

# miR-939-5p Contributes to the Migration and Invasion of Pancreatic Cancer by Targeting ARHGAP4

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**Objective:** Rho GTPase-activating protein 4 (ARHGAP4) is a GTPase-activating protein for the small GTPases of the Rho family that is involved in tumorigenesis. We recently reported that ARHGAP4 can mediate Warburg effect and malignant phenotype of pancreatic cancer. However, the regulation of ARHGAP4 remains unclear.

**Methods:** ARHGAP4 and miR-939-5p expressions in pancreatic cancer tissues and cell lines were measured by real-time PCR or Western blotting. Pancreatic cancer cells were transfected with miR-939-5p inhibitor, miR-939-5p mimic and/or lentivirus expressing ARHGAP4, and the cell viability, invasion and migration were measured by CCK-8 and Transwell assay, respectively. The suppression of ARHGAP4 expression by miR-939-5p was revealed by luciferase reporter assay, real-time PCR or Western blotting.

**Results:** ARHGAP4 expression was decreased, while miR-939-5p was increased in pancreatic cancer tissues compared with adjacent-normal pancreatic tissues. Higher miR-939-5p expression was correlated with advanced pathological stages and poor prognosis of pancreatic cancer patients. miR-939-5p directly targeted ARHGAP4. Either miR-939-5p down-regulation or ARHGAP4 overexpression inhibited viability, invasion and migration of pancreatic cancer cells. However, ARHGAP4 overexpression markedly inhibited the increased viability, migration, and invasion induced by miR-939-5p up-regulation in pancreatic cancer cells.

**Conclusion:** These observations suggested that miR-939-5p regulates the malignant phenotype of pancreatic cancer cells by targeting ARHGAP4, establishing miR-939-5p as a novel regulator of ARHGAP4 with a critical role in tumorigenesis in pancreatic cancer.

**Keywords:** miR-939-5p, ARHGAP4, pancreatic cancer, carcinogenesis

## Introduction

Pancreatic cancer is one of the digestive system tumors in the world, with high invasiveness and malignancy.<sup>1</sup> Although the comprehensive treatment of cancer has made great progress over the decade, the incidence and mortality of pancreatic cancer are increasing worldwide, seriously endangering human life and health. Currently, the treatment is not effective, and the 5-year survival rate is still very low, only about 5%.<sup>2</sup> It is predicted that pancreatic cancer will surpass colorectal, prostate, and breast cancers to become the second leading cause of cancer-related death by 2030.<sup>3</sup> As the early clinical symptoms of pancreatic cancer are not typical, most of the patients were diagnosed with advanced stage or distant metastasis, so the optimal time for surgical treatment is missed.<sup>4</sup> Meanwhile, only 10% to 20% of the patients have the opportunity to receive radical resection,<sup>4</sup> and 80% of patients

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will have recurrence and metastasis within 1–2 years after surgery.<sup>5</sup> Consequently, it is sensible to the importance of studying the pathogenesis of pancreatic cancer and finding new markers closely implicated in the malignant progression and prognosis of pancreatic cancer, so as to improve the early diagnosis and improve the prognosis.

Recently, Rho GTPase activating proteins (RhoGAPs) have been identified as tumor suppressors in some human cancers, including Rho GTPase-activating protein 17 (ARHGAP17),<sup>6</sup> ARHGAP6,<sup>7</sup> ARHGAP24,<sup>8</sup> and ARHGAP30.<sup>9</sup> Besides, evidences have been reported that ARHGAP4 inhibits cell motility and axon outgrowth,<sup>10</sup> associates with nephrogenic diabetes insipidus and intellectual disability,<sup>11</sup> correlates with pathological stages, vascular invasion and prognosis of pancreatic cancer patients, and regulates glycolysis, invasion and migration in pancreatic cancer.<sup>12,13</sup> Nevertheless, the regulation of ARHGAP4 in pancreatic cancer is still undiscovered. microRNAs (miRNA) are small endogenous non-coding RNA molecules with a length of about 18–25 nucleotides that repress protein translation through binding to the 3'-untranslated region (UTR) of their target mRNA and are significantly involved in various cancers, including pancreatic cancer.<sup>14–16</sup> High expression of miR-196a, miR-27a, miR-221, miR-143, miR-135b, miR-199b-5p and miR-21, but low expression of miR-744, miR-455-3p and miR-655, in pancreatic cancer samples were found compared with normal samples and associated with poor prognosis.<sup>17</sup> In addition, the expression profile of miRNAs varies even in the different locations,<sup>18</sup> which further confirms that miRNAs have high application value in the diagnosis, prognosis evaluation and treatment of pancreatic cancer. Recently, a widely reported miRNA, miR-939, has been pointed out to be pivotal in the development and procession of cancers, such as hepatocellular carcinoma,<sup>19</sup> tongue squamous cell carcinoma,<sup>20</sup> gastric,<sup>21</sup> lung,<sup>22</sup> colorectal,<sup>23</sup> and ovarian cancer.<sup>24</sup> However, the clinical significances and roles of miR-939 in pancreatic cancer are still unknown.

