# Original Article miR-27a regulates biological function of patients with pancreatic cancer via PSMA1

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**Abstract:** Objective: To explore whether miR-27a regulates the biological function of patients with pancreatic cancer (PC) via PSMA1. Methods: Seventy-two patients with PC admitted to our hospital were screened as the miR-27a inhibitor group, and 61 healthy persons were recruited into the control group (miR-NC group) at the same time. QRT-PCR was applied to measure miR-27a and PSMA1 expressions in the samples, and WB was applied to measure PSMA1 protein expressions in the cells. Proliferation, invasion, migration, and apoptosis rate were detected using CCK-8, transwell, wound healing assay and flow cytometry. The correlation between miR-27a and PSMA1 was analyzed through the dual-luciferase reporter assay. Results: miR-27a showed high expression in patients' tissues and serum, while PSMA1 showed low expression in their tissues and serum. MiR-27a and PSMA1 expressions in serum were positively correlated with their expressions in tissues, while miR-27a and PSMA1 were negatively correlated in tissues. PSMA1 overexpression and miR-27a silencing inhibited PC cells' proliferation and invasion and elevated apoptosis rate. After co-transfection with miR-27a-inhibitor or si-PSMA1 to cells of BxPC3 and PANC-1, proliferation and invasion abilities were remarkably inhibited, and apoptosis rate was elevated. MiR-27a inhibition increased PSMA1 expression, thereby decreasing proliferation ability of PC cells, and increasing the apoptosis rate. Conclusion: MiR-27a can reduce proliferation and invasion ability as well as elevate apoptosis rate of PC cells via regulating PSMA1.

Keywords: miR-27a, PSMA1, pancreatic cancer, cell biology

#### Introduction

Pancreatic cancer (PC) is a life threatening cancer and a main cause of death in developed countries [1, 2]. Currently, prevention and early diagnosis in the curable stage are impossible [3], and most patients are in advanced stages but show no symptoms [4]. Surgery for PC is usually only offered to patients in early stage, so its surgical resection rate is low and is likely to cause poor prognosis. Generally, the 5-year overall survival (OS) is 6% [5]. With the development of molecular biological diagnosis, the diagnosis of tumors has been greatly improved [6]. We urgently need to find new potential targets for treatment and early diagnosis.

miRNAs can inhibit translation by targeting translation of mRNA complementary sequences, so as to regulate the physiological and pathological processes [7, 8]. More studies have

pointed out that miRNAs participate in the progress of cancer [9, 10]. MiR-27a is regarded as an "onco-miRNA" that may be related to tumor progression [11], and is involved in cancer cell proliferation [12]. Its abnormal expression was observed in many cancers, including PC, breast cancer, etc. [13]. A previous survey has indicated that miR-27a inhibition inhibits PC cells' growth [14].

PSMA1 is a substructure of the proteasome complexes, an important structure for protein degradation catalyzed by ubiquitination in eukaryotic cells [15]. Bortezomib can exert antitumor effects by inhibiting PSMA1 in patients with myelodysplastic syndromes [16]. Studies also have shown that PSMA1 is the direct target gene of miR-27a in PC [17].

At present, there are few researches centered on miR-27a and PSMA1 mechanisms in PC. Therefore, our study aimed to investigate the effects of miR-27a regulating PSMA1 on biological functions in patients with PC to find out new therapeutic targets.

#### Materials and methods

#### Subjects enrollment

With the consent of the patient and hospital, we collected the cancerous tissues and adjacent tissues from 72 patients with PC. And 61 healthy persons at the same time were recruited into the control group. This study was approved by the Ethics Committee of the First People's Hospital of Fuyang Hangzhou.

Inclusion criteria: Patients who were diagnosed as having pancreatic ductal adenocarcinoma with pathology or imaging examinations.

Exclusion criteria: 1) Patients with pancreatic tumors other than ductal adenocarcinoma of the pancreas, such as pancreatic intraductal papillary myxoma (I PMN), neuroendocrine tumors, etc. 2) Patients with other malignant tu mors diagnosed at the same time. 3) Patients with incomplete data.

## Cell sourcing

Normal human pancreatic duct epithelial cells (hTERT-HPNE) and PC cells including BxPC3, PANC-1, AsPC-1, CFPAC-1 (Beijing Fubo Biote-chnology Co., Ltd., cat No. CSC-C1366, CSC-C2530, GTX14882, CSC-C0114, CSC-C0110).

