and enhanced chemosensitivity in oral squamous cell carcinoma [8]. These studies indicated that Rab14 is a potential oncogene in human cancer development and progression. However, the expression pattern of Rab14 in human pancreatic cancers has not been explored. In addition, its biological roles and mechanism have not been investigated.

In this study, we explored the clinical significance and biological characteristics of Rab14 in human pancreatic cancer tissues and cell lines. Our data indicated that Rab14 may act as an oncoprotein in human pancreatic cancers. Rab14 regulates gemcitabine sensitivity through regulation of mitochondrial membrane potential and Bcl-2 in pancreatic cancer cells.

Materials and Methods

Patients and specimens

This study protocol was approved by the ethics review board of The First Affiliated Hospital of China Medical University. Informed consent was obtained for the collection of patient tissues. Primary tumor specimens were obtained from 103 patients diagnosed with pancreatic ductal adenocarcinoma (PDAC) between 2013 and 2017. Clinical characteristics of PDAC samples were listed in Table 1. Of the 103 cases, 67 were males and 36 females (ratio 1.86:1). Seventy-four cases were located in the head, and 29 cases were located in the body/tail of pancreas. There were 82 cases in stage I, and 21 cases in stages II and III. Seventy-seven of 103 (74.7%) cases have lymph node metastasis. The histological diagnosis was evaluated according to the World Health Organization (WHO) classification guidelines. Fresh pancreatic cancer samples and corresponding normal tissue samples were obtained from surgical treatment and stored at − 80 °C. All samples were diagnosed as adenocarcinomas and were collected without any previous neoadjuvant treatment.

Immunohistochemistry

Tumor blocks were obtained from the pathology archive of the First Affiliated Hospital of China Medical University. Tumor specimens were fixed with 10% neutral formaldehyde and embedded in paraffin. Four micrometer thick paraffin tissue sections were made. Immunostaining was performed using the Elivision kit from Maixin (MaiXin, Fuzhou, China). After antigen retrieval in citrate buffer (pH 6.0) for 2 min in an autoclave, 0.3% hydrogen peroxide was used for 10 min. Sections were incubated with goat serum (ready to use, MaiXin, Fuzhou, China) to reduce non-specific binding. Then, sections were incubated with Rab14 antibody at 4 °C overnight (1:300 dilution, Proteintech, USA). Then HRP-

Table 1 Distribution of Rab14 in pancreatic carcinoma according to clinicopathological characteristics

Characteristics	Number of patients	Rab14 low expression	Rab14 high expression	*p*
Age				
< 60	59	37	22	0.5455
≥ 60	44	25	19	
Gender				
Male	67	42	25	0.4808
Female	36	20	16	
Tumor location				
Head	74	44	30	0.8077
Body/tail	29	18	11	
Histological grade				
1	14	12	2	0.0434
2	46	29	17	
3	43	21	22	
TNM stage				
I	82	54	28	0.0204
II III	21	8	13	
T stage				
T1 + T2	54	35	19	0.3146
T3 + T4	49	27	22	
Lymph node metastasis				
N0	26	20	6	0.0439
N1	77	42	35	

conjugated polymers were applied to sections. DAB plus kit (MaiXin, Fuzhou, China) was used to development staining.

For analysis of staining intensity, five views were examined per slide. The staining intensity was graded as 0 (none), 1 (weak), and 2 (moderate/strong). Percentage was graded as 1:1–25%, 2:26–50%, 3:51–75%, and 4:76–100%. Intensity and percentage scores were multiplied to give a final score of 0 to 8. Rab14 was designated as low expression, score < 4 or high expression (overexpression), score ≥ 4.

Cell culture and transfection

BxPC-3 and Capan-2 cell lines were purchased from Shanghai cell bank of the Chinese Academy of Sciences (Shanghai, China). Capan-2 carries KRAS mutation in codon 12, leading to oncogenic RAS protein that is constitutively activated in its GTP-bound state, while the KRAS status in BxPC-3 is wild-type. In addition, Capan-2 has been reported to be wild-type for TP53 while BxPC-3 has Y220C mutant TP53 [9]. We picked these two cell lines so we can check if the biological function of Rab14 was dependent on the molecular background (such as KRAS and TP53) of PDAC cells. Cells were cultured in PRMI-1640 with 10% fetal bovine serum under the conditions of 37 °C, 5% CO2, and 90% humidity. Empty vector (pCMV6-Entry) and Rab14 plasmid were purchased from Origene (USA). Transfection was performed using Attractene transfection reagent (Qiagen, Germany). Rab14 siRNA and negative control siRNA were obtained from Dharmacon (GE healthcare, USA). Dharmafect1 was used for siRNA transfection (GE healthcare, USA). Cells were transfected with siRNA or plasmid 24 h after plating. Cells were used for the following assaying after 60 h of transfection.

Western blotting

Proteins were separated by SDS-PAGE and transferred to PVDF membrane (Millipore, Billerica, MA, USA) and blocked with 5% bovine serum albumin in TBS-T. Membranes were incubated overnight at 4 °C with antibody against Rab14 (1:1000; Proteintech, USA), p-Rb, cyclin A, cyclin D1, cyclin E, p21, Bcl-2, cytochrome c, caspase3, cleaved caspase3, PARP, cleaved PARP (1:1000; Cell Signaling Technology, USA), and GAPDH (1:3000; Santa Cruz, USA). After incubation with HRP-linked anti-mouse/rabbit IgG (1:3000, Santa Cruz, USA)

