miR-23a acts as an oncogene in pancreatic carcinoma by targeting FOXP2

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ABSTRACT

MicroRNAs have been reported to play an important role in tumor development and progression by targeting oncogenes and tumor suppressors. miR-23a has been described as significantly upregulated in multiple cancers and involved in tumorigenesis. The aim of this study was to evaluate the potential roles of miR-23a in pancreatic ductal adenocarcinoma (PDAC). We found that miR-23a level was significantly increased in tissues of PDAC compared with that in the control by real-time PCR. FOXP2 expression was downregulated and inversely correlated with miR-23a. miR-23a directly targeted the 3′-untranslated region of FOXP2 mRNA and repressed its expression. Mechanistically, enhancement of miR-23a by transfection with mimics in Aspc-1 cells significantly promoted cell proliferation and invasion, while miR-23a inhibitors transfection in SW1990 cells induced an inhibitory effect. Moreover, restoration of FOXP2 impaired the pro-proliferation and proinvasion effects of miR-23a, indicating FOXP2 is a direct mediator of miR-23a functions. In conclusion, our findings suggest a novel miR-23a/FOXP2 link contributing to PDAC development and invasion. It may be a potential diagnostic and therapeutic target for PDAC.

Significance of this study

What is already known about this subject?

- Pancreatic carcinoma is one of the most aggressive solid malignancies, but treatment options are very limited and mechanism is not fully understand.
- miR-23a was significantly upregulated in multiple types of cancer and required for tumorigenesis.
- FOXP2 was a member of Forkhead box protein family, but its potential role in altering cell fate and even promoting tumorigenesis has only been reported in few literatures.

What are the new findings?

- In this study, we found that miR-23a level was significantly increased in tissues and serum of pancreatic ductal adenocarcinoma (PDAC) compared with normal control.
- FOXP2 is a novel target of miR-23a.
- miR-23a is negatively correlated with FOXP2 expression.
- Moreover, miR-23a could promote PDAC cell growth and invasion by targeting FOXP2.

How might these results change the focus of research or clinical practice?

- These results suggest a novel link between miR-23a and FOXP2. It may be a potential diagnostic and therapeutic target for PDAC.

INTRODUCTION

Pancreatic carcinoma is always referred to pancreatic ductal adenocarcinoma (PDAC), and one of the most aggressive solid malignancies. Its recent 5-year overall survival is only 7.7% and the median survival time is about 6 months.[1] PDAC is a silent disease. When diagnosed, it is often in advanced stages. The treatment options are very limited and results are disappointing.[2 3] Therefore, searching efficient early prognosis biomarkers and therapeutic targets is critical and may benefit the survival rate of patients with PDAC.

Recently, growing evidence has supported the cancer-related effects of microRNAs (miRNAs), clusters of endogenous and small non-protein coding RNA molecules, which consist of 21–23 nucleotides.[4 5] The sequences of miRNAs are evolutionarily highly conserved and specific in the tumor context and circulation. It functions through negatively regulating the transcription of target genes by binding to their 3′-untranslated region (3′-UTR) and inhibiting translation or inducing mRNA degradation.[6] Many studies have identified that specific miRNAs are abnormally expressed in multiple cancers. They play a role similar to the tumor suppressors or oncogenes depending on cellular contexts and the target genes they regulate, and influence many biological behaviors, including cell differentiation, proliferation, apoptosis, invasion and metastasis.[7–9] Among these miRNAs, miR-23a has been confirmed to be notably upregulated in various cancer types and involved in tumorigenesis. For instance, miR-23a was significantly upregulated in tissues of non-small cell lung cancer compared with matched adjacent lung tissues. The high expression was associated with tumor size; lymph node metastasis; tumor, node, metastases stage; and tumor differentiation.[10] In laryngeal carcinoma, miR-23a and

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APAF-1 were reported to be synchronously upregulated and downregulated. Upregulated miR-23a and knockdown of APAF-1 significantly promoted cell proliferation and colony formation, and suppressed early apoptosis in laryngeal cancer cells.[11] Moreover, miR-23a is required for pathological angiogenesis in a laser-induced choroidal neovascularization mouse model by promoting angiogenic signaling through targeting Sprouty2 and Sema6A proteins.[12] In 2015, Frampton *et al*[13] implied the involvement of miR-23a in PDAC growth and invasion using an integrated data analysis. But the underlying mechanism has not been comprehensively characterized.

Forkhead box (FOX) proteins are a superfamily of evolutionarily conserved transcriptional regulators that control a wide spectrum of biological processes, including metabolism, development, differentiation, proliferation, apoptosis, migration and invasion.[14] FOXP2 was primarily identified as a transcriptional repressor regulating speech and language development.[15] However, its potential role in altering cell fate and even promoting tumorigenesis has only been reported in few literatures to date. In this study, we investigated the regulation of miR-23a to FOXP2 in PDAC cells. We provided evidence that miR-23a could promote PDAC cell growth and invasion by targeting FOXP2. Furthermore, alterations of the levels of miR-23a and FOXP2 in tissues were determined, and data showed that the expression level of miR-23a is negatively correlated with FOXP2 in patients with PDAC.

