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ORIGINAL ARTICLE

Expression of miR-214 in pancreatic cancer and its effect on the biological function of pancreatic cancer cells

Shikang Deng[1,2], Junfeng Wang[2], Jing Xu[2], Jiao Li[1], Li Zhang[2], Yan Jin[2]

[1]Medical School, Kumming University of Science and Technology, Kumming, China. [2]Department of Hepatobiliary and Pancreatic Surgery, First People's Hospital of Yunnan Province, affiliated Hospital of KMUST, Kumming, Yunnan, China.

Summary

Purpose: *To study the expression and biological function of microRNA 214 (miR-214) in pancreatic cancer.*

Methods: *101 patients with pancreatic cancer who came from First People's Hospital of Yunnan Province from December 2013 to December 2016 were selected. 101 pancreatic cancer tissues and 101 adjacent tissues were resected and collected. The miR-214 expression was detected by qRT-PCR. Then the pancreatic cancer cell line AsPC-1 and SW1990 were transfected. MTT assay was used to detect cell viability and flow cytometry was used to detect apoptosis. Transwell chamber assay was used to detect the invasion and migration of cells in vitro. The protein expressions of ING4 in AsPC-1 and SW1990 cells were detected by Western blot.*

Results: *The relative expression of miR-214 in pancreatic cancer was significantly higher than that in adjacent tissues ($p<0.05$). There was a statistically significant difference between the expression level of miR-214 and T stage of pancreatic cancer ($p<0.05$). The relative expression of ING4 protein in SW1990 cells of miR-214 mimics group was significantly lower than that of miR-214 control mimics group ($p<0.05$), and that in AsPC-1 cells of the miR-214 inhibitors group was significantly higher than that in the miR-214 control inhibitors group ($p<0.05$).*

Conclusion: *In conclusion, the expression of miR-214 is highly expressed in pancreatic cancer tissues, and the down-regulation of ING4 protein expression can inhibit the proliferation, invasion and migration of pancreatic cancer cells, promote their apoptosis, and can be used as a new molecular target for the diagnosis and treatment of pancreatic cancer.*

Key words: *miR-214, pancreatic cancer, expression, biological function*

Introduction

Pancreatic cancer is one of the most common and fatal malignant tumors at present. It is usually difficult to be diagnosed in the early stage, the grade of malignancy is high, the 5-year survival rate after routine treatment is low, and the specific pathogenesis is not completely clear, which greatly increases the difficulty of diagnosis and treatment. Therefore, how to improve the diagnosis and treatment of pancreatic cancer is difficult and a hotspot at present. Although the diagnosis and treatment of pancreatic cancer have been improved and updated, the effect is still not ideal [1-3], so it is urgent to explore suitable, reliable and sensitive biomarkers for early diagnosis and treatment of this malignancy [4]. A significant difference was found in the expression profiles of miRs in pancreatic cancer tissues and other normal non-cancer tissues. For example, in pancreatic ductal carcinoma, the difference of the expression level of miRs can be detected by filtration biopsy, which can accurately distinguish malignant and benign pancreatic tissues. It was also found that the expression pattern of miRs can be used to identify pancreatic cancer tissue, chronic pancreatitis tissue, and normal pancreatic tissue [5-7].

Corresponding author: Jin Yan, MD. Department of Hepatobiliary and Pancreatic Surgery, First People's Hospital of Yunnan Province, Affiliated Hospital of Kunming University of Science and Technology, Kunming, China.
Tel: +86 13700692358, Fax: +86 65333801, Email: 1197189367@qq.com
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miRs are non-coding endogenous RNAs with a length of about 20 nucleotides, and can regulate the expression of a gene by targeting the corresponding mRNA [8]. It has been proved that the biological functions of miRs in tumor cells include mainly cell proliferation, migration and invasion, which play an important role in the regulation of gene expression [9,10]. A study [11] found that disorders of miRs are more common in the recurrence of ovarian cancer, and the miR-214 in ovarian cancer mainly acts on the cell survival function and induces cisplatin resistance by targeting the PTEN/Akt pathway. It has also been found that miR-214 is related to pancreatic cancer and has a high expression level in this malignancy. Overexpression of miR-214 can accelerate the proliferation and differentiation of pancreatic cancer cells and accelerate its development [12].

However, there are few reports about the role of miR-214 in the development of pancreatic cancer. Therefore, qRT-PCR was used to detect the difference in the expression level of miR-214 between pancreatic cancer tissues, adjacent tissues and normal pancreatic ductal epithelial cells, as well as the effect of regulating related proteins on the proliferation, apoptosis and other biological functions of pancreatic cancer cells, so as to provide a more complete molecular biological basis and a new molecular direction for the diagnosis and treatment of this disease.