In the present study, our data revealed that the expression of miR-939-5p had a negative pattern as ARHGAP4 in pancreatic cancer tissues and cell lines and was relevant to pathological stages and survival time of patients. Down-regulation of miR-939-5p in pancreatic cancer inhibited cell viability, invasion and migration, while its up-regulation demonstrated an inverse effects which was reversed by overexpression of ARHGAP4, suggesting that miR-939-5p contributes to the invasion and migration in pancreatic cancer by targeting ARHGAP4.

## Materials and Methods

### Clinical Samples

Pancreatic cancer tissues were collected from 70 pancreatic cancer patients who went through tumor resection at Fudan University Shanghai Cancer Center between October 12, 2014 and April 8, 2017. Fresh surgical specimens of primary pancreatic cancer and adjacent-normal pancreatic tissues were collected, stored in liquid nitrogen, and later used for Real-time PCR analysis. This study was approved by the ethic commitment of Fudan University Shanghai Cancer Center. Written informed consent was obtained from all patients, and that this was conducted in accordance with the Declaration of Helsinki.

### Cell Culture

Pancreatic cancer cell lines BxPC-3, Capan-1, PANC-1, MIA PaCa-2 and SW1990 were purchased from Shanghai Institute of Pharmaceutical Industry (Shanghai, China). SW1990, MIA PaCa-2 and PANC-1 were cultivated in DMEM (Gibco, USA), while BxPC-3 and Capan-1 cells were cultivated in RPMI-1640, complementing with 10% fetal bovine serum (FBS), 100 µg/mL streptomycin, and 100 U/mL penicillin under humidified conditions (37°C, 5% CO<sub>2</sub>).

### Cell Transfection

The miR-3150a-3p, miR-939-5p, or miR-762 inhibitor, miR-939-5p mimic and negative control (NC) were purchased from RiboBio (Guangzhou, China). Transfection was performed using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's protocol. ARHGAP4 overexpression constructed by cloning full-length human ARHGAP4 into the lentiviral expression vector pLVX-Puro (Addgen, USA) or blank lentivirus pLVX-Puro (Vector) was transfected into 293T cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol and then transduced into pancreatic cancer cells.

### Luciferase Reporter Assay

The 3'-UTR of ARHGAP4 and the mutated target site of ARHGAP4 3'-UTR were cloned into a pGL3-Promoter vector (Promega, Madison, WI, USA) to constitute ARHGAP4-wild-type (ARHGAP4-WT) and ARHGAP4-mutated-type (ARHGAP4-Mut) reporter plasmid. Pancreatic cancer cells were co-transfected with the reporter plasmid and miR-939-5p mimic, inhibitor, or negative control (NC) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. The Dual-Luciferase Reporter Assay

System (Promega) was used to assay for luciferase activity at 48 h after transfection. Relative luciferase activity was calculated as the ratio of Firefly/Renilla luciferase activity.

## Cell Viability Assay

Cell viability was examined using a Cell Counting Kit (CCK)-8 (Dojindo Lab, Kumamoto, Japan). Cells were transfected with miR-939-5p inhibitor, mimic, or negative control (NC) and/or transduced with lentivirus expressing ARHGAP4 or blank lentivirus (Vector). After treatment, 10  $\mu$ L/well Cell Counting Kit-8 (CCK-8) solution was added, and cells were further incubated for 1 h at 37°C. Absorbance values at 450 nm were measured using a microplate reader (Tecan Safire, Crailsheim, Germany).

## Transwell Assay

The cell migration and invasion were measured by Transwell assay as previously described.<sup>12</sup>

## Real-Time PCR

Total RNA was extracted using RNeasy Plus Universal Kits (QIAGEN, USA). cDNA was synthesized from 1  $\mu$ g of total RNA via thermal cycler system (Bio-Rad, USA). The Real-time PCR was carried out with a LightCycler (Roche Diagnostics GmbH, Mannheim, Germany) using SYBR Green Supermix (Bio-Rad). ARHGAP4 expression was normalized to GAPDH, and miRNAs were normalized to U6. The primer sequences are present as follows: ARHGAP4-forward 5'-CTACAACCTGGCCGTGTGCTTC-3'; ARHGAP4-reverse 5'-CTTCCAGCTCCGGCTCATGTGC-3'; GAPDH-forward 5'-AATCCCATCACCATCTTC-3'; GAPDH-reverse 5'-AGGCTGTTGTCATACTTC-3'; miR-3150a-3p-forward 5'-CGCTGGGGAGATCCTCGA-3'; miR-3150a-3p-reverse 5'-AGTG CAGGGTCCGAGGTATT-3'; miR-939-5p-forward 5'-TGGGGAGCTGAGGCTCTG-3'; miR-939-5p-reverse 5'-AGTG CAGGGTCCGAGGTATT-3'; miR-762-forward 5'-GGGGCTGGGGCCGGGG-3'; miR-762-reverse 5'-AGTGCAGGGTCCGAGGTATT-3'; U6-forward 5'-CTCGCTTCGGCAGCA CA-3'; U6-reverse 5'-AACGCTTCACGAATTTGCGT-3'. Analyses of relative gene expression were performed using the  $2^{-\Delta\Delta CT}$  method.