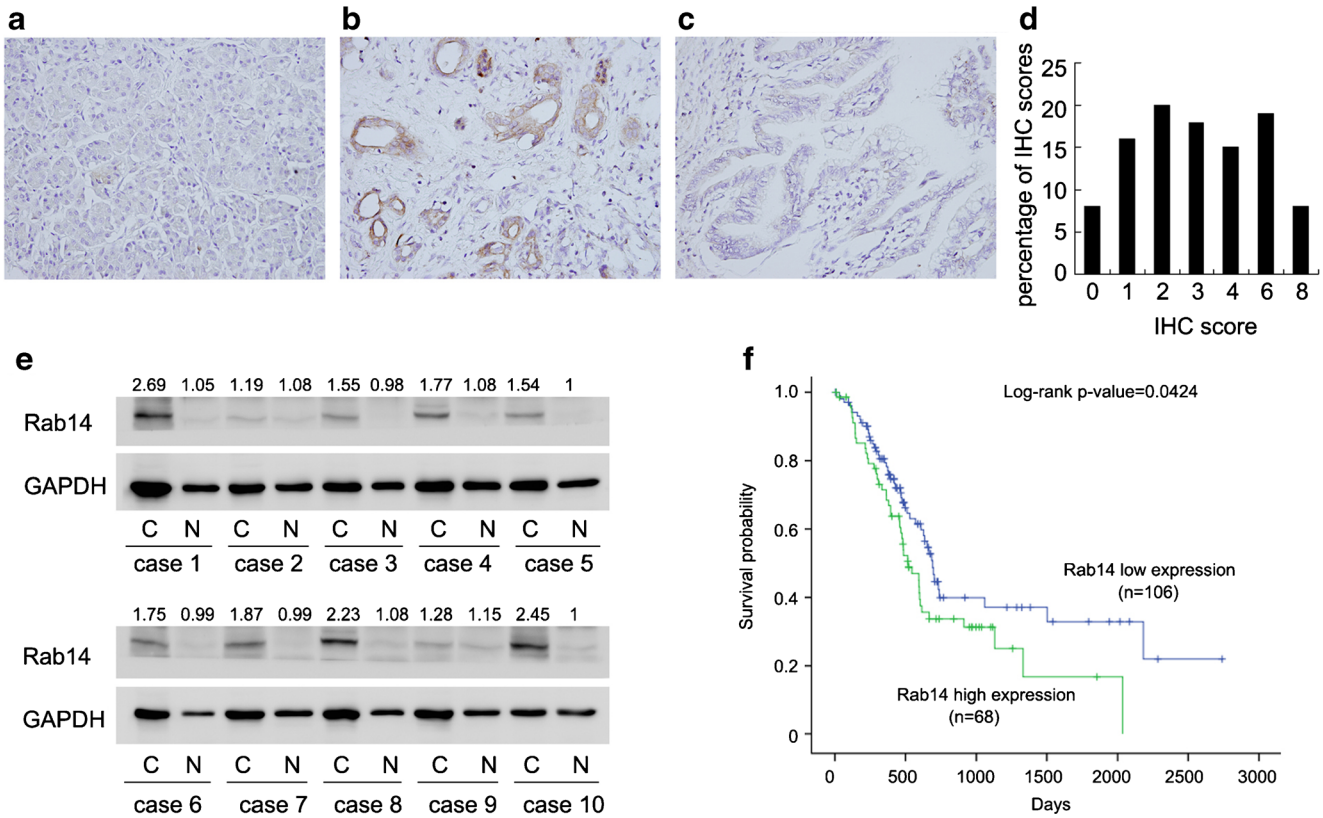
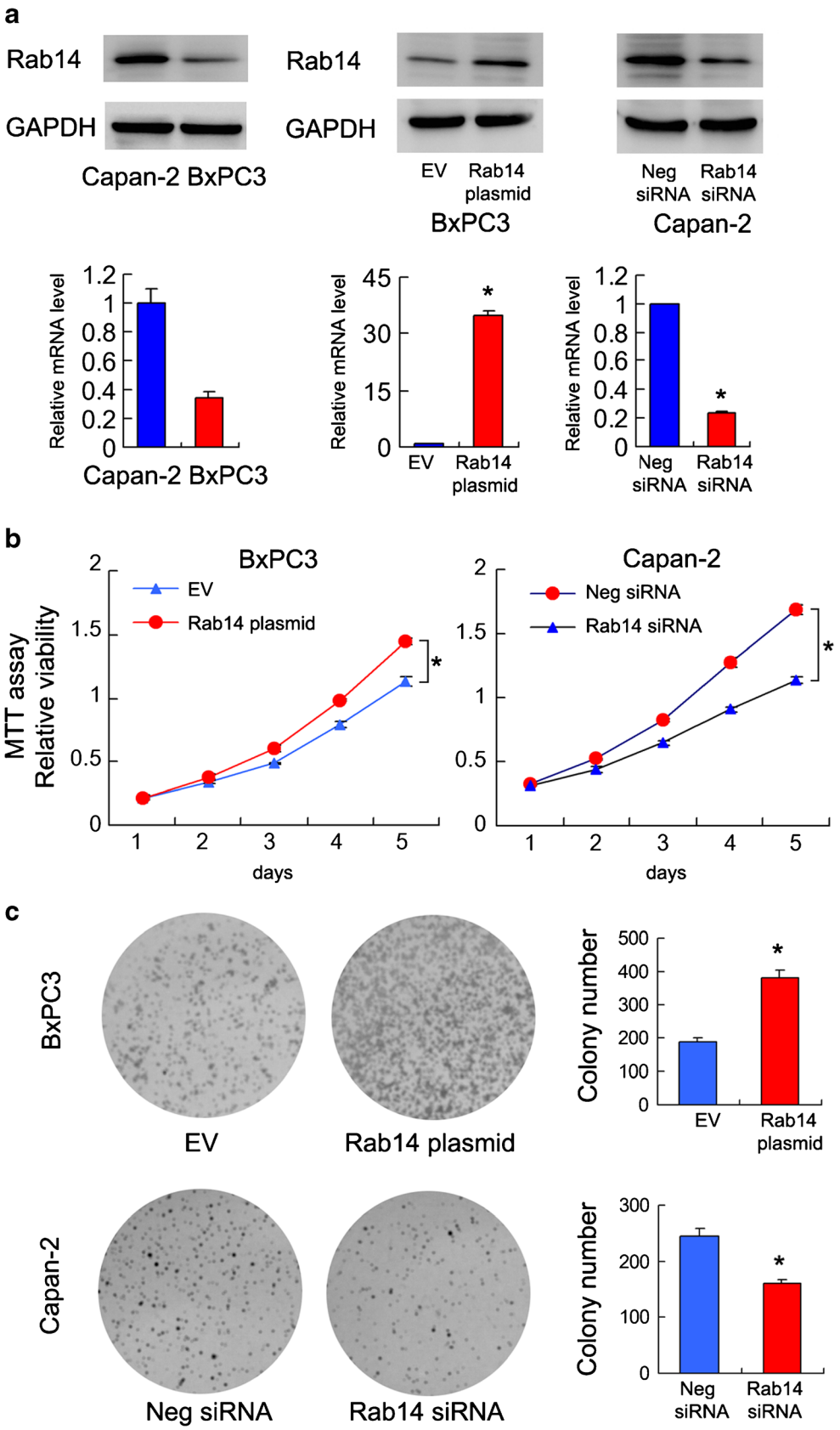