Methods

General materials

A total of 101 patients with pancreatic cancer who came from First People's Hospital of Yunnan Province from December 2013 to December 2016 were enrolled as the subjects of the study. During the operation, 101 pancreatic cancer tissues and 101 adjacent tissues were resected and collected with the consent of the patient. The tissues were immediately stored in a liquid nitrogen container after resection. There were 46 females and 55 males with an average age of 60.2 ±11.4 years.

The inclusion criterion was patients pathologically diagnosed with pancreatic cancer.

The exclusion criteria were as follows: patients with other malignant tumors; patients with metastasis; patients with cognitive impairment or communication disorders; and patients with poor compliance. All patients and their families agreed to participate in the study and signed the informed consent form. This study has been approved by the Hospital Ethics Committee.

Experimental reagents and materials

Human normal pancreatic ductal epithelial (HPDE) cell line and human pancreatic cancer cell lines AsPC-1, Panc-1, MIApaca-2 and SW1990 were purchased from ATCC in the United States. Trizol reagent was purchased from Shanghai Haoran Bio technologies Co., Ltd. Ultraviolet Spectrophotometer was purchased from Shanghai INESA Analytical instrument Co., Ltd. cDNA reverse transcription kit was purchased from Beijing TransGen Biotech Co., Ltd. Real-time quantitative PCR instrument was purchased from Beijing Zhongke Keer instrument Co., Ltd. qRT-PCR kit was purchased from Epitomics Inc. Human pancreatic cancer cell line AsPC-1 was purchased from Shanghai Beinuo Biotechnology Co., Ltd. Fetal bovine serum (FBS) was purchased from Jiangsu Kejing Biotechnology Co., Ltd. 0.25% of pancreatin was purchased from Sigma Inc. Lipofectamine 2000 transfection reagent and the sterile tip were purchased from Thermo Fisher Scientific (China). MTT solution was purchased from Shanghai Yuanmu Biotechnology Co., Ltd. Dimethyl sulfoxide (DMSO) was purchased from Changzhou Hengyu Chemical Co., Ltd. Enzymatic marker was purchased from Beijing Potenov Technology Co. Ltd. FACS Calibur Flow Cytometry was purchased from BD Inc. Annexin V-FITC/propidium iodide (PI) cell apoptosis kit was purchased from Jiangsu KeyGEN BioTECH Corp., Ltd. PI dye and crystal violet were purchased from Shanghai yuanye Bio-Technology Co., Ltd. Transwell chamber was purchased from Beijing Unique Biotechnology Co., Ltd. DMEM (Dulbecco's Modified Eagle's Medium) was purchased from Shanghai Shifeng Biotechnology Co., Ltd. All primers and transfected plasmids were synthesized and designed in Shanghai GenePharma Co., Ltd. Bicinchoninic acid (BCA) kit was purchased from Nanjing SenBeiJia Biological Technology Co., Ltd. Rabbit anti-human ING4 monoclonal antibody was purchased from Sigma company, rabbit anti-human monoclonal antibody GAPDH was purchased from Santa Cruz Biotechnology, electrochemilumine (ECL) developer was purchased from Best Bio company.

Experimental methods

RT-PCR detection

Total RNA was extracted from pancreatic cancer tissue specimens and adjacent tissues and AsPC-1, Panc-1, MIApaca-2 and SW1990 cells by referring to the instructions of Trizol kit. The purity and concentration of RNA were detected by ultraviolet spectrophotometer. RNA

Table 1. Sequence table of related primers

Factor	*Upstream primer*	*Downstream primer*
miR-214	5'-GGACAGGACGCACAGTCA-3'	5'-CAGACGAGGCTCCGTGGT-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'

was reversely transcribed to cDNA by reverse transcription kit. SYBR Green I dye was used for RT-PCR amplification and the reaction system was 20 μL. Amplification conditions were as follows: 90°Cfor 5 min; denaturation at 90°Cfor 20 s; annealing at 65°Cfor 30 s; extension at 72°Cfor 45 s; U6 was used as internal reference for PCR. After 40 cycles of this step, the expression level of miR-214 was finally determined (Table 1).

Culture, transmission and transfection of AsPC-1 and SW1990

Human pancreatic cancer cell lines AsPC-1 and SW1990 were taken out and placed in a medium containing 10% of fetal bovine serum (FBS) and cultured in an incubator of 5% of CO_2 at room temperature. When the cell adherent growth reached 60%, the cells were rinsed with phosphate buffered saline (PBS), then digested with 0.25% trypsin. After digestion, 10% of the culture medium was added to continue to culture at 37°C with 5% CO_2. The cells growing in logarithmic phase were transfected. The SW1990 group was transfected with miR-214 mimics and the corresponding negative control group was transfected with miR-214 control mimics, and the AsPC-1 group was transfected with miR-214 inhibitors and the corresponding negative control group was transfected with miR-214 control inhibitors The transfection steps were as follows: first, the cells were inoculated in 6-well plates with a density of 3×10^5 cells/per well; then, lipofectamine 2000 and DNA were diluted and mixed strictly according to the instructions of Lipofectamine 2000 transfer kit, and was placed at 37°C for 5min; the mixture was mixed with the cells and transfected at 37°C with 5% CO_2; the expressions of miR-214 in AsPC-1 and SW1990 cells were detected by real-time quantitative PCR 48 h after transfection (the methods were the same) and the cells were collected for follow-up experiments.