## Western Blot

Total protein was extracted and separated by electrophoresis on 10% sodium dodecyl sulfonate (SDS) polyacrylamide gels and transferred to Immobilon-P polyvinylidene fluoride membranes (Millipore Corp., USA). Primary antibodies were added to the blocking solution, diluted to the desired

concentration according to the instructions (ARHGAP4 1: 500; GAPDH 1: 2000), and incubated overnight at 4°C. Finally, the protein bands were visualized using enhanced chemiluminescence system (Bio-Rad).

## Statistical Analysis

Data were expressed as mean  $\pm$  standard deviations (SD). Statistical comparisons were conducted using the GraphPad Prism (version 7.0; GraphPad Software, USA) through paired two-sided Student's *t*-test for two-group or one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test for multiple comparisons. Statistical significances were defined as  $P < 0.05$ .

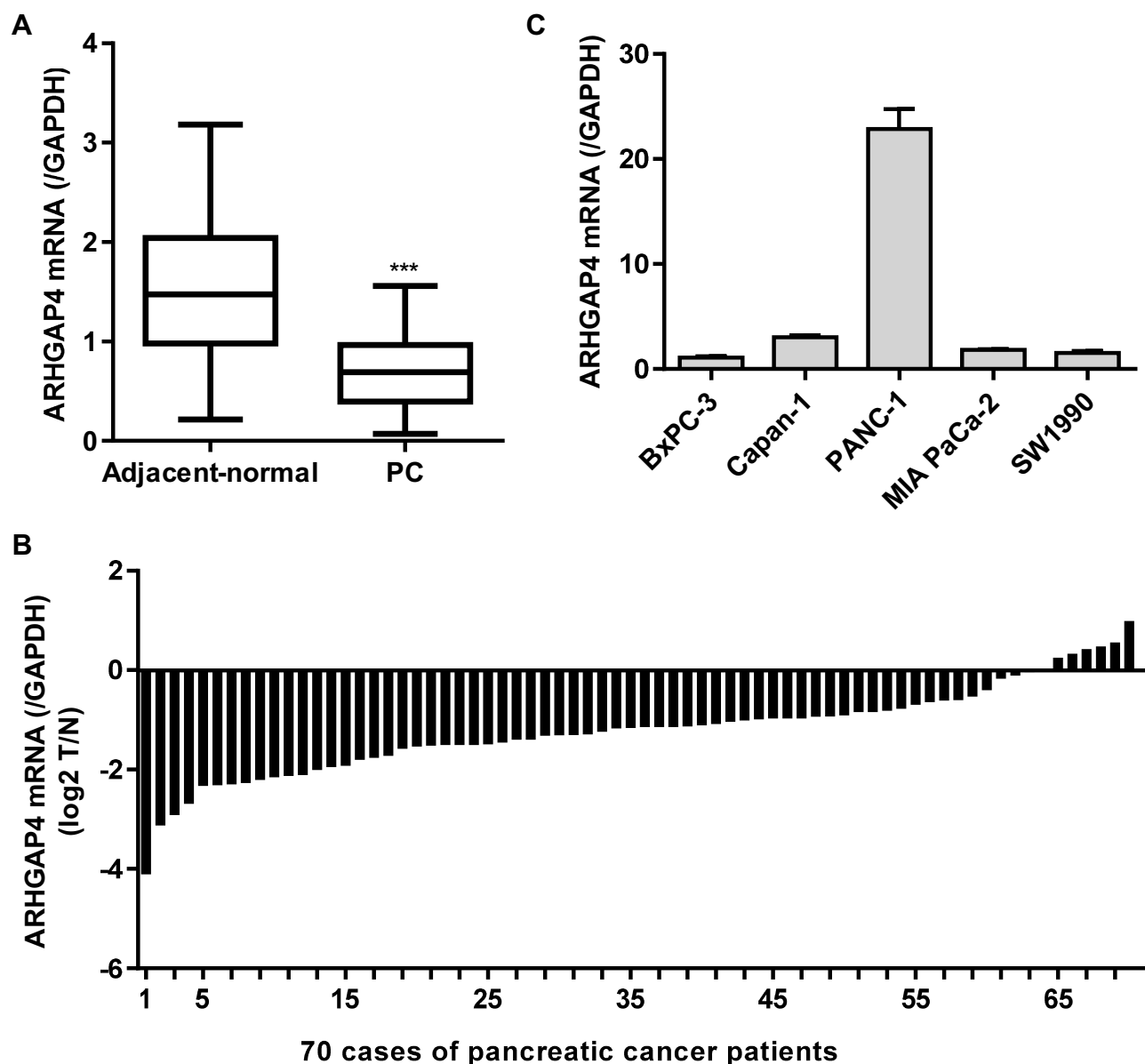
## Results

### ARHGAP4 Expression in Pancreatic Cancer

Our previous studies have shown decreased ARHGAP4 protein expression in pancreatic cancer tissues compared with the non-tumor tissues by immunohistochemistry.<sup>12,13</sup> Here, the ARHGAP4 mRNA expression was also measured by Real-time PCR analysis in pancreatic cancer tissues. ARHGAP4 mRNA levels in pancreatic cancer tissues were markedly decreased compared with the adjacent-normal tissues (Figure 1A). The ARHGAP4 mRNA levels were also shown in 70 cases of pancreatic cancer patients, in which decreased ARHGAP4 mRNA expression levels ( $\log_2 T/N$ ) were found in 91.4% (64/70) of the pancreatic cancer patients (Figure 1B). Moreover, the level of ARHGAP4 mRNA in pancreatic cancer cell lines was also measured. It demonstrated that PANC-1 cells demonstrated highest ARHGAP4 mRNA level, whereas SW1990 and BxPC-3 cells demonstrated lower ARHGAP4 mRNA levels, compared with the other pancreatic cancer cell lines (Figure 1C).

### miR-939-5p Directly Targeted ARHGAP4 in Pancreatic Cancer Cells

Studies have shown that miRNA can be used as a new biomarker for a variety of diseases through regulating gene expression by mediating gene silencing at transcriptional and post-transcriptional levels. According to TargetScan prediction, we found that miR-3150a-3p, miR-939-5p and miR-762 target ARHGAP4 (Figure 2A). To confirm the correlation of ARHGAP4 with miR-3150a-3p, miR-939-5p and miR-762 in pancreatic cancer, the corresponding inhibitor was transfected into the BxPC-3 cells which showed lowest ARHGAP4 mRNA levels

