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RESEARCH

MicroRNA-424-5p Suppresses the Expression of SOCS6 in Pancreatic Cancer

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Abstract MicroRNAs (miRNAs) are a group of small non-coding RNA molecules predicted to control the activity of about 30 % of all protein-coding genes in mammals. The expression of microRNA-424-5p (miR-424-5p) has been shown to vary in multiple hematological and solid organ malignancies, such as pancreatic cancer. This study aimed to characterize the function of upregulated miR-424-5p in pancreatic cancer and show how downstream suppressor of cytokine-induced signaling 6 (SOCS6) is negatively regulated by miR-424-5p. MiR-424-5p and SOCS6 expression was detected using quantitative real-time PCR (qRT-PCR) in pancreatic cancer tissues and adjacent non-tumorous ductal epithelium tissues. Luciferase reporter assays were used to assess SOCS6 as a target of miR-424-5p. The downstream effect of SOCS6 was measured by qRT-PCR after miR-424-5p inhibition and SOCS6 upregulation. The functions of miR-424-5p in vitro in pancreatic cancer cells were measured by migration and invasion assays and flow cytometry. Results suggested miR-424-5p was significantly upregulated in pancreatic cancer and suppress the expression of SOCS6, and miR-424-5p increased proliferation, migration and invasion of pancreatic cancer cells, while inhibited cell apoptosis. It was concluded that miR-424-5p is frequently upregulated in pancreatic cancer and modulates ERK1/2 signaling pathway by negatively regulating SOCS6.

Keywords Pancreatic cancer · miR-424-5p · SOCS6 · ERK1/2 signaling pathway

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Introduction

Pancreatic cancer is an aggressive malignancy with high mortality rates. It is the sixth leading cause of death from malignant disease in China and the fourth leading cause of cancer-related death in the United States [1–3]. Despite recent advances in the treatment of cancer patients, pancreatic cancer is often still fatal, with 5-year overall survival rates of <5 % [4]. Because of its aggressive tumor biology, current treatment regimens do not greatly prolong patient survival, and surgical resection offers the only chance of cure. However, after a complete resection, the 5-year survival rate still remains low and is reported to be no more than 25 % [5, 6]. These factors have led to the need to discover unique molecular targets and biologic therapies for pancreatic cancer.

MicroRNAs (miRNAs) are small (18–22 nucleotides) non-coding RNAs that have critical functions in various biological processes, such as proliferation, development, apoptosis, and differentiation [7]. More importantly, it is suggested that the development and progression of cancer are associated with aberrant upregulation or downregulation of specific miRNAs and their targets in various types of cancer; indeed, certain cancer histotypes can be classified based on miRNA expression profiles [8–10]. It has been shown that miRNAs are negative regulators of gene expression

through imperfect base-pair interactions to sequences within the 3′ untranslated region of protein-coding mRNAs [7]. However, there are more than 15,000 miRNA gene loci in over 140 species [11], and most of their functions have not yet been defined and validated in clinical therapy.

Multiple miRNAs and their molecular target genes have been implicated in the development of pancreatic cancer. MiR-424-5p has been previously reported to be deregulated in colorectal cancer [12], non-small cell lung cancer [13], esophageal squamous cell carcinoma [14], diffuse large B-cell [15], and cervical cancer [16]. High expression levels of miR-424-5p have also been found in pancreatic cancer; however, the biological characteristics, molecular mechanisms, and targets of miR-424-5p are not well-understood [17]. In the present study, we found that miR-424-5p was upregulated in pancreatic cancer and promoted tumor proliferation, migration, invasion, and apoptosis inhibition. We also demonstrated that suppressor of cytokine-induced signaling 6 (SOCS6) may be negatively modulated by miR-424-5p, and that SOCS6 may play a role in several signaling pathways. In previous studies, SOCS6 was shown to play an important role in stomach, colon, and breast cancers [18–21]. Consistent with these studies, we suggest that miR-424-5p modulates the ERK pathway by targeting SOCS6, which in turn influences the behavior of pancreatic cancer [22]. Our study of the mechanism of action of miR-424-5p may provide insights that will lead to better diagnosis, prognosis, and therapeutic opportunities for pancreatic cancer patients.

Materials and Methods

Tissue Samples and Cell Lines

Pancreatic ductal adenocarcinoma (PDAC) tissues and respective adjacent normal ductal epithelium tissues from 24 patients were obtained postoperatively at Xiangya Hospital, Central South University, Changsha, China. Six normal pancreatic ductal epithelium tissue samples were acquired at the Xiangya Hospital through an organ donor procurement program whenever there was no suitable recipient for pancreas transplantation. Patients with other pancreatic malignancies, such as intraductal papillary mucinous adenocarcinoma, acinar cell carcinoma, and endocrine tumor, were excluded. Tissues were obtained before chemotherapy and radiation therapy, and were immediately snap-frozen in liquid nitrogen and stored at −80 °C for RNA extraction. All patients gave signed, informed consent for their tissues to be used for scientific research. The study was approved by the Ethics Committee of Central South University, China. All diagnoses were based on pathological and/or cytological evidence. The human pancreatic cancer cell lines PANC-1, AsPC-1, BxPC-3 and MIAPaCa-2 were obtained from the Central Experiment Laboratory of Xiangya Medical School in Central South University, and were maintained in DMEM supplemented with 10 % FBS and antibiotics (100 units/ml penicillin G Sodium Salt, 100 units/ml streptomycin sulfate; Gibco, Grand Island, NY, USA). Cells were grown in a 37 °C incubator with 5 % CO_2.