MTT detection of proliferation

Forty eight h after transfection, the cells in each group were inoculated in 96-well plates, and the cells with 50 μl of FBS (10%) were completely cultured in each well, at a cell density 3×10^4 cells per well. At 0 h, 24 h, 48 h and 72 h, the cell suspensions of each group were incubated at room temperature with MTT 5 mg/mL for 4 h, and then 150 ul of dimethyl sulfoxide (DMSO) were added for dissolution and precipitation. The optical density (OD) value of each well was measured at 490 nm wavelength by microplate reader. Cell proliferation was assessed in each group. The experiment was repeated 3 times. Cell proliferation=(absorbance value of experimental group-absorbance value of control group) / (absorbance value of control group-absorbance value of blank group).

Detection of apoptosis by flow cytometry

AsPC1 and SW1990 cells in the logarithmic growth phase were inoculated in 6-well plates. The cell concentration was adjusted to 3×10^5 cells per well for inoculation, and after 24 h incubation, the cells were rinsed twice with PBS. Five μl of Annexin V-FITC were added after rinse and 10 μl of PI were added after reaction of 10 min at room temperature. Light avoidance dyeing was performed for 30 min at room temperature. The flow cytometry was used for the detection and analysis of cell apoptosis. The experiment was repeated 3 times.

Detection of invasiveness and migration of cells in vitro by Transwell chamber assay

The transfected cells in each group were digested and centrifuged, and a line was drawn in each hole with the sterile tip of 10 μL and marked. The cells were then resuspended in DMEM medium without FBS and their density was controlled to be 2×10^5 cells/ml. About 100 μl of cell suspension were added into the Transwell upper chamber (The upper chamber of the invasion experiment was covered with Matrigel, and the upper chamber of the migration experiment was not covered with Matrigel). About 600 μl of DMEM medium containing 10% FBS were added to the lower chamber, and then incubated at room temperature for 6 h. After culture, the supernatant in the chamber was cleaned with cotton swabs, and then rinsed with PBS. The cells in the lower chamber were fixed with 95% of ethanol solution for 15 min. The cells were removed and rinsed again with PBS, and then dyed with 0.1% of crystal violet. After dyeing, the number of cell penetration in 6 wells was calculated randomly by microscope, and the mean value was considered to represent the value of migration force. The experiment was repeated 3 times. At the beginning of scratch (W0) and 48 h after culture (W48), images were taken with inverted phase contrast microscope (200x) to observe the remaining distance after the migration of each group of cells from the edge of the scratch to the center of the scratch. Cell migration index (M1) = (W0-W48) / W0 × 100%.

The protein expression of ING4 in the transfected AsPC-1 and SW1990 cells detected by Western blot

Forty-eight h after transfection, cells of each group were washed with 0.01 mol/L PBS for 3 times. The tissues were put on ice and RIPA was added to fully lyse the tissues. Then, tissues were centrifuged at 10 000 r/min for 20 min and the supernatant was taken to collect the total protein. The total protein concentration was determined by BCA method. The loading buffer was added, boiled for 5 min, then the protein samples were separated with SDS-PAGE, transferred to PVDF membrane, sealed with 50 g/L skim milk powder at room temperature for 1 h, added with ING4 (1:1000) primary antibody and GAPDH primary antibody (1:1000) at 4°Covernight. VDF membrane was washed three times with tris buffered saline tween (TBST), and the corresponding secondary antibody was added for incubation at 37°Cfor 2 h. Finally, the color was stained with ECL developer, and the gray value of each band was analyzed by ImageJ image analysis software to obtain the relative expression of ING4 protein in cells. The experiment was repeated 3 times.

Statistics

SPSS 19.0 software (from Bizinsight (Beijing) Information Technology Co., Ltd.) was used in this study for

statistical analyses. Measurement data were reported as mean ± standard deviation. T-test was used to analyze the data between two groups, and single factor variance analysis was used to compare data among groups, repeated measures of variance were used between the two groups at different time points of treatment, and Kaplan-Meier survival analysis and log-rank test were performed to determine the relationship between miR-214 expression and prognosis in pancreatic cancer patients using repeated measures of variance analysis between the two groups at different time points of treatment. GraphPad Prism 8 software was used to visualize the graphics in this study. Statistical significance was set at $p<0.05$.

Results

Expression of miR-214 in pancreatic carcinoma and adjacent tissues

The expression of miR-214 in pancreatic cancer tissues was significantly higher than that in adjacent tissues ($p<0.05$) (Table 2).