MATERIALS AND METHODS

Tissue samples

Surgical specimens of PDAC and adjacent normal pancreatic tissue were obtained from 30 patients with PDAC who underwent surgical resection from January 2010 to December 2016 before receiving any chemotherapy. The 30 patients comprised 17 men and 13 women with a mean age of 58.8 years (range 42–75 years). The fresh samples were frozen in liquid nitrogen, then stored at −80°C for further extraction. All the cancer specimens were histologically classified as adenocarcinomas.

Cell culture

Six pancreatic carcinoma cell lines including Aspc-1, MIA Paca-2, Bxpc-3, Capan-2, SW1990 and Panc-1 (purchased from ATCC, Manassas, Virginia, USA) and normal pancreatic duct epithelial cell line (HPDE-6) were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, California, USA) containing 10% calf serum (Gibco, Carlsbad, California, USA). Cells were cultured in a 37°C humidified atmosphere of 5% CO_2. The Bxpc-3, Panc-1 and MIA-Paca-2 were p53 mutant cell lines as documented by the ATCC website (https://www.atcc.org/Products/All/TCP-2060.aspx); thus, they might have higher tumorous capacity than p53 wild-type cell lines, including Aspc-1 and Capan-2. The SW1990 cells were established from a spleen metastasis of a grade II pancreatic adenocarcinoma derived from the exocrine pancreas.[16] Thus it might be the most highly tumorigenic cells compared with other PDAC cells. Upregulated and downregulated expressions of miR-23a in cells were obtained by transfection with miR-23a mimics or inhibitors, respectively (Genepharma, Shanghai, China). Knockdown of FOXP2 was performed using FOXP2 siRNA (Genepharma). Human cDNA encoding FOXP2 was cloned into the pcDNA 3.0 vector and transfected into cells to induce pcDNA3.0(+)-FOXP2 plasmid. Cells were transfected with the recombinant plasmid using Lipofectamine 2000 reagent (Invitrogen). After 24 hours or 48 hours, transfected cells were collected and used in further assays or RNA/protein extraction.

Quantitative real-time PCR analysis

One milliliter of Trizol (TaKaRa, Tokyo, Japan) was directly added to 10^6 pancreatic carcinoma cells or 1 mg of pancreatic carcinoma and para-carcinoma tissues. Total RNA was then isolated according to the manufacturer's instructions. RNA quality was confirmed by calculating the OD260/280 ratio using absorbances measured by a spectrophotometer. For miR-23a detection, total RNA was reverse-transcribed using the TaqMan miRNA Reverse Transcription Kit and miR-23a-specific primer, and PCR was performed with TaqMan Universal Master Mix II. For FOXP2 mRNA expression detection, cDNA was synthesized from total RNA using the reverse transcription kit, and PCR was performed using SYBR Green PCR Master Mix. U6 snRNA or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was used as an endogenous control, respectively. All the detections were performed in triplicate. Relative expression was calculated using the $2^{-\Delta\Delta}$CT method and normalized to control.

Western blot

Cells were collected and lysed in radio-immunoprecipitation assay (RIPA) buffer with protease inhibitors. Lysates were centrifuged at 20 000 g for 30 min at 4°C. Concentration of protein was determined by the Bradford method. Protein was subjected to a 10% sodium dodecyl sulfate (SDS)-acrylamide gel and transferred onto polyvinylidene fluoride (PVDF) membrane, followed by 1 hour of 5% skim milk blocking at room temperature. The blots were probed with 1:500 diluted anti-FOXP2 (Abcam, Cambridge, Massachusetts, USA) and 1:1000 diluted anti-GAPDH (Santa Cruz Biotechnology, Dallas, California, USA) antibody at 4°C overnight, and subsequently incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000). Signals were visualized using enhanced chemiluminescent method of western blotting (ECL) substrates followed by the quantification of band intensity using the ImageJ software.

Dual-luciferase reporter gene experiment

The wild-type 3'-UTR of FOXP2 gene, which contained the putative miR23a binding site, was amplified by PCR, and then cloned into a pGL3-basic vector downstream to the luciferase gene sequence. A corresponding pGL3 construct containing 3'-UTR of FOXP2 with a mutation of miR-23a was also generated. HEK293T cells were plated in 96-well plates, and cotransfected with 0.1 µg generated plasmid with or without miR-23a mimics. Cells were collected 48 hours post-transfection. Luciferase activity was detected using a dual-luciferase reporter assay according to