Cell Transfections

The miR-424-5p inhibitor, an inhibitor negative control precursor (NC), and the SOCS6 vector pGFP-SOCS6 plasmid were obtained from GenePharma (GenePharma, China). These transfections were performed using Lipofectamine 2000 (Invitrogen, USA) in accordance with the manufacturer's procedure. Cells were grown in 24-well culture plates to 70–80 % confluence. For each well, 7.5 μl human miR-424-5p (has-miR-424-5p) inhibitor or 4 μg pGFP-SOCS6 vector were added to 250 μl Opti-MEM medium. Next, 5 μl of Lipofectamine 2000 was added to 250 μl Opti-MEM medium and then either the inhibitor or vector was mixed with Lipofectamine 2000. The mixture was added to the cells and incubated for 24–48 h. Total RNA and protein were used for quantitative real-time PCR (qRT-PCR) or western blot analysis, respectively, after transfection.

Luciferase Reporter Assays

The pMIR-report luciferase vector was used for the construction of the pMIR-SOCS6 or pMIR-SOCS6-mut vectors. The pMIR-SOCS6-mut vector was built with SOCS6 that had undergone site-directed mutagenesis of the miR-424-5p target site using the Stratagene Quik-Change site-directed mutagenesis kit (Stratagene, Germany). PANC-1 cells were transfected in 24-well plates using Lipofectamine 2000 transfection reagent. Each well was transfected with either 100 ng pMIR-SOCS6 or 100 ng pMIR-SOCS6-mut vectors together with 30 pmol has-miR-424-5p or NC. pRL-TK (Promega, USA) was also transfected as a normalization control. Forty-eight hours after transfection, luciferase activity was measured using the Dual Luciferase Reporter Assay (Promega).

Quantitative Real-Time PCR

Total RNA was extracted from tissues or cells with Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). MiR-424-5p levels were quantified by qRT-PCR using Taqman assay kits (Applied Biosystems, Foster City, CA, USA), with U6 small nuclear RNA as an internal normalized reference. For mRNA detection, reverse transcription was performed according to the protocol provided with the RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas). GAPDH mRNA levels were used for normalization. Their relative levels

were measured in triplicate on a Prism 7900 real-time PCR machine (Applied Biosystems) according to the instructions. The primers used were as follows: for miR-424-5p, forward 5′-CAGCAGCAATTCATGT -3′, reverse 5′- TGGTGTCGTGGAGTCG-3′; for SOCS6, forward 5′-TGGACTCACTGGCACAGAAG -3′,reverse 5′-TGATTGGTCCCCAGTACCAT -3′.

Western Blotting

Total protein was extracted from tissues or cells, and samples were run on 10 % SDS-PAGE acrylamide gel electrophoresis at 80 V, followed by transfer to polyvinylidene difluoride (PVDF) membranes at 300 mA. The transferred membranes were blocked for 1 h with 5 % non-fat powdered milk, and incubated with primary antibodies for 2 h at room temperature. The primary antibodies included rabbit SOCS-6 antibody (1:500; Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA) and mouse GAPDH antibody (1:800; Santa Cruz Biotechnology, Inc.). The membranes were then incubated for 30 min at room temperature with the respective appropriate secondary antibodies and visualized using an ECL chemiluminescent kit (Beyotime Biotechnology, Shanghai, China) following the manufacturer's instructions, and then exposed to X-ray film. GAPDH levels were used to standardize protein loading, with the assumption that the level of GAPDH would be similar in all tissues or cells.

MTT Assays

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma) was dissolved in phosphate-buffered saline (PBS) at 5 mg/mL and used to measure either cell proliferation or viability. Approximately 10^4 cells per well were incubated in culture medium for 96 h in 96-well plates, followed by the addition of 10 μL of the MTT solution. After 4 h incubation, 100 μL of solubilization solution [20 % sodium dodecyl sulfate (SDS)] was added, and the mixture was incubated at 37 °C for 16 h. In this assay, MTT is cleaved to an orange formazan dye by metabolically active cells. The absorbance of the formazan product was measured with an enzyme-linked immunosorbent assay reader at 570 nm.

Flow Cytometry Assays

Cells were collected and washed with PBS containing 2 % ethylene diamine tetraacetic acid. Then, each sample

Table 1 Summary of clinical details and miR-424-5p, SOCS6 expression in pancreatic cancer patients

Case	Gender	Age(years)	Tumor size(cm)	TNM stage	Normalized miR-424-5p change (fold)	Normalized SOCS6 mRNA change (fold)
1	M	56	3.8×4.5×3.3	II	8.5703	0.9114
2	F	70	2.0×3.0×2.2	II	1.2148	0.7165
3	M	61	2.6×3.4×3.5	II	11.8823	1.4182
4	M	66	2.5×3.5×4.4	III	1.8201	0.6311
5	M	63	4.7×4.8×4.6	II	1.7912	0.6880
6	F	75	1.2×1.8×1.6	I	3.8750	0.7426
7	F	57	3.8.×4.0×3.6	II	1.7234	0.8312
8	M	48	3.4×3.8×4.5	III	0.9515	0.7973
9	M	63	3.0×3.0×3.1	II	1.7050	1.3818
10	F	59	4.0×4.2×4.6	II	0.4873	0.7982
11	F	65	2.7×3.5×3.0	II	2.6376	0.7654
12	F	73	2.5×3.2×3.3	IV	1.8985	0.7205
13	M	38	2.8×3.6×4.4	III	17.6805	0.8835
14	M	62	5.0×5.0×5.1	III	5.4497	1.5882
15	F	64	4.2×4.5×4.4	III	4.0097	0.7668
16	M	77	3.0×4.0×4.0	III	4.5687	1.0230
17	M	71	5.7×5.4×6.0	III	2.9662	0.6853
18	F	60	3.6×3.9×4.5	III	1.5355	0.6964
19	F	57	2.5×3.8×3.6	III	0.4552	0.9077
20	M	58	3.9×3.4×5.0	IV	4.8656	0.9740
21	F	69	3.1×4.3×3.7	IV	0.2567	1.2687
22	M	73	2.5×2.9×3.6	III	1.6883	0.7586
23	M	77	3.2×4.2×4.1	III	0.3959	0.9655
24	F	56	2.3×3.0×2.2	II	1.0556	0.6449