The relationship between miR-214 expression and clinicopathological features of pancreatic cancer patients

According to the average expression level of miR-214 in pancreatic cancer tissues (3.46±1.23),

Table 2. Expression of miR-214 in pancreatic carcinoma and adjacent tissues

Factor	*Pancreatic cancer tissue (n=101)*	*Adjacent tissue (n=101)*	*t*	*p*
miR-214	3.46±1.23	2.42±1.13	6.258	<0.001

Table 3. The relationship between miR-214 expression and clinicopathological features of pancreatic cancer patients

Clinicopathological features	*n*	*miR-214*		x^2	*p*
		High-expression group (n=54)	Low-expression group (n=47)		
		n	n		
Gender (case)				0.026	0.871
Male	55	29	26		
Female	46	25	21		
Age (years)				0.166	0.684
≤60	43	24	19		
>60	58	30	28		
Tumor size (cm)				0.226	0.634
≤2	32	16	16		
>2	69	38	31		
T stage				11.711	<0.001
T1-T2	42	14	28		
T3-T4	59	40	19		
TNM stage (case)				0.300	0.584
I-II	77	40	37		
III-IV	24	14	10		
CA19.9 (ng/mL)				0.752	0.386
≤37	26	12	14		
>37	75	42	33		
Lymph node metastasis (case)				2.746	0.098
Yes	41	26	15		
No	60	28	32		
Blood vessel invade (case)				0.222	0.638
Yes	62	32	30		
No	39	22	17		
Lymphatic vessel invasion (case)				0.120	0.729
Yes	34	19	15		
No	67	35	32		
Neural invasion (case)				0.176	0.675
Yes	71	37	34		
No	30	17	13		

patients with higher-than-average expression were divided into the high-expression group (n=54), and patients with lower-than-average expression were divided into the low-expression group (n=47). There was a statistically significant difference between the expression level of miR-214 and T stage of pancreatic cancer ($p<0.05$), but without statistically significant difference between miR-214 and gender, age, tumor size, TNM stage, and CA19.9 expression ($p>0.05$; Table 3).

The relationship between miR-214 expression and prognosis of pancreatic cancer patients

Kaplan-Meier survival analysis showed that the 3-year overall survival rate of pancreatic cancer patients with high miR-214 expression was significantly lower than that of patients with low miR-214 expression ($p<0.05$; Figure 1).

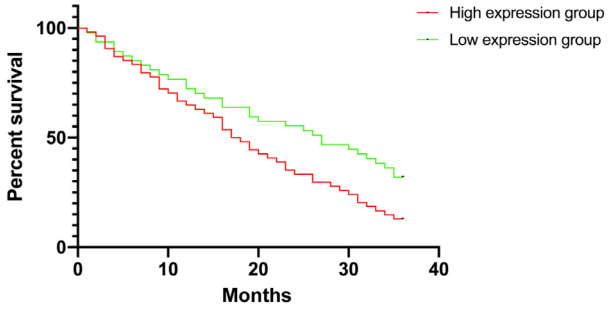

Figure 1. The relationship between miR-214 expression and the 3-year overall survival rate of pancreatic cancer patients. Kaplan-Meier survival analysis showed that the 3-year overall survival rate of pancreatic cancer patients with high miR-214 expression was significantly lower than that of patients with low miR-214 expression ($p<0.05$).

Relative expression levels of miR-214 in HPDE and human pancreatic cancer cell lines AsPC-1, Panc-1, MIApaca-2 and SW1990

The relative expressions of AsPC-1, Panc-1, MsIApaca-2 and SW1990 were significantly higher than that of HPDE, with the highest relative expression of AsPC-1 and the lowest relative expression of SW1990 ($p<0.05$; Figure 2).

Comparison of expression levels of miR-214 in AsPC-1 and SW1990 after transfection

The expression of miR-214 of miR-214 mimics group in SW1990 cells was significantly higher than that of miR-214 control mimics group, and the

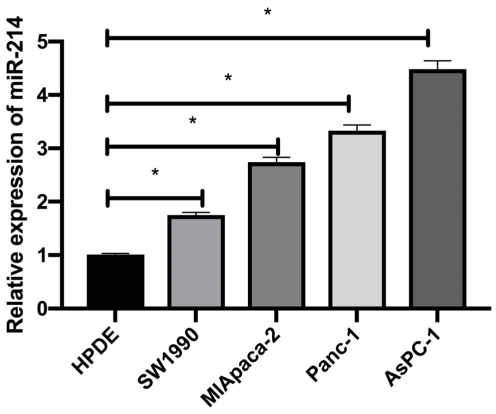

Figure 2. Relative expression levels of miR-214 in HPDE and human pancreatic cancer cell lines AsPC-1, Panc-1, MIApaca-2 and SW1990. The relative expressions of AsPC-1, Panc-1, MIApaca-2 and SW1990 were significantly higher than that of HPDE, with the highest relative expression of AsPC-1 and the lowest relative expression of SW1990 (*$p<0.05$).