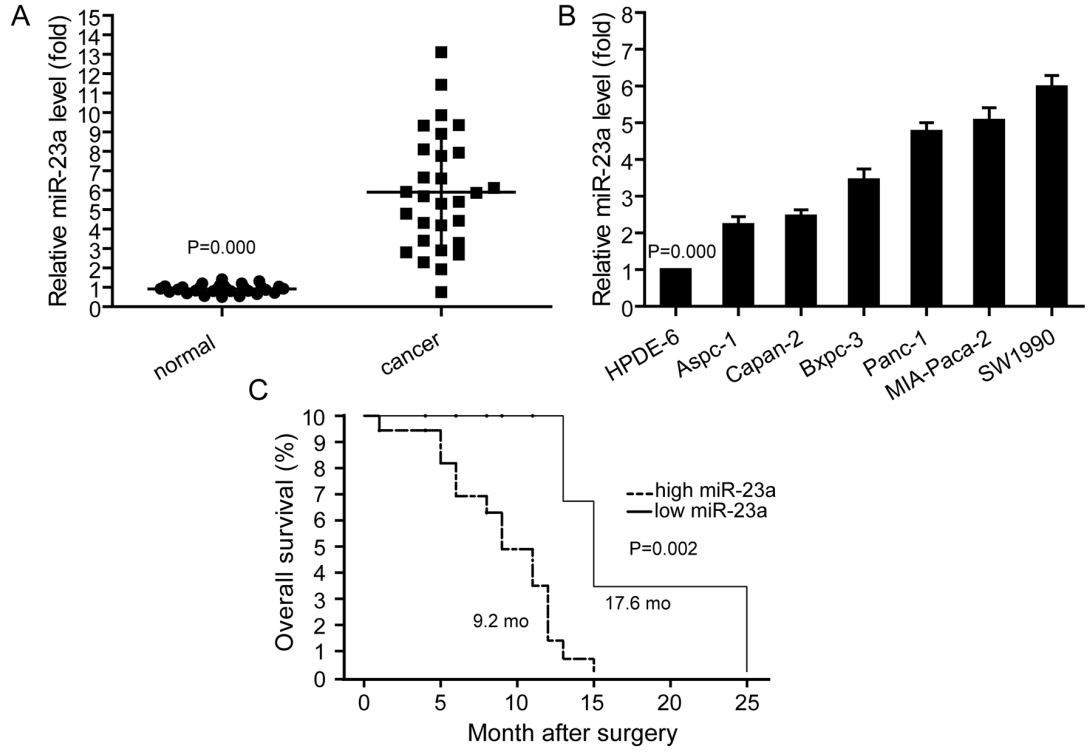

Figure 1 Increased expression of miR-23a in both pancreatic ductal adenocarcinoma (PDAC) tissues and cell lines. (A) Mean expression of miR-23a in the human PDAC tissues and matched adjacent normal pancreatic tissues. (B) Mean expression of miR-23a in six PDAC cell lines and normal pancreatic duct epithelial cell line (HPDE-6). (C) High expression of miR-23a was associated with poor survival. Patients were dichotomized into high-miR-23a or low-miR-23a expression groups using the median value (Kaplan-Meier analysis, P=0.002).

the manufacturer's instructions and normalized to Renilla activity.

MTT detection

Cell proliferation assay were examined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay performed as described previously.[17] Briefly, after incubation for the indicated different time, the unattached cells were removed by gentle washing with phosphate buffered saline (PBS) buffer. Then, 80 µL RPMI-1640 medium and 20 µL MTT (5 mg/mL) (Sigma-Aldrich, St. Louis, Missouri, USA) were added to each well. After incubation at 37°C for 4 hours, the medium was discarded. Then, 200 µL of 0.04 mol/L hydrochloric acid in isopropanol was added to each well. The amount of MTT formazan product was determined by measuring absorbance with a microplate reader at a test wavelength of 570 nm and a reference wavelength of 630 nm.

Transwell assay

The Transwell assay was conducted according to the manufacturer's instructions. Briefly, the cells were digested with 0.05% pancreatin/EDTA. A total of 2×10^5 cells resuspended in 200 µL serum-free RPMI-1640 medium were added into each upper insert precoated with Matrigel matrix. Then, 500 µL medium containing 20% fetal bovine serum (FBS) was added into a matched lower chamber. After a 48-hour incubation, a swab was used to wipe out the remaining Matrigel and cells in the upper chamber. The invaded cells on the lower membrane were observed by fixing in methanol and staining with 0.1% crystal violet.

Statistical analysis

All data were shown as mean±SD. All the statistical analyses were processed using the SPSS V.18.0 software. $P<0.05$ was considered statistically significant. All the experiments were performed at least three times. Student's t-test or one-way analysis of variance was used to compare the differences among the groups. The Mann-Whitney U test and Spearman's correlation analyses were used to estimate the relationship between miR-23a and FOXP2 expression. Overall survival curve was estimated using a Kaplan-Meier analysis and compared using the stratified log-rank test.

RESULTS

Expression of miR-23a in PDAC tissues and cell lines

We performed quantitative PCR analysis to detect the endogenous expression of miR-23a in PDAC tissues and cell lines. miR-23a was significantly enhanced in PDAC tissues compared with their matched adjacent normal tissues (figure 1A, P=0.000). We then extended the test to six human PDAC cell lines—Aspc-1, Capan-2, Bxpc-3, Panc-1, MIA Paca-2 and SW1990— and we observed a notable increase of miR-23a expression in PDAC cells compared with the normal pancreatic duct epithelial cell line HPDE-6 (figure 1B, P=0.000). Because Aspc-1 and SW1990 cells expressed relatively lower and higher levels of miR-23a, respectively, they were selected for further functional