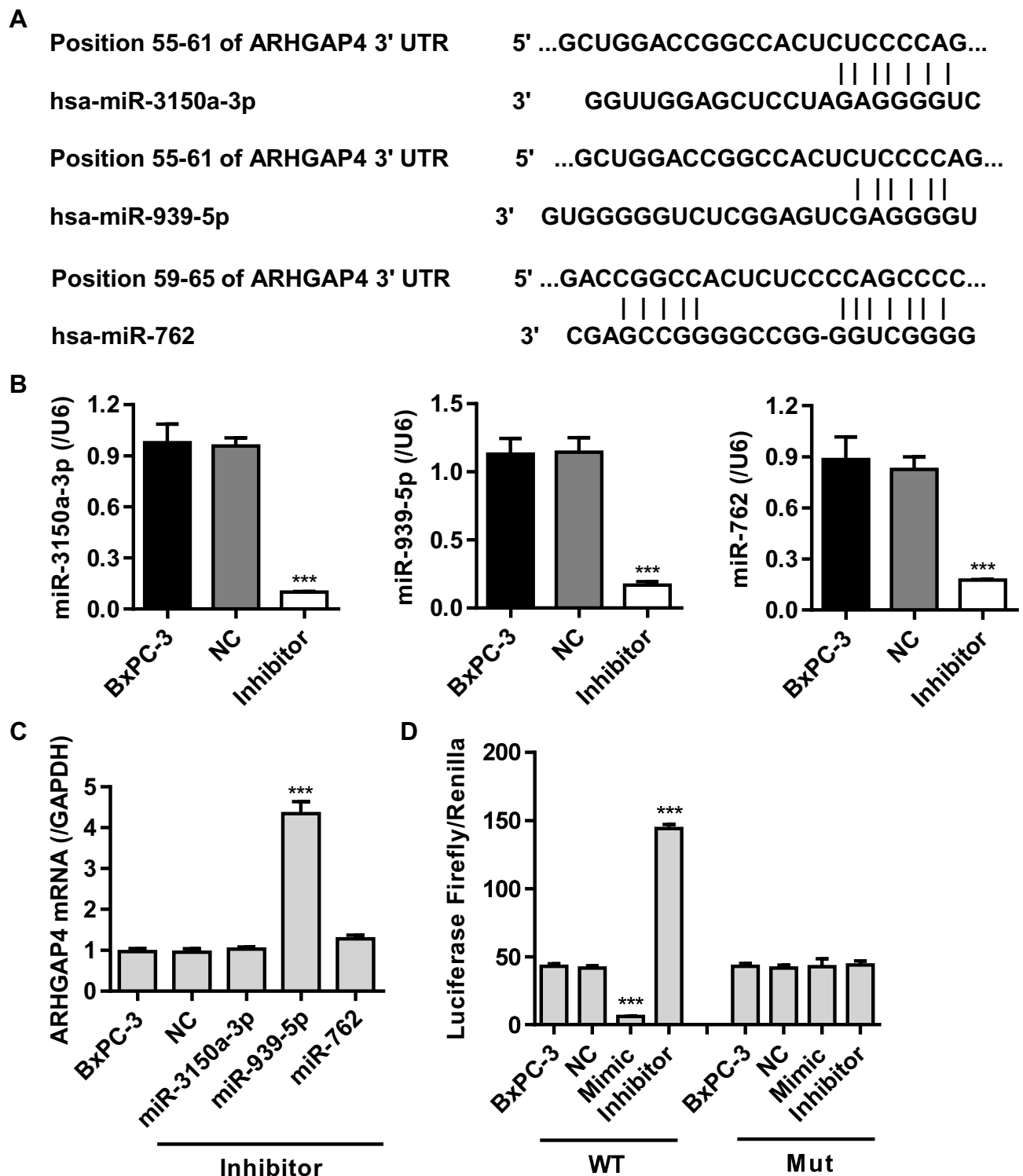


**Figure 1** ARHGAP4 expression in pancreatic cancer tissues. **(A)** ARHGAP4 mRNA levels were decreased in pancreatic cancer tissues (n=70) compared with the adjacent-normal tissues (n=70). **(B)** ARHGAP4 mRNA levels in 70 cases of pancreatic cancer patients ( $\log_2$  T/N). **(C)** ARHGAP4 mRNA expression in five pancreatic cancer cell lines, including BxPC-3, Capan-1, PANC-1, MIA PaCa-2 and SW1990. \*\*\* $P < 0.001$  compared with adjacent-normal.

**Abbreviations:** PC, pancreatic cancer; N, adjacent-normal pancreatic tissues; T, pancreatic cancer tissues.

(Figure 1B). And the expression of miR-3150a-3p, miR-939-5p, miR-762 and ARHGAP4 was measured (Figure 2B and C). Compared to miR-3150a-3p and miR-762 inhibitors, BxPC-3 cells transfected with miR-939-5p inhibitor significantly increased the expression of ARHGAP4 mRNA compared with negative control (NC). The similarly results were also found in SW1990 and PANC-1 cells which demonstrated lower and highest ARHGAP4 mRNA