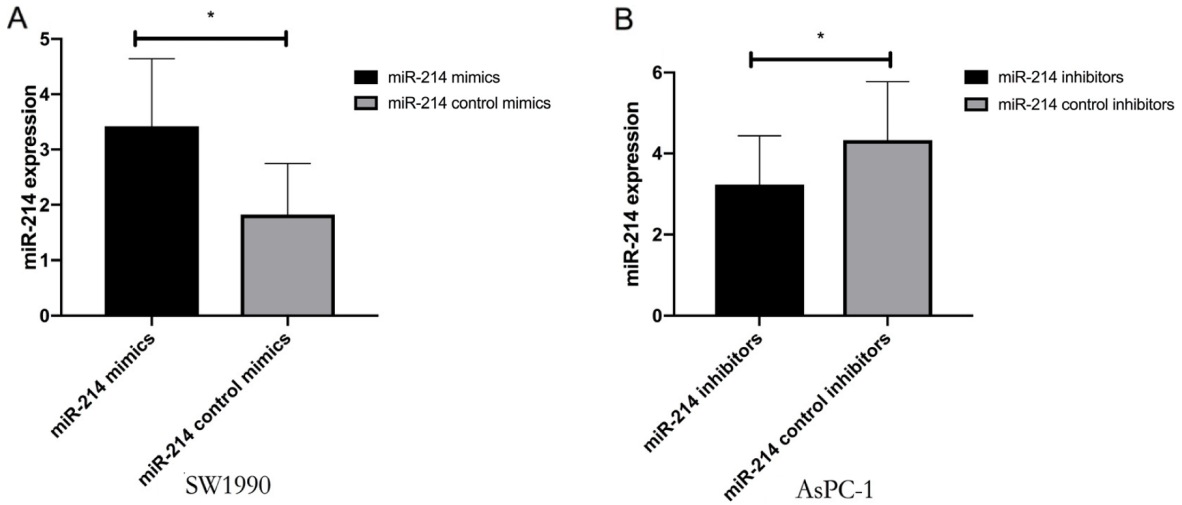

Figure 3. Comparison of expression levels of miR-214 in cells of each group after transfection. **A:** The expression of miR-214 mimics group in SW1990 cells was significantly higher than that of miR-214 control mimics group. **B:** The expression of miR-214 inhibitors group in AsPC-1 cells was significantly lower than that of miR-214 control inhibitors group (*$p<0.05$).

expression of miR-214 of miR-214 inhibitors group in AsPC-1 cells was significantly lower than that of miR-214 control inhibitors group (p<0.05; Figure 3).

Effect of miR-214 on the proliferation of AsPC-1 and SW1990 cells

The proliferation of SW1990 cells in miR-214 mimics group 24 h, 48 h and 72 h after culture was significantly higher than that in miR-214 control mimics group, while the proliferation of AsPC-1 cells in miR-214 inhibitors group 24 h, 48 h and 72 h after culture was significantly lower than that in miR-214 control inhibitors group (p<0.05; Figure 4).

Comparison of apoptosis rate of each group

The apoptosis rate of SW1990 cells in miR-214 mimics group was significantly lower than that in the miR-214 control mimics group, while the apoptosis rate of AsPC-1 cells in miR-214 inhibitors group was significantly higher than that in the miR-214 control inhibitors group (p<0.05; Figure 5).

Comparison of cell invasion and migration index in each group

The migration and invasion of SW1990 cells in miR-214 mimics group were significantly higher than those in miR-214 control mimics group, while the migration and invasion of AsPC-1 cells in miR-214 inhibitors group were significantly lower than those in miR-214 control inhibitors group (p<0.05; Figure 6).

Effect of miR-214 on the expressions of ING4 protein in AsPC-1 and SW1990 cells

The relative expressions of ING4 protein in SW1990 cells of miR-214 mimics group and miR-214 control mimics group were 0.27±0.04 and 1.01±0.01, respectively. The relative expression of