Normalized miR-424-5p and SOCS6 change show that miR-424-5p and SOCS6 expression in pancreatic cancer tissues performed by the $2^{-\triangle\triangle Ct}$ method with adjacent non-tumor pancreatic ductal epithelium tissues as a calibrator. $\triangle Ct$ Cobtained from real-time PCR was subject to Student's *t* test. Data show the means from three independent analyses

containing 10^5–10^6 cells/ml was stained with annexin V-FITC and 10 μL PI (Annexin V-FITC/PI Apoptosis Detection Kit, ADL) for 15 min. Afterwards, the cells were diluted using binding buffer and analyzed with a BD FACSAria.

Cell Migration and Invasion Assays

The migration and invasion assays were performed using 6-well transwell chambers (8 μm; Corning) and Matrigel (BD Biosciences). For the migration assays, PANC-1 cells transfected with miR-424-5p inhibitor or NC were suspended in N,O-Bis(trimethylsilyl) acetamide (BSA), and the cell concentration was adjusted to 5×10^4 cells/ml, then 2 ml aliquots of the suspension were seeded into the upper chambers and 1 ml MEM containing 10 % FBS was added to the bottom chambers. After 24 h, the migrated cells were fixed with 95 % ethanol and stained with hematoxylin for 10 min. The invasion assay protocol was similar to that of the migration assay except that the upper chambers were first covered with 50 mg/ml Matrigel.

Statistical Analysis

Data from at least three independent experiments are expressed as means ± SD. Differences between groups were analyzed using the two-tailed Student's *t*-test after fitting the normal distribution by F test. All tests performed were two-sided. Reported *P*-values were considered to be statistically significant at <0.05. Statistical calculations were executed using SPSS software (v.18.0).

Results

The Expression of miRNA-424-5p is Aberrantly Increased in Human PDAC Tissues and PDAC Cell Lines

To identify the importance of miRNA-424-5p in the development of PDAC, especially in Chinese patients, we collected three different human clinical specimens. Then we used qRT-PCR to detect the expression of miR-424-5p in PDAC tissues, respective adjacent normal ductal epithelium tissues, and normal pancreatic ductal epithelium tissues. The results in Table 1 show that miR-424-5p expression increased in about 79 % of the PDAC samples (19 of 24 cases), with a median upregulation of 3.48-fold (range 1.06- to 17.68-fold). The expression of miR-424-5p in PDAC tissues was higher than in the adjacent normal ductal epithelium tissues (0.07407± 0.03385 vs. 0.04601±0.03143, $P<0.05$); the same results were found between adjacent normal pancreatic ductal epithelium tissues and normal pancreatic ductal epithelium tissues (0.04601±0.03143 vs. 0.02792±0.01801, $P<0.05$; Fig. 1a).

We also detected the level of miR-424-5p in PDAC cell lines. We found that the mature form of miR-424-5p was overexpressed by almost 7–11-fold in PANC-1, AsPC-1, BxPC-3 and MIAPaCa-2 cells compared with in normal human pancreatic ductal epithelium tissues; and PANC-1 cells exhibited the highest levels of miR-424-5p expression (Fig. 1c). These results suggested that miR-424-5p may be a novel factor associated with the development of pancreatic cancer.

The Expression of SOCS6 mRNA is Reduced and Inversely Correlated with miR-424-5p in Human PDAC

To explore the mechanism(s) by which miR-424-5p executes its function in pancreatic cancer, we first applied four bioinformatics algorithms (miRbase, TargetScan, PicTar and miRanda) to identify its potential target genes. Among these many candidates, SOCS6 was selected for further analysis. We used qRT-PCR to detect the expression of SOCS6 mRNA in clinical specimens. The results showed that the expression of SOCS6 mRNA in PDAC tissues were lower than in the adjacent normal ductal epithelium tissues (0.00752±0.00123 vs. 0.00883±0.00227, $P<0.05$); but no significant differences were found between the adjacent and normal tissues (0.00883 ±0.00227 vs. 0.01102±0.00108, $P>0.05$; Fig. 1b).

To further address the biological relationship between SOCS6 and miR-424-5p in PDAC, Pearson correlation analysis was applied to compare their relative expression levels in these human clinical specimens. We obtained a statistically significant inverse correlation ($R=-0.455$, $P=0.026$) in a total of 24 tumors (Fig. 1d). Thus, the expression of miR-424-5p is inversely correlated with SOCS6, indicating that this miRNA may function as a novel SOCS6 repressor in pancreatic cancer and accelerates tumorigenesis and metastasis.