Fig. 1 Expression of Rab14 in pancreatic cancers. **A** Negative Rab14 staining in normal pancreatic tissues. **B** Positive Rab14 staining in pancreatic carcinoma. **C** Negative Rab14 staining in pancreatic carcinoma. **D** Distribution/percentage of IHC scores in 103 samples. **E** Western blot of Rab14 protein status in ten paired PDAC with adjacent normal tissues. Densitometry analysis of the western blots was performed as indicated. Rab14 protein levels were significantly higher in cancer tissues than in normal tissues of most cases. **F** Kaplan-Meier plot of patients with PDAC; data was obtained from TCGA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◀ **Fig. 2** Rab14 promotes cancer cell proliferation. **A** Western blot of Rab14 expression in BxPC-3 and Capan-2 pancreatic cancer cell lines. Plasmid transfection significantly upregulated Rab14 protein and mRNA levels in BxPC-3 cells, and siRNA treatment downregulated Rab14 protein and mRNA expression in Capan-2 cells. **B** MTT assay showed that Rab14 overexpression promoted proliferation of BxPC-3 cell line. Rab14 depletion inhibited proliferation of Capan-2 cell line. **C** Colony formation assay demonstrated that Rab14 overexpression upregulated colony numbers in BxPC-3 cell line while Rab14 depletion downregulated colony numbers in Capan-2 cell line. $*p < 0.05$

at 37 °C for 2 h, visualization was performed using ECL (Thermo-Fisher, USA) and DNR BioImaging Systems (DNR, Israel).

MTT assay and colony formation

For MTT assay, cells were plated in 96-well plates in medium containing 10% FBS at approximately 5000 cells per well 24 h after transfection. Twenty microliters of 5 mg/ml MTT (thiazolyl blue) solution was added to each well and incubated for 4 h at 37 °C. The MTT formazan was dissolved in DMSO. Then the plate was measured at 490 nm using a plate reader.

For colony formation assay, cells were seeded in 6-cm culture plates and cultured in the incubator. After 2 weeks, the plates with cell colonies were stained using Giemsa. Colony numbers were counted manually, and colonies with > 50 cells were scored.

Quantitative real-time PCR

Quantitative real-time PCR was performed using SYBR Green master mix kit from Applied Biosystems. PCR was performed using 7500 real-time PCR system (Applied Biosystems) as follows: 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 60 s. GAPDH was used as the endogenous control. The fold change of target gene was calculated using the $2^{-\Delta\Delta Ct}$ Method. The sequences of the primer pairs are as follows: Rab14 for CATGGCAA CTGCACCATACAAC; Rab14 rev, GCAAGATTTTCCTA CTCCCATGTC; GAPDH for CCTGCACCACCAAC TGCTTA; and GAPDH rev, GGCCATCCACAGTC TTCTGAG.

Cell cycle analysis

Cells were fixed with 1% paraformaldehyde, which was stained with propidium iodide in PBS with RNase A for 30 min. Cell cycle analysis was analyzed using ACEA flow cytometer with NovoExpress.

Annexin V/PI staining

Annexin V/PI staining kit from BD bioscience was used to determine the rate of apoptosis according to the manufacturer's protocol. Negative control was prepared by incubating cells in the absence of inducing agent. Flow cytometry was performed using ACEA flow cytometer and NovoExpress software. Compensation matrix was calculated using the auto compensation function of NovoExpress software.

Matrigel invasion assay

Cell invasion assay was performed using a 24-well transwell chamber coated with 20-μl Matrigel (1:6 dilution). Cell suspension with serum-free medium was transferred to the upper matrigel chamber. Medium supplemented with 10% fetal bovine serum was added to the lower chamber. After 20–24 h, invading cells that invaded through the membrane were fixed and stained with hematoxylin.

Detection of mitochondrial membrane potential

The mitochondrial membrane potential (MMP) was detected by using the JC-1 staining method. Briefly, cells were harvested, washed with PBS, and incubated with 5-μM JC-1 (Cell Signaling Technology) for 30 min in the incubator. Then, cells were washed and analyzed using the ACEA flow cytometer (ACEA, USA). Data were analyzed using the NovoExpress software (ACEA, USA).

Statistical analysis

SPSS version 16 was used for all analyses. χ^2 test was carried out to examine the correlation between Rab14 and clinical parameters. Rab14 data from 174 cases of pancreatic ductal adenocarcinoma was obtained from The Cancer Genome Atlas (TCGA) (downloaded from http://www.oncolnc.org/). The Kaplan-Meier method was used to estimate the probability of patient survival, and differences in the survival of subgroups of patients were compared by using Mantel's log-rank test. Student's *t* test was performed to compare data between the control and experimental group. $P < 0.05$ was considered as statistically significant.