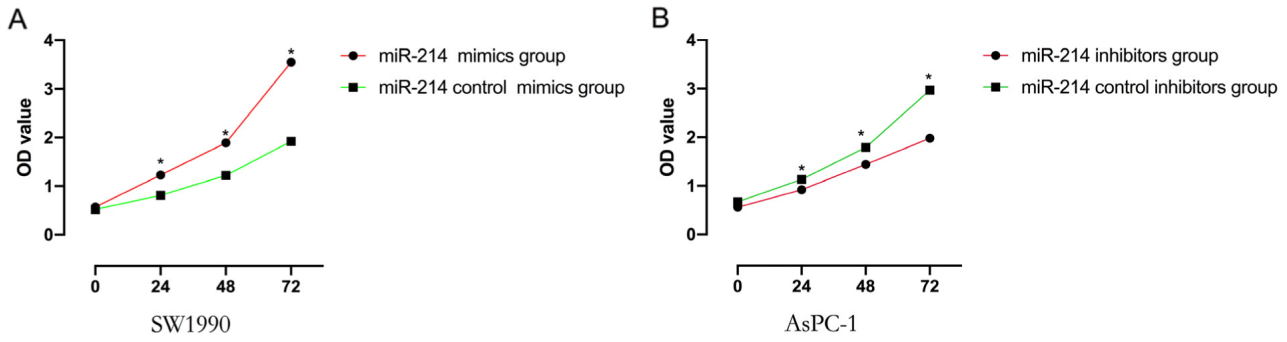

Figure 4. Effect of miR-214 on the proliferation of AsPC-1 and SW1990 cells. **A:** The proliferation of SW1990 cells in miR-214 mimics group 24 h, 48 h and 72 h after culture was significantly higher than that in miR-214 control mimics group. **B:** The proliferation of AsPC-1 cells in miR-214 inhibitors group 24 h, 48 h and 72 h after culture was significantly lower than that in miR-214 control inhibitors group (*p<0.05).

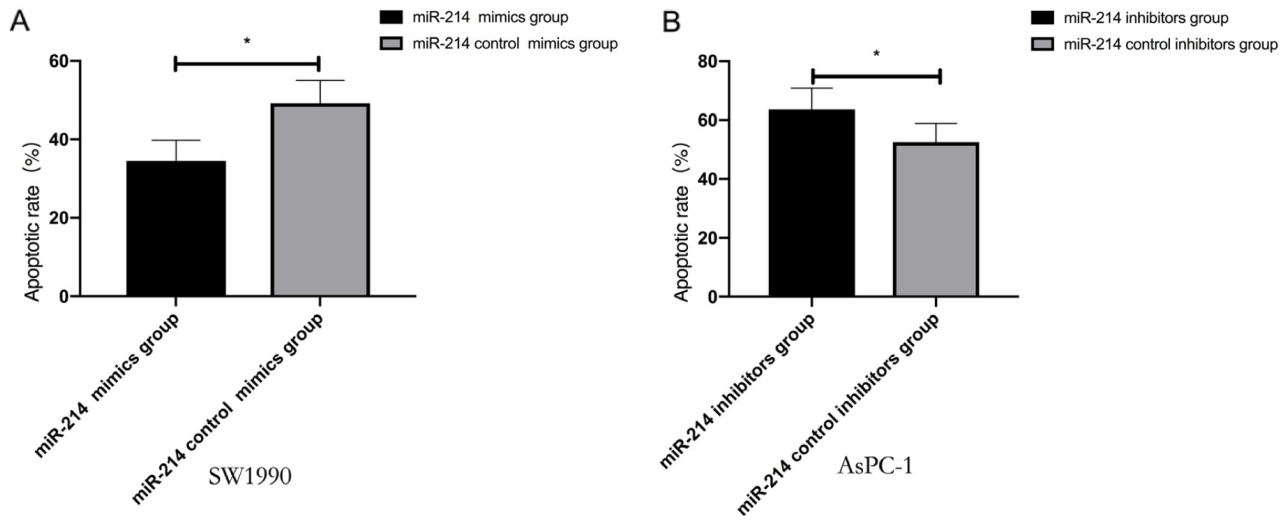

Figure 5. Comparison of apoptosis rate of each group. **A:** The apoptosis rate of SW1990 cells in miR-214 mimics group was significantly lower than that in the miR-214 control mimics group. **B:** The apoptosis rate of AsPC-1 cells in miR-214 inhibitors group was significantly higher than that in the miR-214 control inhibitors group (*p<0.05).

ING4 protein in SW1990 cells of miR-214 mimics group was significantly lower than that of miR-214 control mimics group ($p<0.05$). The relative expressions of ING4 protein in AsPC-1 cells in the miR-214 inhibitors group and the miR-214 control inhibitors group were 3.74±0.14 and 1.01±0.02, respectively. The relative expressions of ING4 protein in AsPC-1 cells of the miR-214 inhibitors group were significantly higher than that in the miR-214 control inhibitors group ($p<0.05$; Figure 7).

Discussion

The development of pancreatic cancer is silent and has highly malignant biological behavior. After chemotherapy, radiotherapy, surgical treatment and other traditional treatments, there is still no significant effect on the survival rate of patients with pancreatic cancer [13,14]. Tumor cells detached from the lesion enter the blood or lymphatic system and circulate in the body, and then gradually proliferate in the transferred organs to form a metastasis. Tumor proliferation, invasion and metastasis are still important causes of treatment failure and death, which is the same pathological mechanism of pancreatic cancer cells [15,16]. In recent years, many reports have pointed out that the abnormal expression of multiple miRs are involved in the occurrence and progression of different types of malignant tumors. The expression of tumor suppressor gene mR can be changed with the change of miRs expression level or activity, which can induce and regulate the occurrence and development of tumor [17-19]. It has been found