## Experimental reagents and instruments

ABI Stepone Plus Real-Time PCR System; Lipofectamine<sup>™</sup>2000 transfection kit. ECL luminescence kit, BCA protein assay kit, flow cytometer, microplate reader, and DR5000 UV-visible spectrophotometer were used. All primers were synthesized by Bao Biological Engineering Co., Ltd. (China).

## Cell transfection experiments

Lipofectamine<sup>™</sup>2000 kit was used to transfect miR-27a-mimics (overexpression sequence), RNA negative control (Si-NC), miR negative control (miR-NC), and PAK4 RNA targeted inhibition (si-PAK4). The procedures were strictly performed according to the instructions.

#### qRT-PCR test

We used TRIzol kit to obtain total extraction of RNA from cells, tissues and serum. We used UV spectrophotometer and agarose gel electrophoresis to test the purity, concentration and integrity of total RNA.

The total system was 10  $\mu$ L, with 2× Talent qPCR premix 5.0  $\mu$ L, 1.25  $\mu$ l of upstream and 1.25  $\mu$ l of downstream primers, 100 ng cDNA, and added water to prepare a 20  $\mu$ l solution. The qPCR data were analyzed by double delta Ct.

## Western blot test

We performed PIPA lysis method to obtain the total protein extraction of cells. The protein samples were prepared for SDS-PAGE electrophoresis, and we transferred the separated proteins to the PVDF membrane. We added and sealed  $\beta$ -catenin, cyclin D1, c-myc (1:1000) primary antibody, and  $\beta$ -actin (1:3000) at 4°C overnight. After ECL light emission process, we scanned the protein strips, and analyzed the gray values through Quantity One software.

#### CCK-8 detects cell proliferation

Each well was added with CCK-8 solution (Cat: HY-K0301-100T, MedChem Express, Monmouth Junction, NJ, USA, 15  $\mu$ L/well) at 0 h, 12 h, 24 h, 36 h, 48 h, 60 h, and 72 h. After incubating for 3 h, we determined the optical density (OD) value at 450 nm by a microplate reader (Bio-Tek, Winooski, VT, USA). We conducted colony formation for 14 d, fixed the colonies in 70% ethanol for 10 min, and stained it with 1% crystal violet solution at room temperature for 10 min. The photos were taken by Nikon camera (Nikon 5300, Nikon Corporate, Tokyo, Japan).

## Transwell method detects cell invasion

We tested invasion ability of cells by Transwell assays. We coated Transwell chamber with Matrigel at 37°C for 30 min. After that, we inoculated 200  $\mu$ L treated cells in the upper chamber with a density of 2 × 10<sup>3</sup> cells/mL, and then added 700  $\mu$ L complete medium to the lower chamber. We cleared the uninvaded cells after 48 h. We rinsed the cells on the lower layer with PBS for three times, fixed in 4% paraformaldehyde for 30 min, and then dyed the cells with

Indexes	n	miR-27a	t	Р	PSMA1	t	Р
Gender			1.477	0.144		0.753	0.454
Male	45	5.09±0.71			1.55±0.44		
Female	27	4.64±0.67			1.93±0.43		
Age (years)			1.489	0.141		0.704	0.484
≤ 55	40	5.19±0.66			1.83±0.65		
> 55	32	4.96±0.64			1.72±0.67		
TNM stage			5.588	< 0.001		6.466	< 0.001
I, II	41	4.43±0.61			2.36±0.60		
III, IV	31	5.23±0.59			1.43±0.61		
Degree of differentiation			5.575	< 0.001		6.891	< 0.001
Moderate/High	47	4.48±0.54			2.14±0.53		
Low	25	5.23±0.55			1.23±0.54		
Lymph node metastasis			3.588	< 0.001		3.332	< 0.001
Yes	44	4.63±0.58			1.87±0.57		
No	28	5.12±0.54			1.42±0.54		

Table 1. Relationship between miR-27a, PSMA1 and clinicopathological characteristics of PC patients (mean  $\pm$  SD)

crystal violet solution for 10 min. We observed cells with a microscope.

## Flow cytometry detects apoptosis

Treated PC cells (2 ×  $10^5$  cell/well) were inoculated in the 24-well plate and were cultured at 37°C overnight. We rinsed the cells with PBS and re-suspended them in 0.5 mL of binding buffer with 5 µL of annexin for 20 min. We performed V-FITC and propidium iodide double staining (BD Biosciences, Franklin Lakes, NJ, USA) in the darkness. We used flow cytometer for detection. We repeated the experiment for three times and took the average value.