SOCS6 is a Direct Target of miRNA-424-5p

To test the above hypothesis, we sought to confirm the possible regulation of SOCS6 by miRNA-424-5p. Based on the bioinformatics prediction that there is only one potential position, 302–309, in the 3′ UTR of SOCS6 mRNA targeted by miRNA-424-5p (Fig. 2a), we constructed the reporter by inserting the wild-type fragments from the 3′ -UTR region of SOCS6 into the downstream of the luciferase coding region (named pGL3-SOCS6). MiR-424-5p was cotransfected with these reporter plasmids into human pancreatic cancer cells PANC-1. As shown in Fig. 2b, the intensity of fluorescence after miR-424-5p cotransfection was remarkably reduced compared with the negative control; however, no significant variation in luciferase activity was observed for either the pGL3-SOCS6-MUT or the negative control miR-424-5p cotransfection. Thus, the luciferase assays revealed that miR-424-5p could bind to the SOCS6 3′-UTR, causing a significant decrease in luciferase activity compared with the negative control. These results suggest that SOCS6 is a direct target of miR-424-5p.

This finding led us to examine whether inhibiting the expression of miR-424-5p could increase endogenous SOCS6 protein levels in human pancreatic cancer cell lines. We ectopically expressed hsa-miR-424-5p inhibitor in PANC-1 and SW1990. Using Western blot assays, we found that the level of SOCS6 protein was increased significantly by miR-424-5p inhibitor compared with control treatment (Fig. 2d).

As SOCS6 is a known negative regulator of the ERK1/2 cascade [22, 23], the repression of SOCS6 by miR-424-5p should impair this signaling pathway. Next, we examined the effect of miR-424-5p on the expression of the transcriptional targets of these pathways in PANC-1 cells. qRT-PCR analysis showed that the expression levels of the ERK1/2 signaling pathway target genes *BCL-2* and *MCL-1* [24], were suppressed by miR-424-5p overexpression (Fig. 2c), suggesting that miR-424-5p can regulate SOCS6-regulated signaling pathways. Herein, we proposed that miR-424-5p may exert its biological function through regulating the expression of its direct target SOCS6.