expression, respectively (Supplementary Figure S1A-C). We then constructed the wild-type and mutated 3'-UTR of ARHGAP4, BxPC-3 cells were co-transfected with miR-939-5p mimic or inhibitor, and wild-type or mutated ARHGAP4. We found that up-regulation of miR-939-5p significantly decreased while down-regulation of miR-939-5p significantly increased the luciferase activity of wild-type ARHGAP4, but did not affect the luciferase



**Figure 2** miR-939-5p targeted ARHGAP4 directly at the 3'-UTR of ARHGAP4 in pancreatic cancer cells. (A) The binding sites of miR-3150a-3p, miR-939-5p and miR-762 on the 3'-UTR of ARHGAP4 were predicted by Targetscan. (B) miR-3150a-3p, miR-939-5p and miR-762 levels in BxPC-3 cells transfected with their corresponding inhibitor or negative control (NC). (C) ARHGAP4 mRNA levels in BxPC-3 cells transfected with miR-3150a-3p, miR-939-5p, or miR-762 inhibitor or NC. (D) The luciferase activity was measured by the Dual-Luciferase Reporter Assay. \*\*\* $P < 0.001$  compared with NC.

activity of mutated ARHGAP4 (Figure 2D). The similarly results were also found in SW1990 and PANC-1 cells (Supplementary Figure S1D). These data indicated that

miR-939-5p directly bound to the 3'-UTR sequence of ARHGAP4 and regulated ARHGAP4 expression in pancreatic cancer cells.