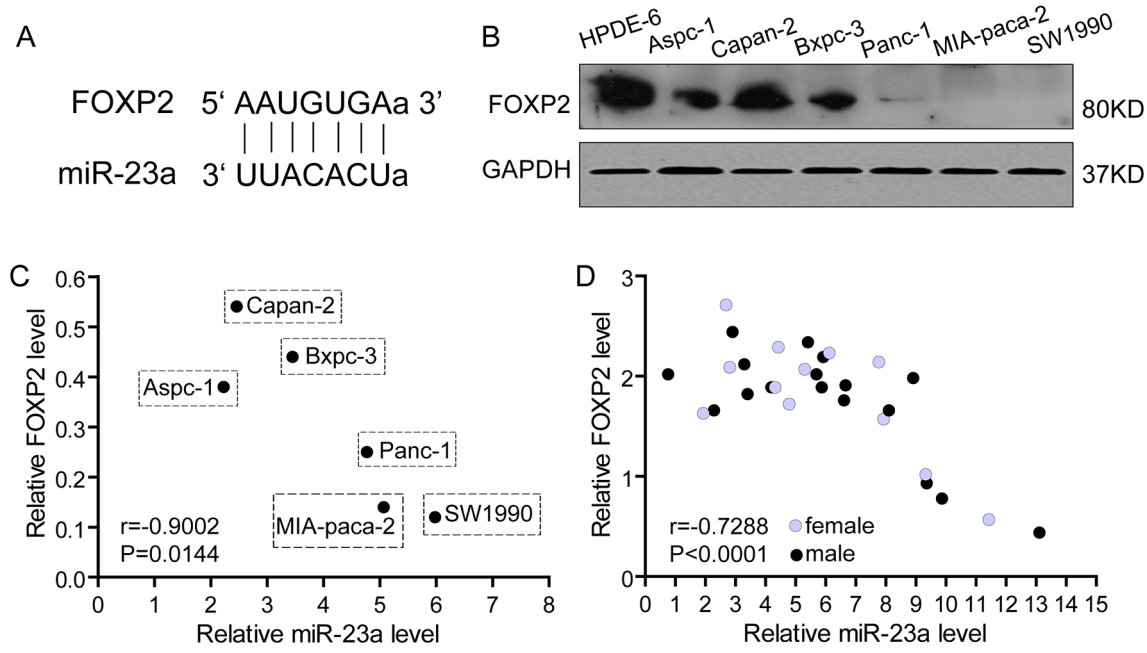

Figure 2 Negative correlation between miR-23a and FOXP2. (A) The predicted miR-23a binding site within FOXP2 3′-untranslated region is shown. (B) Variable FOXP2 level in six pancreatic ductal adenocarcinoma (PDAC) cells and pancreatic duct epithelial cell line (HPDE-6). GAPDH was used as the loading control. (C) An inverse correlation between miR-23a and FOXP2 was observed in six PDAC cell (Spearman's correlation, r=−0.9002, P=0.0144). (D) An inverse correlation between miR-23a and FOXP2 was observed in PDAC tissues (gray dots, female; black dots, male; Spearman's correlation, r=−0.7288, P<0.0001). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

assays. Moreover, Kaplan-Meier univariate analyses of overall survival indicated that high miR-23a level was associated with poor survival (figure 1C, P=0.002, median 17.6 vs 9.2 months).

miR-23a is negatively correlated with FOXP2

By searching online miRNA target prediction databases including TargetScan, PicTar and miRanda, we found that FOXP2 was a potential target gene of miR-23a (figure 2A). We first examined the FOXP2 expression profile in the panel of six PDAC cell lines. Western blot analysis showed that all of the cell lines exhibited lower expression of FOXP2 (figure 2B). Then we investigated the relevance between miR-23a and FOXP2 expression. Expression of miR-23a was inversely correlated with the level of FOXP2 mRNA in all the PDAC cell lines (figure 2C). Moreover, we also observed an inverse correlation between miR-23a and FOXP2 expression in PDAC tissues and their adjacent normal tissues (figure 2D).

FOXP2 is a direct target of miR-23a

We transfected Aspc-1 cells with miR-23a mimics and SW1990 cells with miR-23a inhibitors, respectively, at a concentration of 50 nM. Real-time PCR results showed that expression of miR-23a was significantly increased in the miR-23a mimics group when compared with the control group (figure 3A left, P<0.01). Relatively, miR-23a was significantly downregulated in the miR-23a inhibitors group when compared with the corresponding control group (figure 3A right, P<0.01). Meanwhile, at 48 hours post-transfection, the enhanced miR-23a in Aspc-1 cells significantly repressed FOXP2 mRNA expression compared with cells transfected with scramble control (figure 3B left, P<0.01), and downregulated miR-23a by inhibitors in SW1990 cells led to a remarkable increase of FOXP2 mRNA level (figure 3B right, P<0.01). Consistently, western blot assay also represented apparent changes of FOXP2 protein expression (figure 3C).