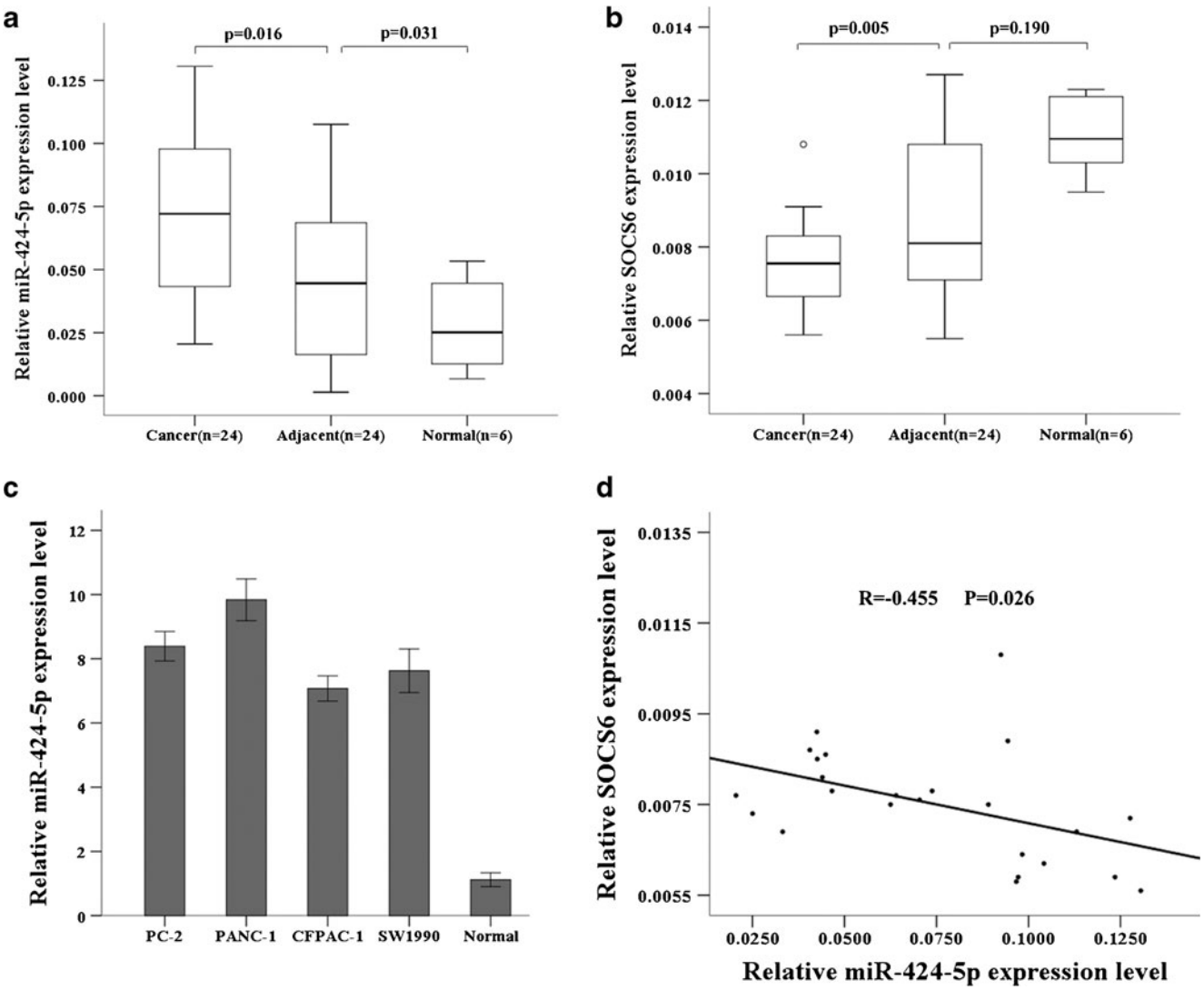

Fig. 1 Comparison of miR-424-5p and SOCS6 levels in pancreatic cancer tissues and cell lines. The expression levels were measured by qRT-PCR. The Student's *t*-test was used to determine statistical significance. Data show the means from three independent analyses. **a** Relative miR-424-5p expression levels in different tissues: 24 paired PDAC and respective adjacent normal ductal epithelium tissues samples; six normal ductal epithelium tissues. *Boxes* represent the medians and inter-quartile ranges of the normalized threshold values. MiR-424-5p expression levels were calculated by the $2^{-\triangle Ct}$ method and normalized to U6 small nuclear RNA. **b** Relative SOCS6 expression levels in different tissues: 24 paired PDAC and respective adjacent normal ductal epithelium tissues samples; six normal ductal epithelium tissues. SOCS6 expression levels were calculated using the $2^{-\triangle Ct}$ method and normalized to GAPDH mRNA. **c** Relative miR-424-5p expression levels in normal pancreatic ductal epithelium tissues, and in PDAC cell lines PANC-1, PC-2, CFPAC-1 and SW1990. **d** The inverse correlation of SOCS6 and miR-424-5p expression levels were examined by Spearman correlation analysis

Inhibition of miR-424-5p Expression Affects PANC-1 Cell Proliferation

The SOCS family is thought to act largely as a negative regulator of signaling by cytokines and some growth factors. We proposed that SOCS6 is a direct target of miRNA-424-5p, thus we believe that miR-424-5p is likely connected with tumor proliferation. To confirm this proposal, we performed MTT assays in a common pancreatic cancer cell line, PANC-1, which has high expression levels of miR-424-5p. As shown in