Results

Rab14 is overexpressed in human pancreatic cancer

To explore the expression pattern of Rab14 in human pancreatic cancer, we examined Rab14 protein expression in 103 cases of pancreatic ductal adenocarcinoma (PDAC) specimens using immunohistochemistry. Negative staining was

observed in most normal pancreatic tissues (16/20; Fig. 1A). In pancreatic cancer tissues, Rab14 showed high expression in 41 out of 103 (39.8%) PDAC tissues (Fig. 1B). The remaining 62 of 103 (60.2%) cases showed low expression (with 11 cases of negative staining) (Fig. 1C). Distribution of IHC scores is shown in Fig. 1D.We analyzed the correlation between Rab14 status and clinical factors. As shown in Table 1, Rab14 overexpression was significantly associated with higher histological grade ($p = 0.0434$) and advanced TNM stage ($p = 0.0204$), as well as nodal metastasis ($p = 0.0439$). We also performed analysis between subgroup (scores 4, 6, and 8) of high expression. As shown in supplementary Table 1, we found that Rab14 score (strong Rab14 staining in > 75% tumor cells) was significantly correlated with advanced TNM stage.

To further confirm the IHC results, we examined Rab14 protein status in ten paired pancreatic cancer tissues with corresponding normal tissues. As shown in Fig. 1E, significant Rab14 protein upregulation was observed in most PDAC tissues examined.

We then used the TCGA data for bioinformatic analyses. RNA-seq data from 174 cases of pancreatic ductal adenocarcinoma with follow-up information was obtained from TCGA database. These 174 cases were ranked from high to low based on mRNA levels of Rab14. The upper 40% cases were defined as high Rab14 expression and lower 60% as low Rab14 expression (according to the IHC results). We found a negative correlation between overall patient survival and Rab14 expression using the Kaplan-Meier plots ($p = 0.0424$ by the log-rank test; Fig. 1F). The results showed that high expression of Rab14 was correlated with poor overall survival in patients with PDAC.

Rab14 promotes cell proliferation, cell cycle progression, and invasion

Rab14 protein expression was examined in two pancreatic cancer cell lines including BxPC-3 and Capan-2. As shown in Fig. 2A, Capan-2 cell line has relatively high endogenous Rab14 expression while BxPC-3 cell line has relatively low Rab14 expression. Thus, BxPC-3 cells were used for plasmid transfection, and Capan-2 cells were used for Rab14 siRNA treatment. As shown in Fig. 2A, transfection and siRNA knockdown efficiency were validated by RT-qPCR and western blotting.

MTT and colony formation assays were carried out to examine the effect of Rab14 on cancer cell proliferation. MTT showed that the proliferation rate of Capan-2 cells decreased significantly after Rab14 depletion while Rab14 overexpression increased the proliferation rate of BxPC-3 cells (Fig. 2B). Colony formation assay demonstrated that Rab14 positively regulated the colony formation ability of BxPC-3 cells, while Rab14 depletion diminished colony formation ability (Fig. 2C). In addition, cell cycle analysis showed that Rab14 transfection increased the percentage of BxPC-3 cells in S phase and promoted G1-S transition in BxPC-3 cells, while Rab14 depletion inhibited the G1-S cell cycle transition (Fig. 3A).

The significant correlation between Rab14 and positive lymph node metastasis suggested it may influence cancer cell invasion. As shown in Fig. 3B, Rab14 overexpression upregulated the invasive capacity of BxPC-3 cells, while Rab14 siRNA downregulated invasion by Capan-2 cells.

Rab14 regulates gemcitabine sensitivity

We also examined the effect of Rab14 on gemcitabine resistance. Gemcitabine at a dosage of 1 μM was used to treat Rab14 overexpressing or depleted pancreatic cancer cells for 24 and 48 h. MTT assay showed that Rab14 transfection upregulated BxPC-3 cell viability after 24 and 48 h of drug treatment (Fig. 4A). In contrast, Rab14 depletion in Capan-2 cells downregulated cell viability, suggesting Rab14 induces gemcitabine resistance. Annexin V/PI analysis was also used to examine apoptosis rate. As shown in Fig. 4B, Rab14 overexpression reduced the rate of gemcitabine-induced apoptosis, while Rab14 knockdown upregulated gemcitabine-induced apoptosis. These results suggest that Rab14 overexpression reduces gemcitabine sensitivity in pancreatic cancer cells.

Rab14 regulates mitochondrial membrane potential

Because sensitivity to chemotherapeutic drugs is closely related to mitochondrial function, we examined whether Rab14 influences mitochondrial membrane potential (MMP). JC-1 staining was used to monitor changes in MMP after gemcitabine treatment. JC-1 staining exhibits red fluorescence under normal conditions but emits green fluorescence when MMP is downregulated after drug treatment. As shown in Fig. 5, Rab14 overexpression in BxPC-3 cells treated with gemcitabine decreased percentage of cells with green fluorescence, suggesting that Rab14 upregulated MMP. In contrast, Rab14 siRNA diminished MMP in gemcitabine-treated Capan-2 cells.

Rab14 regulates cell cycle and apoptosis-related proteins

To elucidate the mechanism of Rab14 underlying gemcitabine sensitivity and proliferation, we checked for changes in related proteins using western blotting. As shown in Fig. 6, Rab14 overexpression upregulated cyclin A, cyclin D1, cyclin E, p-Rb, and Bcl-2 while downregulating p21 in BxPC-3 cells. In contrast, Rab14 downregulated cytochrome c, cleaved caspase3, and cleaved PARP in BxPC-3 cells treated with gemcitabine. Meanwhile, Rab14 siRNA treatment showed the opposite effect in Capan-2 cells.