To further explore if the predicted binding site of miR-23a to 3'-UTR of FOXP2 is responsible for the above regulation, we cloned the 3'-UTR of FOXP2 into a luciferase reporter gene (wt-FOXP2). A mutant version (mut-FOXP2) by the binding site mutagenesis was also constructed. Results showed that the luciferase signal of FOXP2-3'-UTR in the miR-23a mimics group decreased by about 60% (figure 3D, P<0.01). For mutant FOXP2-3'-UTR, the luciferase signals of different groups did not decrease significantly. Taken together, these results demonstrated that miR-23a could directly target FOXP2. miR-23a promotes PDAC cell growth and invasion.

We performed gain of function and loss of function assays on miR-23a upregulated Aspc-1 cells and miR-23a downregulated SW1990 cells to validate whether miR-23a regulates cellular growth and invasion. Through MTT assay, we found that enhanced miR-23a significantly promoted growth of Aspc-1 cells compared with control cells (figure 4A, P<0.01). Correspondingly, miR-23a inhibitors transfected SW1990 cells presented suppressed cell growth (figure 4B, P<0.01). Similarly, we also observed an increase in invasive capacity of PDAC cells to migrate through Matrigel in miR-23a mimics transfected Aspc-1cells (figure 4C, P<0.01), and transfection of inhibitors in SW1990 cells apparently inhibited cell invasion ability (figure 4D, P<0.01).

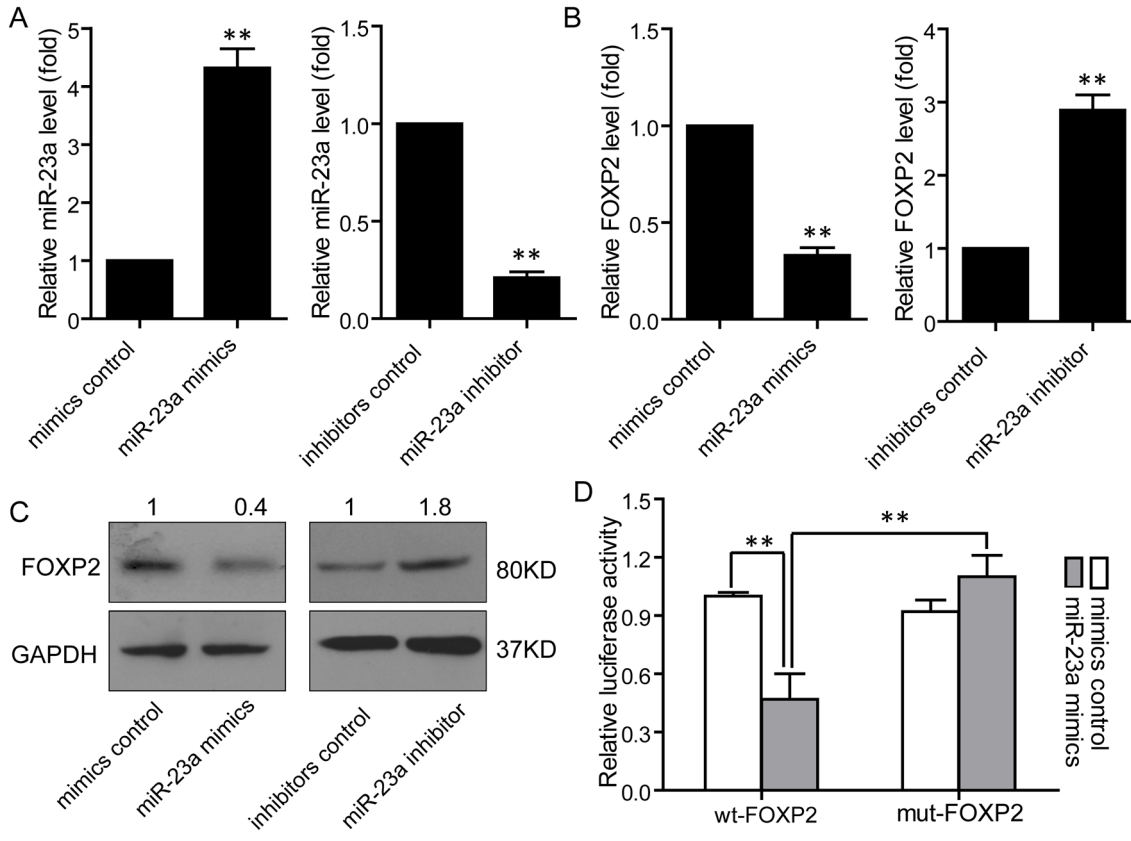

Figure 3 miR-23a directly targets FOXP2. (A) Expression of miR-23a was significantly enhanced by transfecting miR-23a mimics into Aspc-1 cells (left). Expression of miR-23a was significantly silenced by transfecting miR-23a inhibitors into SW1990 cells (right). (B) Upregulated expression of miR-23a inhibited FOXP2 mRNA level (left), while reduction of miR-23a restored FOXP2 mRNA level (right). (C) Upregulated expression of miR-23a inhibited FOXP2 protein level (left), while reduction of miR-23a restored FOXP2 protein level (right). (D) The repression of luciferase activity by FOXP2 3′-untranslated region (3′-UTR) was dependent on miR-23a. Mutated FOXP2 3′-UTR abrogated miR-23a-mediated repression of luciferase activity (**P<0.01). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