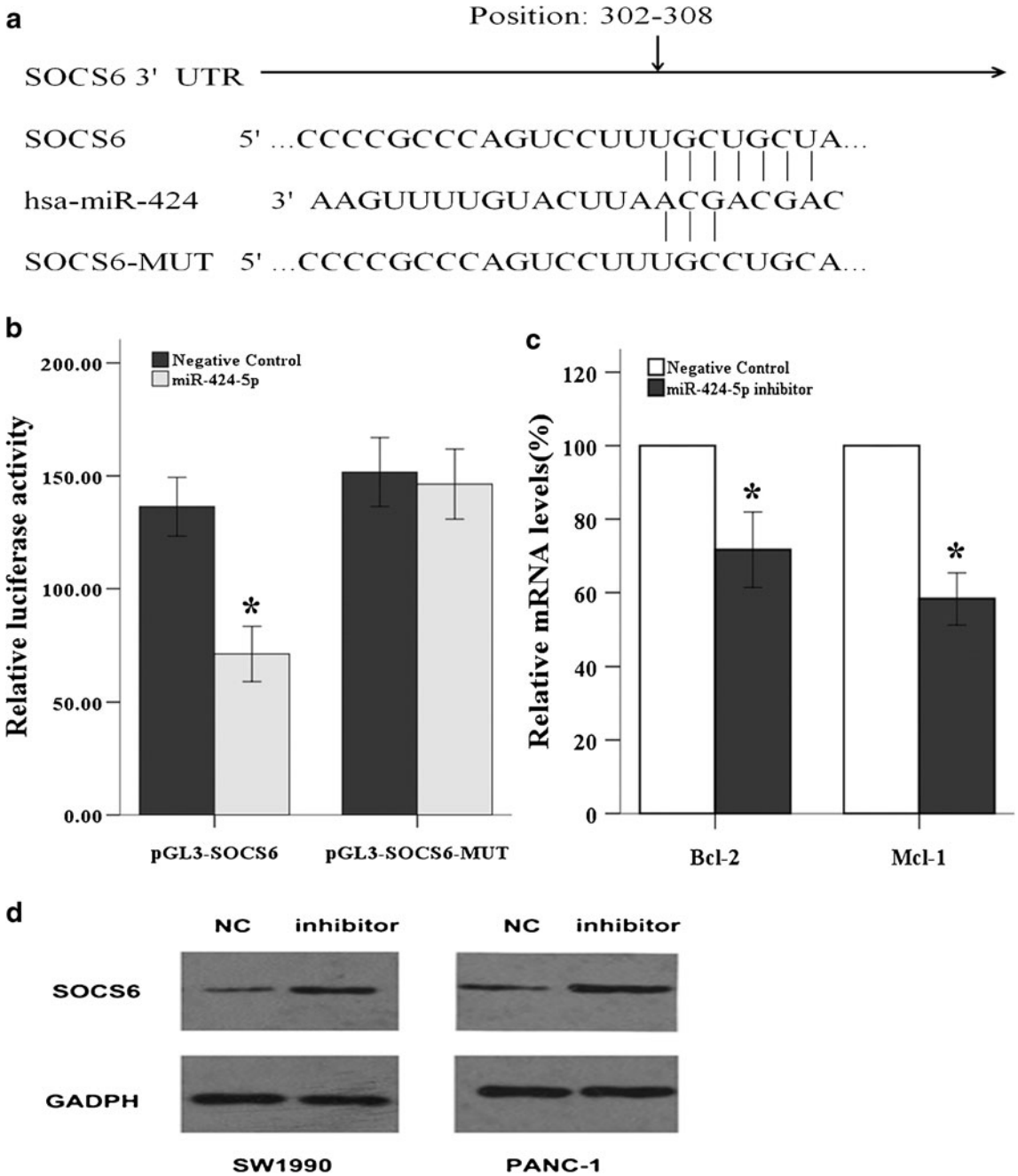

Fig. 2 SOCS6 is a direct target of miR-424-5P. **a** Putative miR-424-5p binding sequences in the 3′ UTR of SOCS6 mRNA. **b** Relative luciferase assay comparing the pGL3-SOCS6 and pGL3-SOCS6-MUT vectors in PANC-1 cells. Firefly luciferase activity was normalized to Renilla luciferase activity. Values are expressed in triplicate, mean ± SD, *$P<0.05$. **c** The expression of target genes of SOCS6-mediated pathways was reduced by miR-424-5p overexpression. qRT-PCR was used to determine the expression levels of indicated targets of Bcl-2 and Mcl-1 in PANC-1 cells 48 h after transfection. The results are presented as means ± SD. *$P<0.05$; **d** miR-424-5p suppresses expression of endogenous SOCS6. Western blot was used to examine the expression levels of endogenous SOCS6 in SW1990 and PANC-1 cells 48 h after transfection

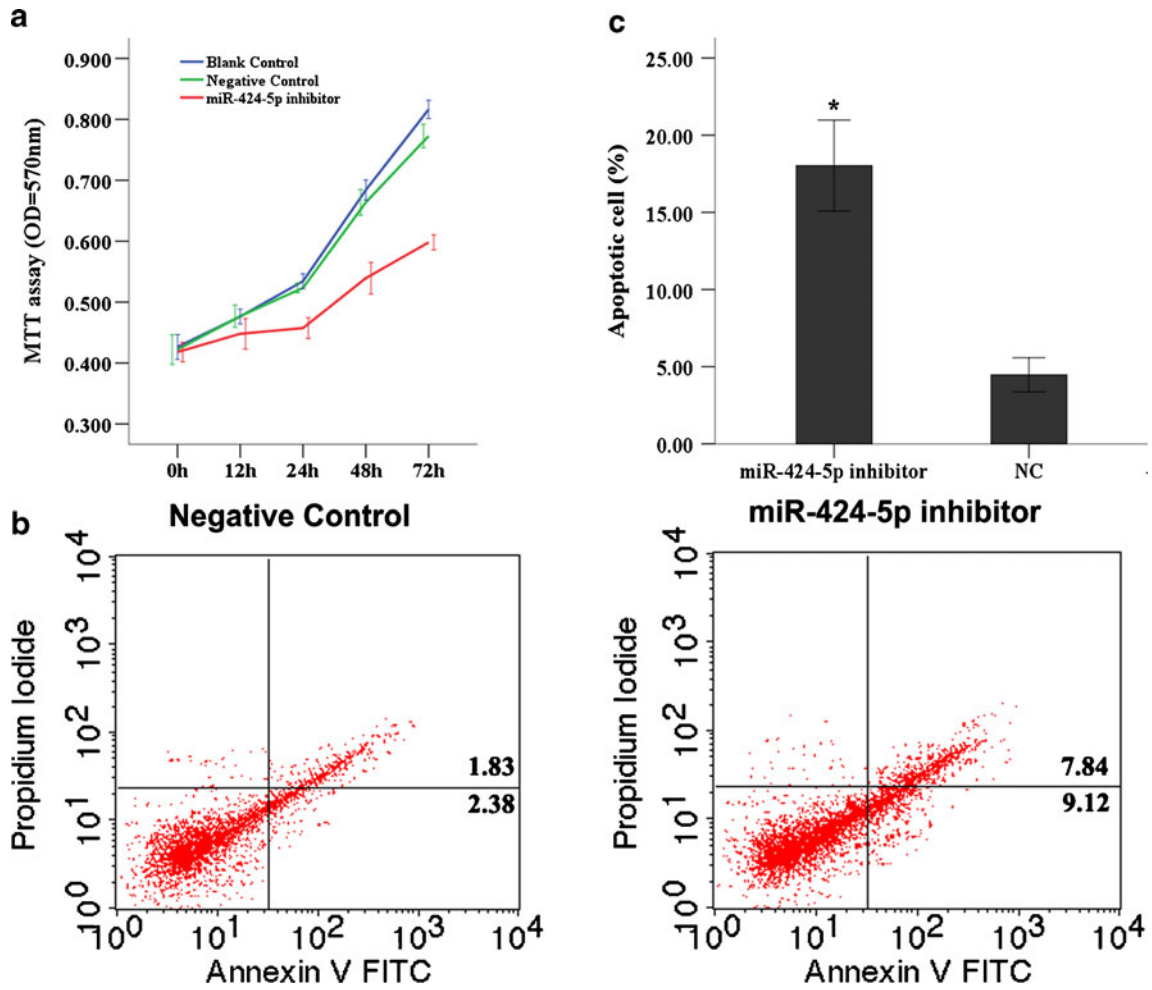

Fig. 3 Role of miR-424-5p in proliferation and in inhibiting apoptosis of pancreatic cancer cells. **a** Cell proliferation was measured by MTT assays in PANC-1 cells transfected with miR-424-5p inhibitor or negative control. The *x*-axis indicates the number of hours after transfection. Data represent the mean ± SD of the three independent experiments performed in triplicate. **b-c** Apoptosis of PANC-1 cells was detected by flow cytometric analysis 72 h after transfection with miR-424-5p inhibitor or NC (*$P<0.05$). The cells were stained with annexin V-FITC. The *right upper quadrant* represents late apoptotic and necrotic cells; the *right lower quadrant* represents early apoptotic cells; the *left upper quadrant* represents cells that were mechanically injured; and the *left lower quadrant* represents normal proliferating cells

Fig. 3a, miR-424-5p inhibitor reduced cell proliferation 24–72 h after transfection compared with the negative control and the blank, both of which remained similar throughout the 72-h assay period. These results indicate that anti-miR-424-5p can suppress the proliferation of PANC-1 cells.