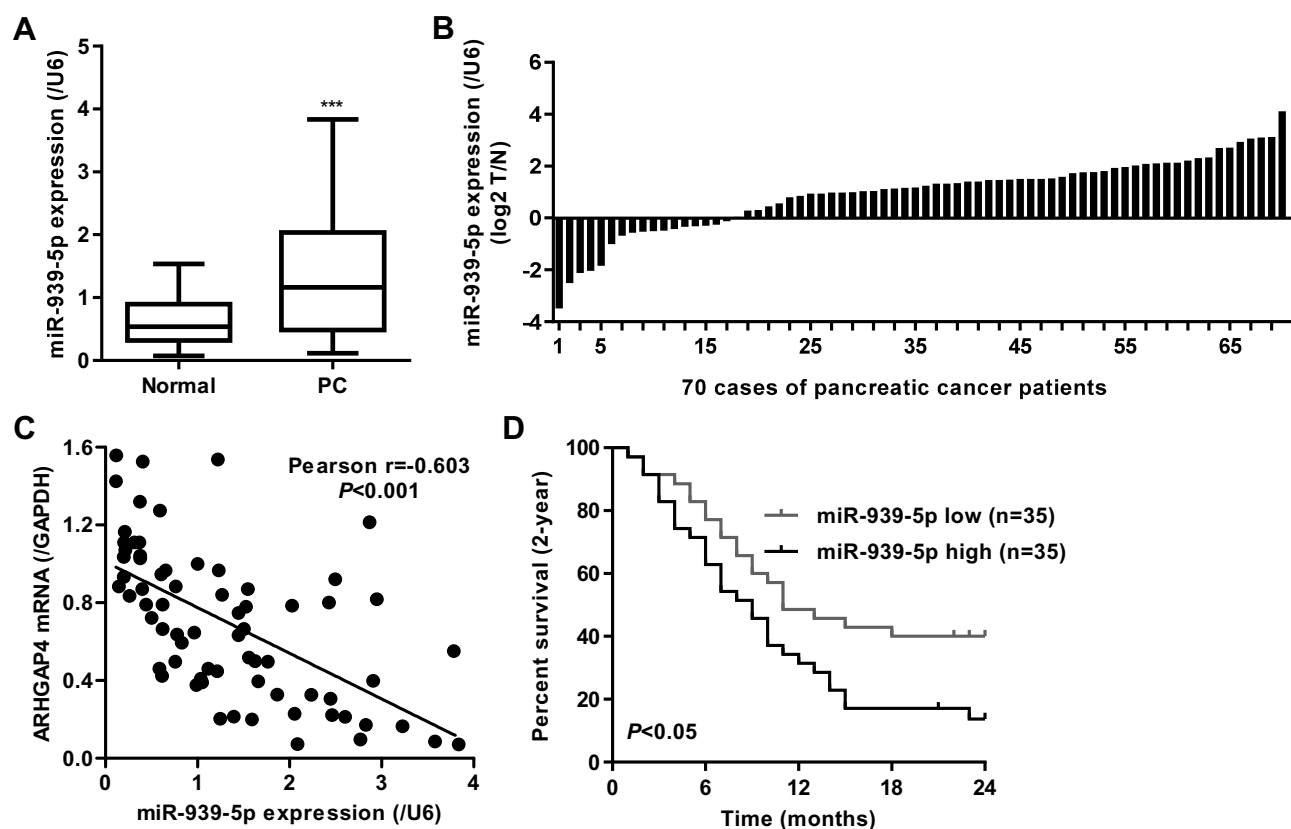
## miR-939-5p Expression in Pancreatic Cancer and Its Correlation with Prognosis

Our previous studies have shown that ARHGAP4 could regulate the invasion and migration in pancreatic cancer cells.<sup>12</sup> Therefore, we suggested that miR-939-5p may also contribute to pancreatic cancer progression. We first investigated miR-939-5p expressions by Real-time PCR analysis in pancreatic cancer. miR-939-5p levels in pancreatic cancer tissues were markedly up-regulated compared with the adjacent-normal tissues (Figure 3A). The miR-939-5p levels were also shown in 70 cases of pancreatic cancer patients, in which increased miR-939-5p levels ( $\log_2 T/N$ ) were found in 75.7% (53/70) of the pancreatic cancer patients (Figure 3B). Pearson correlation analysis demonstrated that the expression of miR-939-5p in pancreatic cancer tissues was negatively correlated with the expression of ARHGAP4 (Figure 3C). To further analyze the correlation of miR-939-5p expression with clinical features of pancreatic cancer patients, 70 pancreatic cancer patients were divided into two groups (high vs low) according to the cutoff

values (median value=0.151) measured by Real-time PCR. Chi-square test indicated that the miR-939-5p expression was correlated with the pathological stages, but not with tumor size and site, gender and age (Table 1). Kaplan-Meier analysis and log rank test showed that the overall survival time of patients with lower miR-939-5p expression were notably higher than that of patients with higher miR-939-5p expression, suggesting that increased miR-939-5p was significantly correlated with poor prognosis of pancreatic cancer patients (Figure 3D).