## Double luciferase activity assay

We transfected PSMA1 3'UTR-Wt, PSMA1'UTR-Mut, miR-27a-inhibitor and miR-NC into BxPC3 and PANC-1 cells through Lipofectamine<sup>™</sup>2000 kit and determined the luciferase activity through Bright-Glo Luciferase Assay System (Promega, WI, USA).

## Statistical analysis

SPSS 20.0 was adopted for statistical analysis. GraphPad 7 (California San Diego, USA) was used to draw figures. The *t*-test was used and data are represented as the mean  $\pm$  SD. P < 0.05 was considered as statistically significant difference.

#### Results

#### MiR-27a and PSMA1 diagnostic value in PC

The relative expression of PSMA1 was remarkably lower than that in normal groups (P < 0.001). The areas under the miR-27a and PS-MA1 curves were 0.905 and 0.932, respectively. Pearson test revealed that miR-27a and PSMA1 expression in PC tissue had a negative correlation (r = 0.000, P < 0.001). Further analysis revealed that miR-27a and PSMA1 were closely associated with lymph node metastasis, pathological differentiation, and TNM stage (Table 1; Figure 1).

miR-27a expression in cells and the impact on cell biological function in patients with PC

MiR-27a in cells of BxPC3, PANC-1, Capan-1, and CFPAC-1 of patients with PC were significantly increased in comparison to hTERT-HPNE cells (P < 0.05).

BxPC3 and PANC-1 cells that had the largest difference in miR-27a expression were transfected, and miR-27a-inhibitor expression after transfection was examined. It was found that miR-27a-inhibitor group had remarkably lower miR-27a expression than miR-NC group (P < 0.01). The proliferation, invasion, and apoptosis rate after transfection were detected. It was shown that miR-27a-inhibitor group showed



**Figure 1.** The diagnosis value of miR-27a and PSMA1 in pancreatic cancer. MiR-27a expression was remarkably elevated in cancer tissues and serum of PC patients, and the serum AUC was 0.911 (A). PSMA1 expression was notably reduced in tissues and serum of PC patients, and the serum AUC was 0.970 (B). MiR-27a and PSMA1 expression in cancer tissues was positively correlated with serum expression (r = 0.619, P < 0.001; r = 0.602, P < 0.001), and miR-27a and PSMA1 expression in cancer tissues had a negative correlation (r = -0.612, P < 0.001) (C). Note: \*\*\*P < 0.001.

remarkably lower proliferation and invasion ability but notably higher apoptosis rate than miR-NC group (**Figure 2**, P < 0.05).

PSMA1 expression in cells and its impact on cell biological function in patients with PC

Cells of BxPC3, PANC-1, AsPC-1, and CFPAC-1 had notably lower PSMA1 than hTERT-HPNE cells (P < 0.05). BxPC3 and PANC-1 with the largest differences in PSMA1 expression were chosen for transfection. The group transfected with sh-PSMA1 showed notably higher miR-27a expression than sh-NC group (P < 0.01). In comparison to cells transfected with sh-NC, BxPC3 and PANC-1, cells transfected with sh-PSMA1 showed lower proliferation ability, and increased apoptosis rate (P < 0.05). After cotransfection with miR-27a-inhibitor or si-PSMA1 to cells of BxPC3 and PANC-1, proliferation and invasion ability were remarkably inhibited, and apoptosis rate was also elevated (**Figure 3**, P < 0.05).

#### miR-27a gene identification

PSMA1 3'UTR-Wt luciferase activity was notably increased after miR-27a expression was suppressed (P < 0.05). Conversely, when miR-27a was overexpressed, PSMA1'UTR-Wt lucifer-



**Figure 2.** MiR-27a expression in cells and the impact on cell biological function in patients with pancreatic cancer. MiR-27a expression in each group of cell lines (A). MiR-27a expression of transfected BxPC3 and PANC-1 cells (B). Cell proliferation of transfected BxPC3 and PANC-1 cells (C, D). Cell invasion of transfected BxPC3 and PANC-1 cells (E). Cell apoptosis of transfected BxPC3 and PANC-1 cells (F). Note: \*P < 0.05.

ase activity was notably reduced (P < 0.05). It was observed in WB detection that PSMA1 expression in BxPC3 and PANC-1 cells was notably increased after transfecting with miR-27a-inhibitor, but it was notably reduced after transfecting with miR-27a-minicis (**Figure 4**, P < 0.05).