Inhibition of miR-424-5p Expression Induces Apoptosis in PANC-1 Cells

To further characterize the growth inhibitory function of anti-miR-424-5p, miR-424-5p inhibitor-transfected cells were subjected to apoptosis assays and assessed using annexin V-FITC. We further showed that the treatment of cells with anti-miR-424-5p induces increased PANC-1 cell apoptosis, but less effect was observed when cells were treated with the NC transfectants (Fig. 3b, c, $p=0.027$). These data suggested that anti-424-5p increased the apoptotic rate in PANC-cells, coinciding with increased levels of SOCS6 protein.

Inhibition of miR-424-5p Expression Suppresses PANC-1 Cell Migration and Invasion

To study the role of the miR-424-5p in cell invasion and migration, we assessed the capacity of invasion and migration in PANC-1 cells after transfection with a miR-424-5p inhibitor. As shown in Fig. 4a, c, miR-424-5p inhibitor reduced the migratory ability of PANC-1 cells to 60 % compared with the NC cells ($P<0.05$). The invasive capacity was also suppressed by nearly 50 % by the miR-424-5p inhibitor compared with the NC cells ($P<0.05$; Fig. 4b, c). These results indicate that downregulation of miR-424-5p inhibited both the migration and invasion of PANC-1 cells in vitro.

Discussion

The expression of miRNAs in various human tumors suggests that they could have regulatory functions in cancer. Several miRNAs have been revealed as 'onco(genic)-miRs' (miR-21 and miR-155) or 'tumor-suppressor miRs' (miR-29b, miR-34 and let-7 families) involved in pancreatic tumor development [25, 26]. However, the molecular mechanisms of miRNAs in pancreatic cancer and their relationship with these regulatory factors are currently poorly understood. In our study, we examined the expression of miR-424-5p in human pancreatic cancer tissues and adjacent non-tumorous tissues. We also observed the effect of miR-424-5p on proliferation, apoptosis, migration and invasion in human pancreatic cancer cell lines. The *SOCS6* gene is a direct target of miRNA-424-5p. In PANC-1 cells, we demonstrated that miR-424-5p was involved in the ERK1/2 signaling pathway by repressing *SOCS6* expression. Our results support the proposal that miR-424-5p is a new and important molecule that is closely related to pancreatic cancer development.

MiRBase (http://www.mirbase.org/index.shtml) shows that miR-424-5p is located on human chromosome Xq26.3, and has overlapping transcripts in the intergenic region. Enhanced levels of miR-424-5p were observed in colon cancer [27], while it was downregulated in chronic lymphatic leukemia [28] and cervical cancer [29]. In