FOXP2 participates in miR-23a-regulated cell proliferation and invasion

To further confirm the functional relationship between miR-23a and FOXP2, we (1) upregulated FOXP2 expression in miR-23-induced Aspc-1 cells by cotransfection of FOXP2 plasmid and miR-23a mimics; and (2) downregulated FOXP2 expression in miR-23a-silenced SW1990 cells by cotransfection of FOXP2 siRNA and miR-23a inhibitors, and then tested changes of cell proliferation and invasion. Once FOXP2 expression was effectively induced by plasmid transfection (figure 5A,B), transfected Aspc-1 cells exhibited decreased cell growth (figure 5C left) and impaired cell invasion (figure 5D left). On cotransfection of miR-23a mimics and FOXP2 cDNA, the proliferation rate (figure 5C left) and invasion ability (figure 5D left) were restored significantly. In contrast, FOXP2 siRNA transfection in SW1990 cells led to a notable decrease in FOXP2 expression both at the mRNA and protein levels (figure 5A,B). Cotransfection with miR-23a inhibitors and FOXP2 siRNA significantly decreased the proliferation and invasion rates compared with FOXP2 siRNA transfection alone (figure 5C,D right). These data indicate that miR-23a inhibits the proliferation and invasion of PDAC cells by targeting FOXP2.

DISCUSSION

miRNA is widely involved in the physiological and pathological regulations of cellular behavior. The dysregulation of specific miRNA can be involved in the initiation and development of cancer and being considered as a novel type of biomarkers and potential therapeutic targets.[18 19] Of these miRNAs, miR-23a was defined as a specific driver in PDAC through detection with tumor tissue and saliva.[20 21] Further studies showed that miR-23a was involved in PDAC growth and invasion.[13] Mechanically, researchers found that miR-21, miR-23a and miR-27a clusters acted as cooperative repressors of tumor suppressor genes including PDCD4, BTG2 and NEDD4L. To induce proliferation of PDAC,[22] miR-23a and miR-24 clusters targeted for FZD5, HNF1B and TMEM92 to induce epithelial-mesenchymal transition (EMT)-like cell shape transformation and integration into mesothelial monolayers of PDAC.[23] Based on these findings, we speculated that miR-23a alone, not contained in a cluster, might be also critical in PDAC progression processes. Finally, through a series of experiments, we showed that miR-23a was aberrantly increased in PDAC cell lines and patient tissues. Restoration of miR-23a promoted cell growth and invasion capability in the gain of function assays and vice versa. Moreover, using a luciferase reporter,

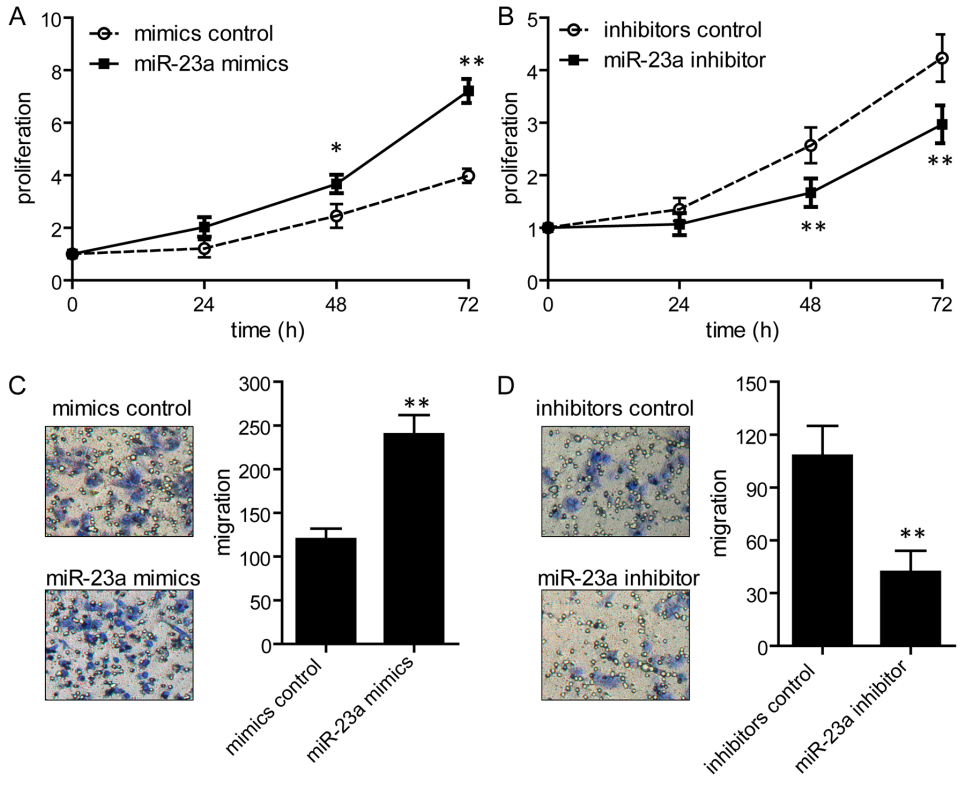

Figure 4 Effects of miR-23a on proliferation and invasion in Aspc-1 and SW1990 cells. (A) Upregulation of miR-23a inhibited proliferation of Aspc-1 cells. (B) Downregulation of miR-23a promoted proliferation of SW1990 cells. (C) Upregulation of miR-23a inhibited invasion of Aspc-1 cells. (D) Downregulation of miR-23a promoted invasion of SW1990 cells. Representative images and statistical analysis are shown. **P<0.01, *P<0.05.