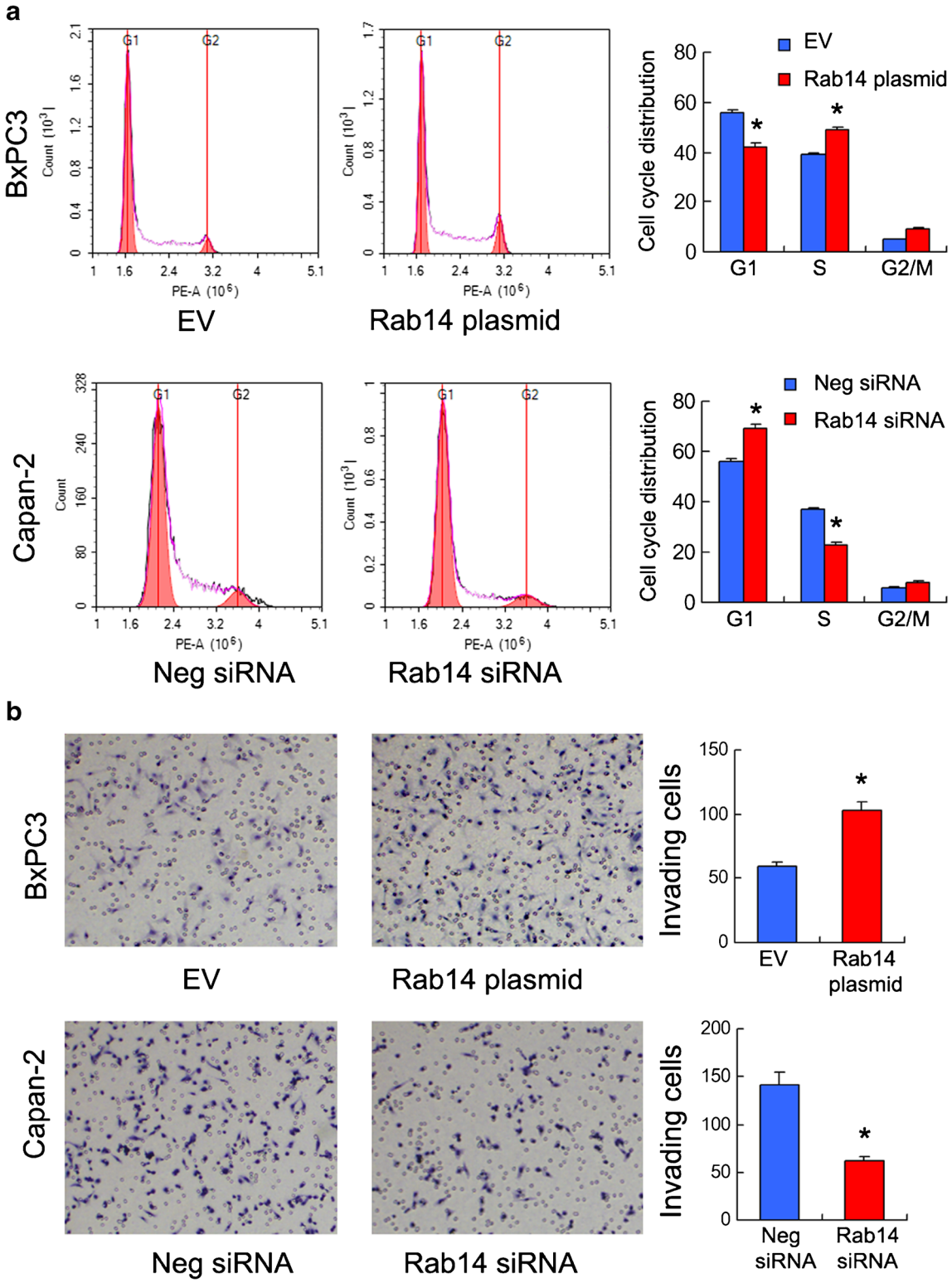

Fig. 3 Rab14 regulates cell cycle and invasion. **A** Cell cycle analysis demonstrated that Rab14 overexpression upregulated and downregulated the percentage of S phase and G1 phase BxPC-3 cells, respectively, while Rab14 depletion upregulated and downregulated the percentage of G1 phase and S phase Capan-2 cells, respectively. **B** Matrigel invasion assay demonstrated that Rab14 overexpression increased the invading number of BxPC-3 cells while Rab14 depletion decreased the number of invading Capan-2 cells.*$p < 0.05$