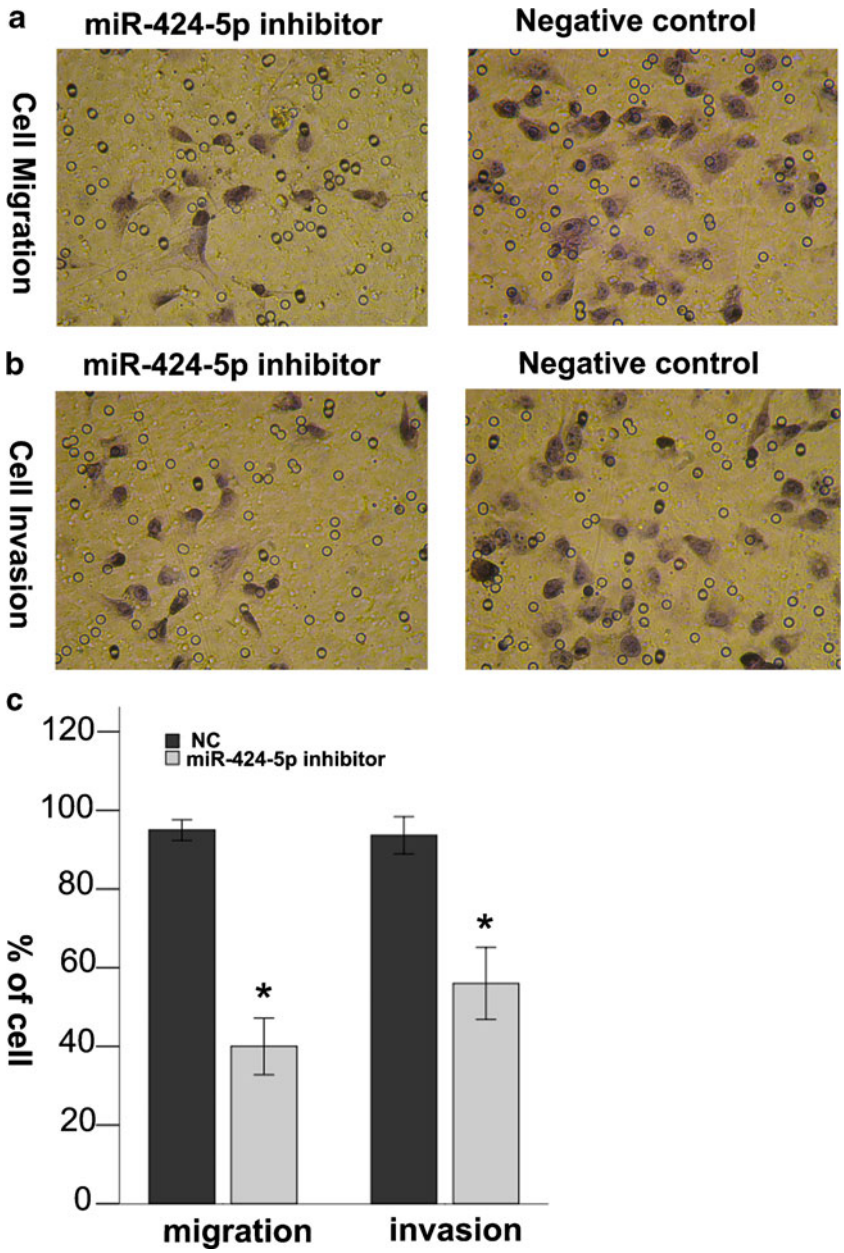

Fig. 4 Role of miR-424-5p in migration and invasion of pancreatic cancer cells. Migration and invasion assays were performed using 6-well transwell chambers after PANC-1 cells were transfected with miR-424-5p inhibitor (*P <0.05)

addition, miR-424-5p is more highly expressed in T-ALL as compared with B-ALL, and may serve as a marker to distinguish these two entities [30]. MiR-424-5p also appears to be involved in cellular differentiation [31, 32]. Interestingly, miR-424-5p is induced by the transcription factor PU.1 during monocyte/macrophage differentiation [33]. Although it has been reported that miR-424-5p was upregulated in pancreatic cancer cell lines via miRNA precursor expression, the biological behavior and mechanisms that were responsible remain largely undefined [17]. Here, we found that miR-424-5p expression was significantly higher in pancreatic cancer than in adjacent tumors and normal pancreatic tissues, and that miR-424-5p was involved in proliferation, migration, invasion, and apoptosis in PANC-1 cells. Thus, miR-424-5p is a candidate oncogene of pancreatic cancer, where it may play an important role in promoting tumor development.

The SOCS family contains eight proteins, SOCS-1 to SOCS-7 and CIS, which are characterized by an amino-terminal (N-terminal) region of variable length, a central SH2 domain, and a carboxyl-terminal (C-terminal) SOCS box [34], and was first identified as an inhibitor of cytokine signaling. Tumor-suppressor function is proposed for SOCS6, and it is downregulated in a variety of cancers and has the capacity to inhibit tumorigenesis when expressed in cell lines derived from gastric cancer (AGS and AZ-521) as well as non-small-cell lung cancer (H1299) and kidney (HEK293) [18]. The ectopic expression of SOCS6 caused a 40 % decrease in SCF-dependent cell proliferation and a similar reduction in signaling through ERK1/2 [22]. In most pancreatic cancers, there are multiple activated pathways, such as ERK1/2, TGF-β, K-RAS, JNK, integrin, and Hedgehog signaling, and altered processes, including control of G1/S phase transition and DNA damage [24, 35]. The ERK signaling pathway is well known to be implicated in the regulation of numerous cellular physiological processes including cell proliferation and differentiation. It has been reported that activation of the ERK pathway functions to protect pancreatic tumor cells from apoptosis as well as to regulate their progression in the cell cycle through regulating the expression of Bcl-2 and Mcl-1 [24]. Here, we find that miR-424-5p directly targets SOCS6 and that inhibition of miR-424-5p expression could increase endogenous SOCS6 protein levels in human pancreatic cancer cell lines. In addition, downregulated miR-424-5p inhibited the expression levels of downstream targets of SOCS6, such as Bcl-2 and Mcl-1, and inhibited the ERK pathway. Moreover, the expression levels of miR-424-5p and SOCS6 are inversely correlated in human clinical specimens of pancreatic cancer. Together, these results indicate that miR-424-5p interacting with the SOCS6 gene modulates ERK pathway activity, thus promoting pancreatic cancer formation and development.

Because the number of PDAC samples was small, we have not shown relationships between miR-424-5p or SOCS6 expression and size, TNM stage, degree of differentiation, genetic background, and prognosis of PDAC patients. Although a survival curve was not drawn, we estimated that higher miR-424-5p and lower SOCS6 levels were associated with lower survival rates. Therefore, miR-424-5p and SOCS6 levels may prove to be new therapeutic targets for the clinical course of PDAC. Next, we plan to investigate miR-424-5p functions in vivo by injecting anti-miR-424-5p lentiviral vector-transfected PDAC cells into athymic nude mice.

In conclusion, we have demonstrated that miR-424-5p is a candidate oncogene that is upregulated in PDAC compared with adjacent normal ductal epithelium tissues. Upregulated miR-424-5p plays an important role in promoting proliferation, migration, and invasion, and in inhibiting apoptosis of pancreatic cancer cells. The results showed that miR-424-5p downregulates the expression of SOCS6, and thus elevates ERK pathway activity. Therefore, miR-424-5p may be useful in the diagnosis and therapy of pancreatic cancer.

Conflicts of interest The authors declare no conflict of interest.

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