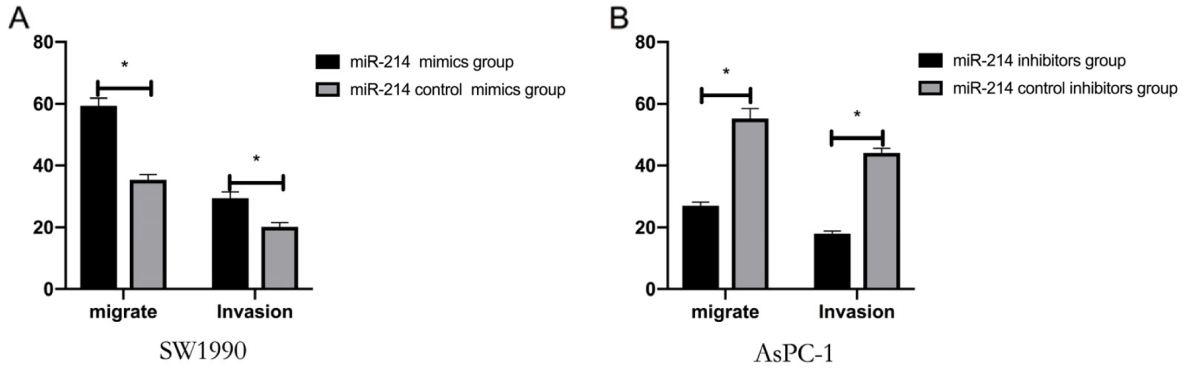

Figure 6. Comparison of cell invasion and migration index in each group. **A:** The migration and invasion of SW1990 cells in miR-214 mimics group were significantly higher than those in miR-214 control mimics group. **B:** The migration and invasion of AsPC-1 cells in miR-214 inhibitors group were significantly lower than those in miR-214 control inhibitors group (*$p<0.05$).

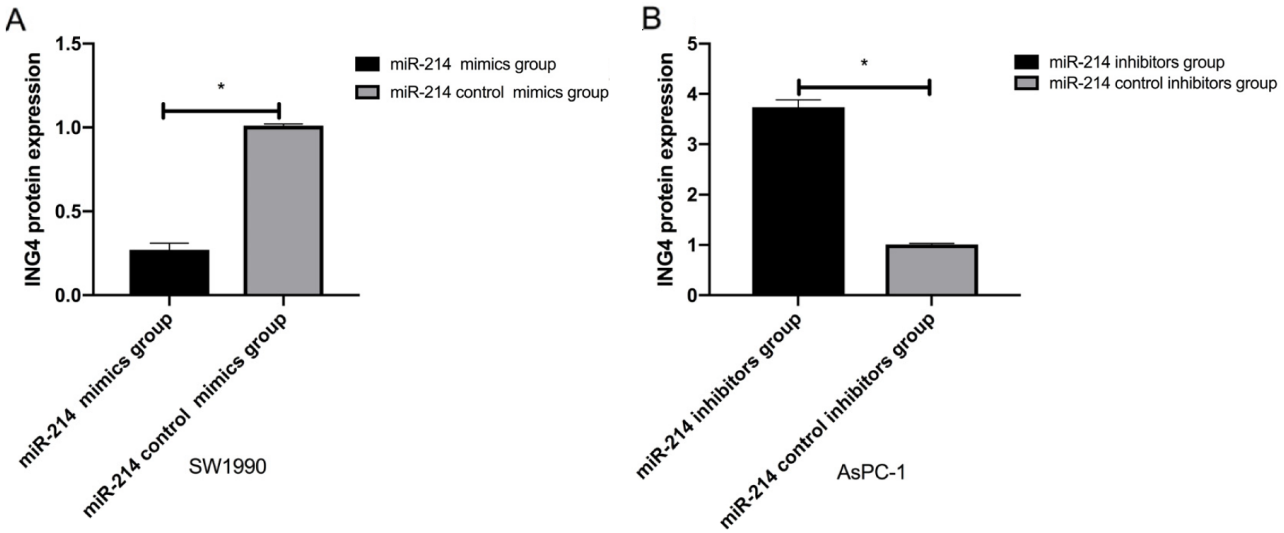

Figure 7. Effect of miR-214 on the expressions of ING4 protein in AsPC-1 and SW1990 cells. **A:** The relative expression of ING4 protein in SW1990 cells of miR-214 mimics group was significantly lower than that of miR-214 control mimics group. **B:** The relative expressions of ING4 protein in AsPC-1 cells of the miR-214 inhibitors group was significantly higher than that in the miR-214 control inhibitors group (*$p<0.05$).