#### Discussion

PC is a highly aggressive malignancy as well as one of the main reasons of cancer death in humans [4, 18]. Because the early symptom of PC is mostly abdominal pain, and there are no other obvious symptoms, patients with clinical stage I are rarely diagnosed [19, 20]. Determining the development, occurrence, prognosis and underlying mechanisms of PC will help clinicians to explore more suitable treatment options for PC.

In our study, miR-27a and PSMA1 expression in PC was observed, and the clinical value in PC was analyzed. We observed that miR-27a expression in cancer tissues was increased remarkably in comparison to the adjacent tissues, and experimental group exhibited higher miR-27a expression in serum than control group, however, PSMA1 expression was opposite to that of miR-27a.

Pearson correlation analysis found that miR-27a and PSMA1 expression in tissues was positively correlated with expression in serum, while miR-27a and PSMA1 expression was negatively correlated in tissues. After performing ROC curve analysis on miR-27a and PSMA1, we found that the AUC of miR-27a > 0.90 and the AUC of PSMA1 > 0.95, indicating that both serum miR-27a and PSMA1 showed good diagnostic performance for PC. Later, we further found that there was a close relationship between patients' lymph node metastasis, pathological differentiation, TNM stage and miR-27a, PSMA1. We speculated that miR-27a and PSMA1 predicate in the occurrence and progress of PC. We thus performed cell biology experiments for further verification.

Some studies have suggested that miR-27a is abnormally upregulated in multiple tumors, including PC, and miR-27a inhibition can suppress the growth, colony formation as well as migration of Panc-1 and Mia PaCa-2 in PC cell



**Figure 3.** PSMA1 expression in cells and the impact on cell biological function in patients with pancreatic cancer. PSMA1 expression in each group of cells (A). PSMA1 expression of transfected BxPC3 and PANC-1 cells (B). Proliferation of transfected BxPC3 and PANC-1 cells (C, D). Invasion of transfected BxPC3 and PANC-1 cells (E). Apoptosis rate of transfected BxPC3 and PANC-1 cells (F). Note: \*P < 0.05.



Figure 4. Identification of miR-27a gene. The binding sites and relative luciferase activity between miR-27a and PSMA1-dual luciferase (A). PSMA1 protein relative expression in BxPC3 and PANC-1 cells after transfection (B). Note: \*P < 0.05.

lines [21, 22]. Other studies have implied that PSMA1 plays a crucial role in cancer cell invasion and shows strong inhibitory effects on tumor growth in human breast cancer, PC, lung cancer and xenografts of prostate cancer cells [23, 24]. We detected miR-27a and PSMA1 in normal pancreatic cells and PC cells, and found that PC cells showed notably higher miR-27a expression than normal pancreatic cells, while PSMA1 was higher than normal pancreatic cells. It was found that proliferation and invasion ability were notably inhibited, and apoptosis rate was remarkably elevated after transfection of miR-27a, indicating that miR-27a expression can inhibit proliferation and invasion as well as promote apoptosis of cells.

We also found through cotransfection experiments that PSMA1 and miR-27a overex-

pression could remarkably increase proliferation and invasion ability of BxPC3, PANC-1 and decrease the apoptosis rate, suggesting that a close relationship may exist between miR-27a and PSMA1. To this end, we further confirmed the regulatory relationship between miR-27a and PSMA1 by a double luciferin report assay. The results revealed that the PSMA1 3'UTR-Wt luciferase activity was notably increased after miR-27a expression was suppressed. In contrast, when miR-27a was overexpressed, the PSMA1'UTR-Wt luciferase activity was remarkably reduced, but miR-27a expression had no influence on the PSMA1 3'UTR Mut luciferase activity. This shows that miR-27a and PSMA1 have a targeted regulatory relationship, that is, miR-27a inhibition can elevate PSMA1 expression, thus decreasing proliferation of PC cells, and increasing apoptosis rate.

This study has initially confirmed that miR-27a can accelerate the PC cells apoptosis by regulating PSMA1 expression. There are still some limitations. For example, tumor formation experiments could be performed in nude mice, confirming that whether 27a-inhibitor can improve tumor formation in rats. Signal pathways can be further analyzed and the relationship between miR-27a, PSMA1 and signal pathways as well as the effect of signal pathways on the biological function of PC cells should be determined. We will design more studies to supplement these deficiencies.

In summary, miR-27a can reduce the ability of proliferation and invasion as well as elevate apoptosis rate of PC cells via regulating PSMA1.

## Disclosure of conflict of interest

None.

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