## miR-939-5p Down-Regulation Inhibited Invasion and Migration in Pancreatic Cancer Cells

We further assumed the role of miR-939-5p in regulating cell invasion and migration of pancreatic cancer. Firstly, the expression of miR-939-5p in pancreatic cancer cell lines was measured. As shown in Figure 4A, PANC-1 cells showed lowest miR-939-5p level, whereas SW1990 and BxPC-3 cells showed higher miR-939-5p levels, compared with the other pancreatic cancer cell lines. SW1990



**Figure 3** miR-939-5p expression in pancreatic cancer tissues. (A) miR-939-5p level was increased in pancreatic cancer tissues (n=70) compared with the adjacent-normal tissues (n=70). (B) miR-939-5p levels in 70 cases of pancreatic cancer patients ( $\log_2 T/N$ ). (C) Pearson correlation scatter plots in pancreatic tissues (n=70). (D) Kaplan-Meier curve of pancreatic patients with high or low miR-939-5p levels analyzed by Log-rank (Mantel-Cox) test. \*\*\* $P < 0.001$  compared with adjacent-normal.

**Abbreviations:** PC, pancreatic cancer; N, adjacent-normal pancreatic tissues; T, pancreatic cancer tissues.

**Table I** Correlation of the Expression of miR-939-5p with Clinicopathological Parameters in Patients with PC

Clinicopathological Parameters	miR-939-5p Expression		P-value
	Low High		
	(n=35, 50%)	(n=35, 50%)	
Gender			
Male	19 (27.1%)	21 (30.0%)	0.6291
Female	16 (22.9%)	14 (20.0%)	
Age (years)			
<60	16 (22.9%)	19 (27.1%)	0.4733
≥60	19 (27.1%)	16 (22.9%)	
Tumor size (cm)			
≤4	20 (28.6%)	16 (22.9%)	0.3388
>4	15 (21.4%)	19 (27.1%)	
Pathological stages			
I	6 (8.6%)	4 (5.7%)	0.0072
II	20 (28.6%)	8 (11.4%)	
III	6 (8.6%)	18 (25.7%)	
IV	3 (4.3%)	5 (7.1%)	
Tumor site			
Pancreatic head	14 (20.0%)	10 (14.3%)	0.5268
Pancreatic body	11 (15.7%)	15 (21.4%)	
Pancreatic tail	10 (14.3%)	10 (14.3%)	

**Note:** Differences between groups were done by the Chi-square test.

and BxPC-3 cells were therefore transfected with miR-939-5p inhibitor, and the cell viability, invasion and migration were measured. We found that miR-939-5p inhibitor markedly inhibited the viability of BxPC-3 and SW1990 cells by 14.4% and 12.9% at 48 h and by 21.9% and 19.7% at 72 h, respectively, compared with negative control (NC) (Figure 4B and C). Moreover, miR-939-5p inhibitor significantly inhibited BxPC-3 cell migration and invasion by 40.2% and 60.7% and that of SW1990 cells by 53.4% and 44.9%, respectively, compared with NC (Figure 4D and E).

### miR-939-5p Up-Regulation Promoted Invasion and Migration in Pancreatic Cancer Cells Through Targeting ARHGAP4

To investigate the mechanism of miR-939-5p affecting the invasion and migration in pancreatic cancer cells, PANC-1 cells which showed lowest miR-939-5p levels (Figure 4A) were transfected with miR-939-5p mimic and/or transduced with lentivirus expressing ARHGAP4. And the expression levels of miR-939-5p and ARHGAP4 were