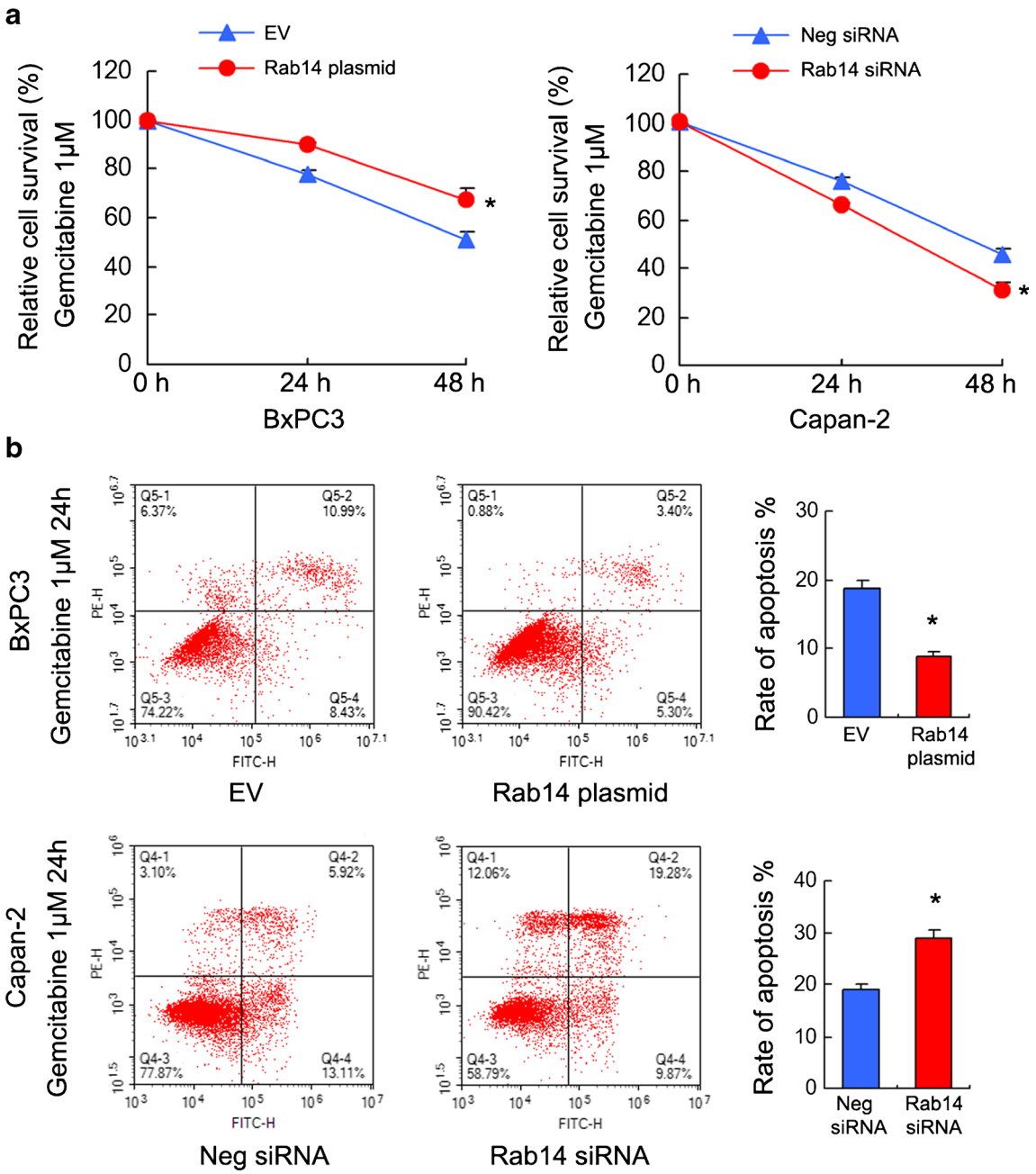

Fig. 4 Rab14 regulates gemcitabine-induced apoptosis in pancreatic cancer cells. **A** MTT assay showed that when treated with gemcitabine, Rab14 overexpression in BxPC-3 cells upregulated cell viability after 24 and 48 h of treatment, while Rab14 depletion downregulated Capan-2 cell viability. **B** Annexin V/PI analysis showed that Rab14 overexpression inhibited gemcitabine-induced apoptosis in BxPC-3 cells, and its depletion enhanced gemcitabine-induced apoptosis rate in Capan-2 cells. *$p < 0.05$

Rab14 regulates Bcl-2 expression through YAP

Bcl-2 is closely related to sensitivity to drugs and MMP status. We sought to determine the mechanism underlying the effect of Rab14 on Bcl-2 regulation. We found that Rab14 could positively regulate YAP, an important effector of the Hippo signaling pathway. Rab14 overexpression upregulated while Rab14 depletion downregulated both total and nuclear YAP protein in pancreatic cancer cells (Fig. 7A). To further validate the association between YAP and Bcl-2 in pancreatic cancer cells, we treated BxPC-3 cells with YAP siRNA and tested the effect of Rab14 plasmid in YAP-depleted cells. As shown in Fig. 7B, YAP depletion significantly downregulated Bcl-2 protein levels. YAP depletion abolished the effect of Rab14 plasmid on Bcl-2 in BxPC-3 cells. YAP depletion also downregulated Bcl-2 protein expression in Capan-2 cells.