we verified that miR-23a directly binds to a specific complementary site within the 3'-UTR of FOXP2 mRNA. Restored miR-23a expression in Aspc-1 cells suppressed FOXP2 expression post-transcriptionally. Therefore dysregulation of miR-23a, at least partly, contributed to the pathological process of PDAC through targeting FOXP2 pathway.

miRNAs show different patterns of expression in patients with cancer. Our results showed that miR-23a was commonly upregulated in 6 PDAC cell lines and 30 PDAC patient tissues, which is consistent with previous studies. For example, expression of miR-23a was higher in breast cancer cells with high metastasis ability and patients with lymph node metastasis.[24] miR-23a was also significantly elevated in microsatellite instability colorectal cells and tissues compared with microsatellite stability cancer cells and tissues, thus is key player in multidrug resistance.[25] On the contrary, He *et al*[26] reported miR-23a was lowly expressed in osteosarcoma cells and tissues compared with normal controls. miR-23a also showed a significant downregulation in prostate cancer cell line and tissue samples.[27] This implies that the regulatory role of miR-23a may be specific to tumor context. miRNA plays a different role in different tumor environments and different stages of tumor development. Thus more study should be carried out on the type of each tumor.

Previous studies have validated that miR-23a target several tumor suppressors including APAF1,[28] STAT3,[29] IRS-1[30] and E-cadherin[31] in different types of cancers. In this study, we sought to reveal the mechanistic link of miR-23a to its novel targets and its contribution to PDAC development. We found that FOXP2, a member of the FOX transcription factor family, is highly predicted as a miR-23a target using several bioinformatic algorithms. In previous studies, FOXP2 was described to regulate speech and language development,[15] neuronal differentiation and neuronal subtype specification,[32] and cell proliferation.[33] FOXP2 in normal pancreatic endocrine cells could facilitate the proliferative capacity postnatally through regulation of cell cycle regulatory genes.[33] In cancer cells, a recent study showed that a low level of FOXP2 is associated with vein invasion, number of tumor nodes, α-fetoprotein (AFP) and poor survival of patients with hepatocellular carcinoma,[34] indicating its clinical value as a prognostic marker. Furthermore, we demonstrated that FOXP2 is downregulated in primary PDAC tissues and PDAC cell lines. Restoration of FOXP2 significantly suppressed cell growth and invasion, indicating the tumor-suppressive role of FOXP2. Since FOXP2 induced p21WAF1/CIP1 activation and regulated a p21-dependent growth arrest checkpoint in osteosarcoma cell line,[35] we hypothesized that loss of FOXP2 may result in reduced p21 protein induction and consequential total Rb downregulation. Further study is needed. And given that miR-23a binds to the 3'-UTR of FOXP2 mRNA and leads to a decreased FOXP2 expression, we thought that aberrantly elevated miR-23a is responsible, at least partly, for the FOXP2 silencing and might increase P21 activity in PDAC.