that miR-214 is closely related to the occurrence and development of tumors and shows abnormal expression in malignancies. The relative expression of miR-214 in pancreatic cancer, cervical cancer and other tumor tissues is significantly higher than that in matched adjacent tissues [20,21]. The content of miR-214 decreased significantly in cervical cancer tissues, suggesting that miR-214 may be regulated by inhibiting downstream mtDNA transcription factor A. It was also found that miR-214 significantly inhibited the migration and invasion of cervical cancer cell lines, and the clinical condition of the patient was inversely related to the expression level of miR-214 [22]. As a new potential therapeutic target for gastric cancer, miR-214 can be used as novel treatment of gastric cancer through targeted therapy [23]. Through the above studies, it can be concluded that the expression of a specific miR in the development of malignant tumors may play different biological roles and effects in different tumors, which is a new idea and direction of screening and treatment of cancer that we need to further explore in the future. However, the effect of miR-214 on the biological function of pancreatic cancer cells was not described in detail. Therefore, the expression of miR-214 in pancreatic cancer cells and its biological function were discussed.

We first detected the content of miR-214 in pancreatic cancer tissues and adjacent tissues of patients with pancreatic cancer. The results showed that the expression of miR-214 in pancreatic cancer tissues was significantly higher than that in adjacent tissues, the difference being statistically significant. It has been also found that miR-214 is highly expressed in pancreatic cancer [24], and miR-214 can regulate the sensitivity of pancreatic cancer cells to chemotherapeutic drugs by inhibiting the expression of ING4 target gene. This indicates that the high expression of miR-214 may be related to the progression of pancreatic cancer. Further analysis of the relationship between its expression and the clinicopathological parameters and prognosis of pancreatic cancer patients revealed that the expression of miR-214 was significantly different when T staging was different, and the basic clinicopathological parameters related to pancreatic cancer, such as gender, age and CA19.9 expression, were not found to be related to miR-214. The 3-year overall survival rate of pancreatic cancer patients with high miR-214 expression was significantly lower than that of those with low miR-214 expression. A study has shown [25] that the up-regulated expression of miR-214 may accelerate the deterioration of pancreatic cancer patients, and the expression of miR-214 was negatively correlated with the survival of pancreatic cancer patients, which is consistent with the data of this study. However, it remains to be further explored whether the shortened survival time of patients is affected by the biological characteristics of tumors and the intervention of chemotherapy. To understand the biological function of miR-214 expression on pancreatic cancer cells, we compared the inhibited miR-214 in AsPC-1 cells after transfection and overexpressed miR-214 in SW1990 cells with the corresponding control group. The proliferation, apoptosis rate, invasion and migration index of pancreatic cancer cells in each group showed that the inhibition of miR-214 expression decreased the proliferation, invasion and migration index of pancreatic cancer cells and increased the apoptosis rate, while the changes in proliferation, apoptosis rate, invasion and migration index of pancreatic cancer cells with increasing miR-214 expression showed the opposite. The above conclusion indicates that the change of miR-214 expression has an effect on the proliferation, invasion and migration of pancreatic cancer cells, and the increased expression has an inhibitory effect on the above biological functions, and can lead to increased apoptosis rate of tumor cells. The direction of regulating the biological function of miR-214 expression in pancreatic cancer was investigated, but the biological functions of pancreatic cancer cells regulated by miR-214 expression and the mechanism between them have not been clarified. Therefore, further study was conducted on the effect of miR-214 expression on the expression of ING4 protein in AsPC-1 and SW1990 cells which found that inhibition of miR-214 expression increased ING4 protein expression in pancreatic cancer patients, whereas overexpression of miR-214 decreased ING4 protein expression. Some authors [26] found that the expression of miR-214 in human osteosarcoma increases more significantly than in adjacent tissues, and the Wnt pathway-related gene Wnt3A may be regulated by miR-214. It has also been pointed out that the expression of miR-214 in nasopharyngeal carcinoma (NPC) is higher than in paracancer tissues, the low expression of miR-214 in NPC cells can promote apoptosis and slow down the proliferation of NPC cells [27]. In malignant melanoma tissues, miR-214 can inhibit tumor proliferation and migration by regulating the expression of TFAP2C anti-oncogene [28]. However, the data on the role of miR-214 in the occurrence and development of pancreatic cancer and the effect of specific biological function are not sufficient. Combined with the results of this study, it was concluded that miR-214 expression may act on the biological

functions of pancreatic cancer cell proliferation, invasion and migration through ING4 protein.

In conclusion, miR-214 is overexpressed in pancreatic cancer tissues. Low expression of miR-214 can inhibit the proliferation and invasion of pancreatic cancer cells. MiR-214 participates in the biological behavior regulation of pancreatic cancer cells. This study provided some theoretical support for the interpretation of regulation behavior and mode of action of miR-214 in pancreatic cancer cells. However, we acknowledge that this study has also some limitations. For example, it is not clear how miR-214 is involved in the regulation of pancreatic cancer, and further experiments are needed for relevant detailed studies and discussion.

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Conflict of interests

The authors declare no conflict of interests.

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