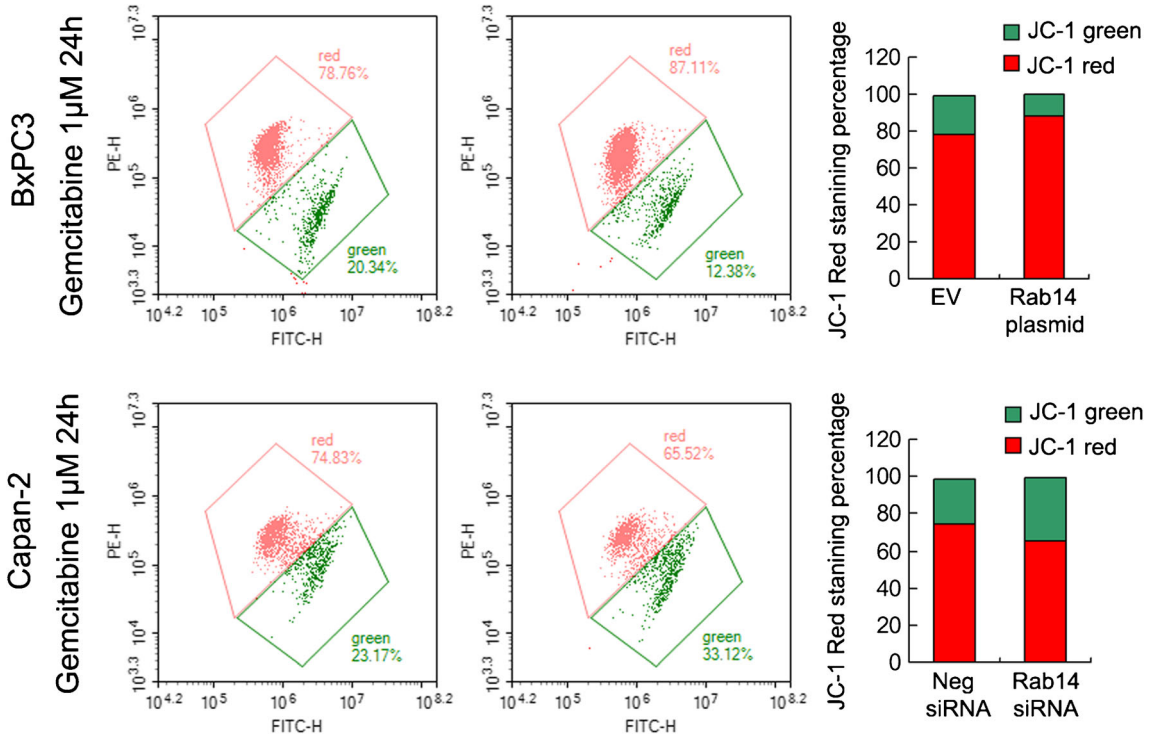

Fig. 5 Rab14 regulates mitochondrial membrane potential. JC-1 staining demonstrated that Rab14 overexpression upregulated mitochondrial membrane potential as indicated by the increased JC-1 (red/green ratio), while Rab14 depletion downregulated mitochondrial membrane potential with decreased JC-1 (red/green ratio)

Discussion

Many researchers have demonstrated that Rab family proteins play crucial functions in the tumorigenesis and cancer development [10]. Rab14 plays a pivotal role in regulating membrane vesicle transport and signal transduction [4]. Recent studies show that Rab14 is upregulated in ovarian and lung cancers [5, 6], yet no study has assessed its exact roles in pancreatic cancer.

To address this issue, we examined Rab14 protein expression patterns and found overexpression in 41/103 (39.8%) pancreatic cancer tissues. High Rab14 status positively correlated with advanced TNM stage as well as positive nodal metastasis. Furthermore, our results were supported by TCGA database, which showed that Rab14 high expression correlated with poor overall survival in patients with PDAC. To our knowledge, this is the first study showing clinical significance of Rab14 overexpression in pancreatic carcinoma.

To examine the putative role of Rab14 as an oncogene in pancreatic cancer, we transfected Rab14 plasmid in BxPC-3 cells and depleted Rab14 in Capan-2 cells. Cancer cell proliferation was examined by MTT, colony formation assay, and cell cycle analysis. Our data showed that Rab14 promoted cancer cell proliferation and colony formation ability, as well

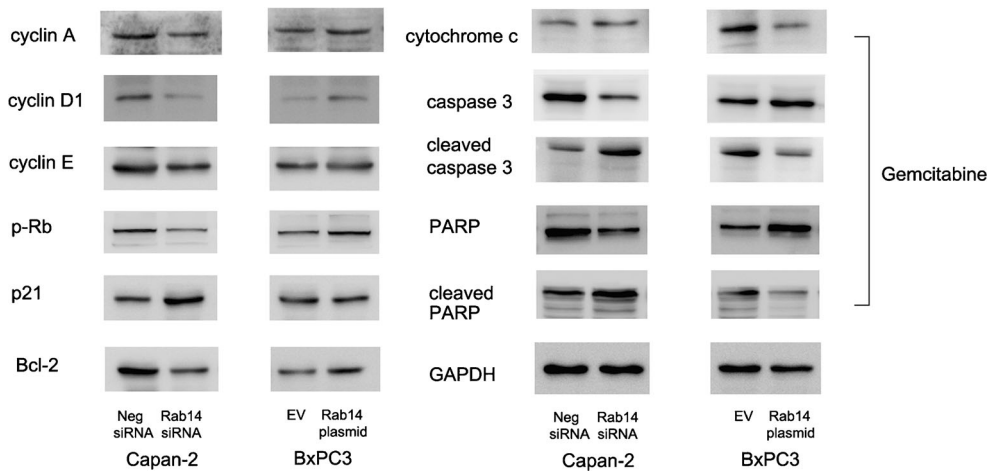

Fig. 6 Rab14 regulates cell cycle and apoptosis-related proteins. Western blot showed that Rab14 overexpression upregulated the levels of cyclin A, cyclin D1, cyclin E, p-Rb, Bcl-2, caspase 3, and PARP and downregulated Bcl-2, cytochrome c, cleaved caspase 3, and cleaved PARP in BxPC-3 cells. Rab14 depletion downregulated showed the opposite effects in Capan-2 cells