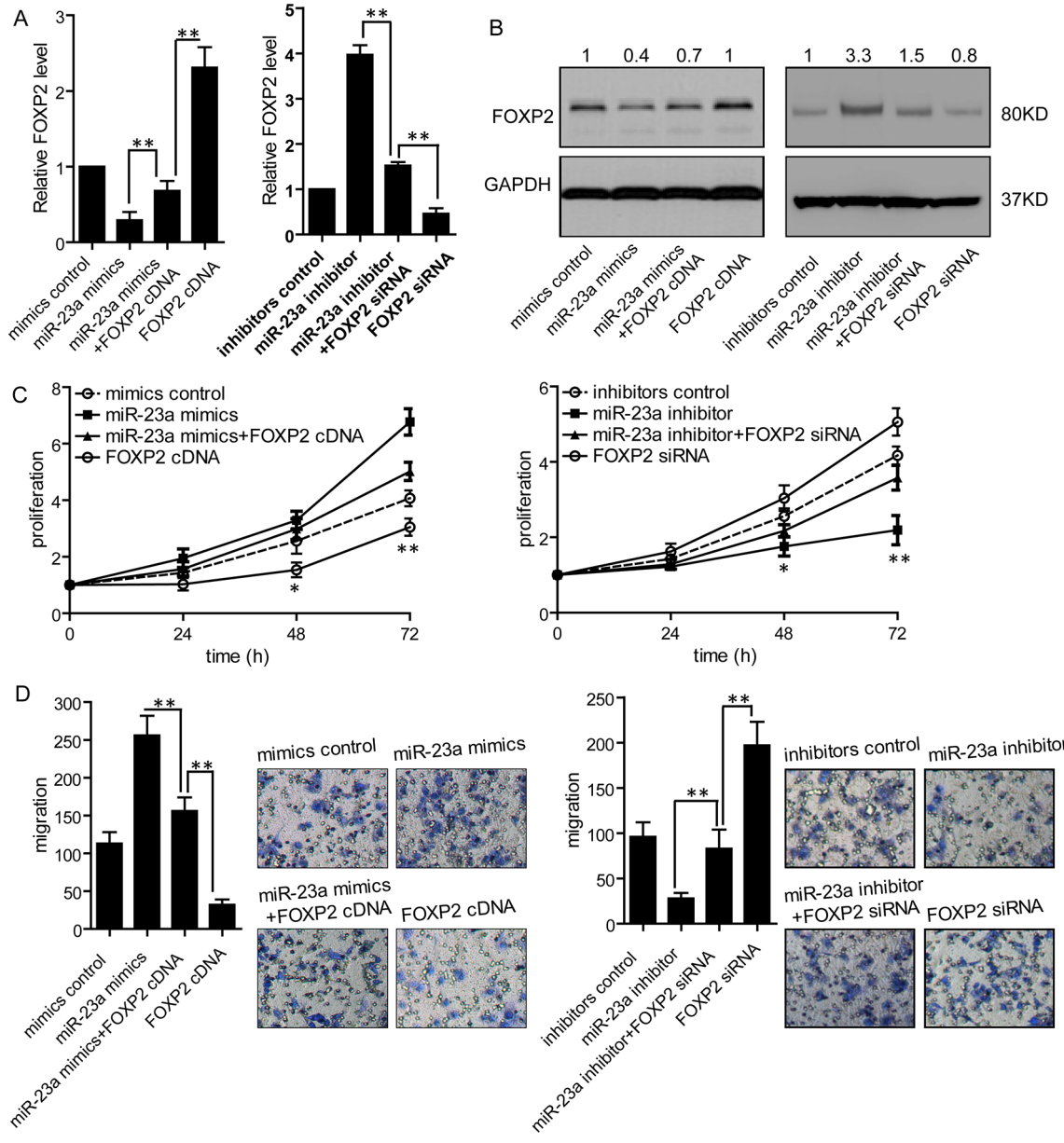

Figure 5 miR-23a induces the proliferation and invasion by suppressing FOXP2 expression. (Left) Aspc-1 cells were transfected with miR-22a mimics and FOXP2 cDNA or cotransfected with miR-23a mimics and FOXP2 cDNA, respectively. (Right) SW1990 cells were transfected with miR-23a inhibitors and FOXP2 siRNA or cotransfected with miR-23a inhibitors and FOXP2 siRNA, respectively. (A) PCR assay for FOXP2 mRNA level determination. (B) Western blot assay for FOXP2 protein level determination. (C) MTT assay for cell proliferation. (D) Transwell assay for cell invasion. Representative images and statistical analysis are shown. **P<0.01, *P<0.05.

Through seed-match analysis, Frampton *et al*[22] found that miR-23a and miR-27a had the same seed sequence and belonged to the same miRNA family. Moreover, combination of miR-21, miR-23a and miR-27a was involved in regulating crucial tumor suppressor network implicated in PDAC progression. They further validated that miR-21 regulated PDCD4; miR-21 and miR-27a regulated BTG2; and miR-23a regulated NEDD4L. By searching online miRNA target prediction databases (miRNA.org and TargetScan), we found that FOXP2 mRNA overlapped between the potential miR-23a and miR-27a targets. Interaction between miR-23a and miR-27a might correlate with FOXP2 synergistically. Our results demonstrated that miR-23a alone could promote PDAC growth much effectively; thus, for this reason, we did not consider this further. miRNAs can serve as potential efficient biomarkers for cancer diagnosis and prognosis. They could be applied to body specimens from biological fluids to tissues, and help to function in clinical context, for example, defining tumor types, susceptibility, prognosis and response.[36 37] Moreover, there are many current clinical trials for miRNA-based therapeutics.[38] Targeting miRNAs may be used directly to target tumor cells, and also to enhance other therapies; for example, they may have a potential use in reducing the drug resistance of tumors. This has been shown by

the chemoresistant properties of miR-100 in small cell lung cancer.[39 40] We showed that miR-23a was commonly highly expressed in patients with PDAC, silencing miR-23a resulted in repressed cell growth and invasion, and high miR-23a level was associated with poor survival of patients, suggesting miR-23a may be a new potential diagnostic factor and target of PDAC. Further investigation will be required to elucidate this question.

In conclusion, we found that the expression of miR-23a was upregulated in tissues and cell lines of PDAC. We further identified that miR-23a directly targets FOXP2 to induce proliferation and metastasis of PDAC cells. This study highlights a new strategy for gene therapy and diagnosis of PDAC through a new link between miR-23a and FOXP2.

Contributors HD and ZY performed the experiments. ZY analysed the data. HD and RQ designed the study and wrote the manuscript.

Competing interests None declared.

Ethics approval The study was performed with the approval of the Medical Ethics Committee of Central Hospital based in the Xinjiang Uygur Autonomous Region and conducted according to the principles expressed in the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement All of the data were published and available when required.

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