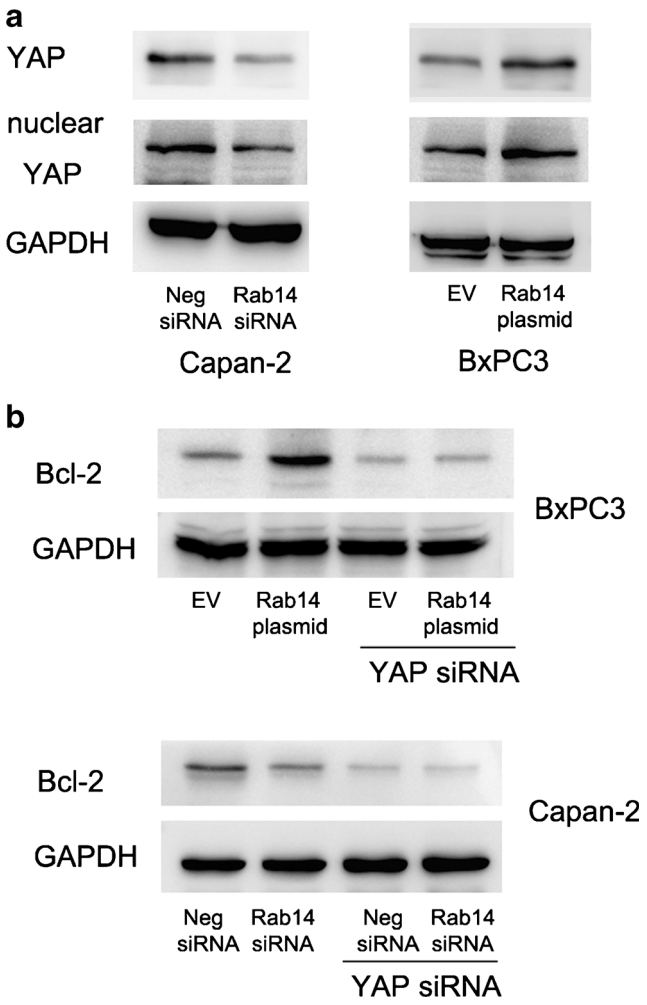

Fig. 7 Rab14 regulates Bcl-2 expression through YAP. **A** Rab14 overexpression upregulated and Rab14 depletion downregulated total and nuclear YAP protein. **B** Bcl-2 protein expression was significantly diminished in BxPC-3 cells treated with YAP siRNA, and Rab14 overexpression failed to upregulate Bcl-2 expression in YAP siRNA-treated cells. YAP siRNA also downregulated Bcl-2 protein expression in Capan-2 cells

as G1-S cell cycle transition. Western blots demonstrated that Rab14 overexpression upregulated p-Rb, cyclin D1, cyclin E, and cyclin A and downregulated p21 protein in BxPC-3 cells. In Capan-2 cells, Rab14 siRNA downregulated p-Rb, cyclin D1, cyclin E, and cyclin A and upregulated p21 protein. All of these proteins are closed associated with cell cycle and cancer cell proliferation. Cyclin D1, cyclin E, and cyclin A are cell cycle regulators which participate in the G1-S transition and in pancreatic cancer progression [11, 12]. p21 is a cell cycle inhibitor, and its depletion facilitates cell proliferation [13, 14]. The retinoblastoma tumor suppressor protein Rb, which prevents excessive cell growth by inhibiting cell cycle progression, is dysregulated in most cancers [15]. When Rb is phosphorylated, it becomes inactive and allows cell cycle progression. Thus, our results supported the hypothesis that Rab14 was a promoter of pancreatic cancer progression. However, we are also aware that the BxPC-3 cell line with lower levels of endogenous Rab14 originated from a poorly differentiated tumor, while Capan-2 with a high level of endogenous Rab14 was derived from a well-differentiated tumor from which the patient had survived for a considerable time. This is not in accord with our IHC results. We believe that this discrepancy was based on selection bias due to a limited number of PDAC cell lines.

The role of Rab14 on cisplatin sensitivity has been demonstrated in oral squamous cell carcinoma [8]. In the present study, we explored the role of Rab14 in gemcitabine sensitivity of pancreatic cancer cell and its possible mechanism. Our data demonstrated that Rab14 overexpression maintained cell viability after treatment with gemcitabine. In addition, the rate of gemcitabine-induced apoptosis was lower in Rab14 overexpressing cells. Accordingly, western blots demonstrated that Rab14 downregulated cytochrome c release and caspase3/PARP cleavage, indicating its role in apoptosis inhibition.

Chemotherapeutic drugs, including gemcitabine, induce apoptosis through the mitochondria-dependent pathway, and mitochondria function is important during gemcitabine resistance. Using JC-1 staining, we found that Rab14 overexpression upregulated mitochondrial membrane potential (MMP) while Rab14 depletion downregulated MMP. Loss of MMP triggers apoptosis through the mitochondria-dependent pathway, which accelerated cytochrome c release with increased mitochondrial membrane permeability [16]. Thus, our results indicated that Rab14 might inhibit apoptosis through positive regulation of MMP.

To search for the possible mechanism underlying the effect of Rab14 gemcitabine sensitivity and mitochondrial function, we screened related proteins and found that Rab14 was able to upregulate Bcl-2, an important anti-apoptotic protein. Bcl-2 was reported to protect cancer cell from drug-induced cell death by preservation of MMP, reducing mitochondrial membrane permeabilization, and maintaining its integrity [17, 18]. Our results suggested that Rab14 downregulates gemcitabine sensitivity by upregulating Bcl-2 and maintaining mitochondrial membrane potential. Next, we investigated the potential mechanism by which Rab14 upregulates Bcl-2 expression. After screening several signaling pathways, we found that Rab14 increased levels of YAP, a key effector of the Hippo signaling pathway. Previous reports indicated that Bcl-2 was a downstream target of YAP in various cells [19, 20]. We further validated the correlation between YAP and Bcl-2 using YAP siRNA. Rab14 overexpression failed to induce Bcl-2 in YAP-depleted cells, which supported the presence of Rab14 regulation on YAP/Bcl-2 in pancreatic cancer cells.

A recent study indicated the potential effect of Rab14 on invasion and metastasis in which it was reported that Rab14 affected EphA2 trafficking to generate cell-cell repulsion events that drove tumor cells apart, promoting efficient invasion and metastasis of pancreatic cancer [21]. Our study mainly focused

on the role of Rab14 in regulating gemcitabine sensitivity, suggesting that Rab14 was a potential biomarker for gemcitabine treatment. However, we only used two cell lines, and further study will be required to validate the role of Rab14 in cell lines with different molecular background and to test the expression and role of Rab14 in gemcitabine resistant cell lines.

In conclusion, the current study has identified Rab14 as an oncoprotein which is overexpressed in human pancreatic cancers. Rab14 promoted pancreatic cancer cell proliferation and inhibited gemcitabine-induced apoptosis by maintaining mitochondrial membrane potential, possibly through YAP/Bcl-2 signaling.

Contributions JG and CG conceived and designed the experiments. JG performed the experiments. CG wrote the manuscript. All authors reviewed and approved the manuscript.

Compliance with ethical standards

This study was carried out according to the recommendations of the ethics committee of the First affiliated hospital of China Medical University.

The study was performed according to the standards set in the Declaration of Helsinki 1975.

Conflict of interest The authors declare that they have no conflict of interest.

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