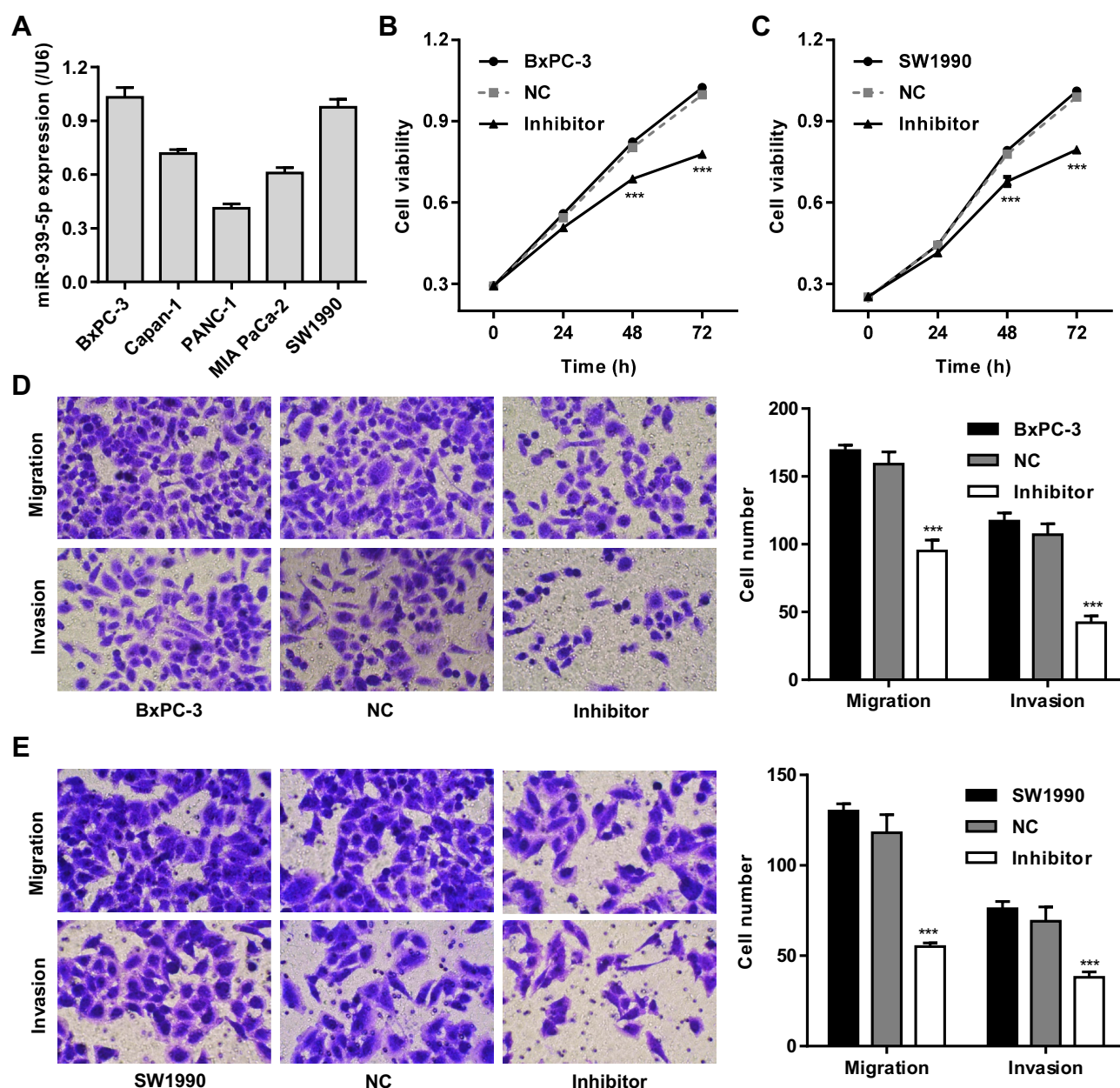
shown in Figure 5A–C. Up-regulation of miR-939-5p significantly increased the miR-939-5p levels and decreased ARHGAP4 levels in PANC-1 cells. Moreover, miR-939-5p mimic markedly increased while ARHGAP4 overexpression markedly suppressed the PANC-1 cell viability, invasion and migration (Figure 5D–F). Importantly, ARHGAP4 overexpression also inhibited the malignant phenotype of PANC-1 cells induced by miR-939-5p mimic, suggesting that miR-939-5p up-regulation promoted invasion and migration of pancreatic cancer cells through targeting ARHGAP4 (Figure 5D–F).

### Discussion

In this study, we identified ARHGAP4 as a down-regulated gene while miR-939-5p as an up-regulated miRNA in pancreatic cancer based on Real-time PCR analysis. Meanwhile, we demonstrated a critical role of miR-939-5p in the progression of pancreatic cancer and proposed an ARHGAP4-mediated mechanism.

ARHGAP4 is a member of the RhoGAPs family that involves in the cancer progression by stimulating GTP hydrolysis to inactivate small GTPases. p190 RhoGAP reduced cell invasion and metastasis of pancreatic cancer.<sup>25</sup> In addition to p190 RhoGAP, only ARHGAP4 has been found to correlate with pathological stages, vascular invasion and prognosis of pancreatic cancer patients and regulate Warburg effect as well as cell invasion and migration.<sup>12,13</sup> Our previous study demonstrated decreased ARHGAP4 protein levels in pancreatic cancer tissues based on the immunohistochemical staining on tissue microarrays. Here, the decreased ARHGAP4 mRNA levels in pancreatic cancer tissues were also observed compared with the adjacent-normal tissues. However, ARHGAP4 was up-regulated in colorectal cancer tissues and cell lines, and its overexpression was correlated with T stage, N stage, clinical stage, metastasis and poor prognosis,<sup>26</sup> which were inconsistent with our present and previous studies.<sup>12</sup> These data suggest that the expression and clinical correlation of ARHGAP4 vary in different cancers.

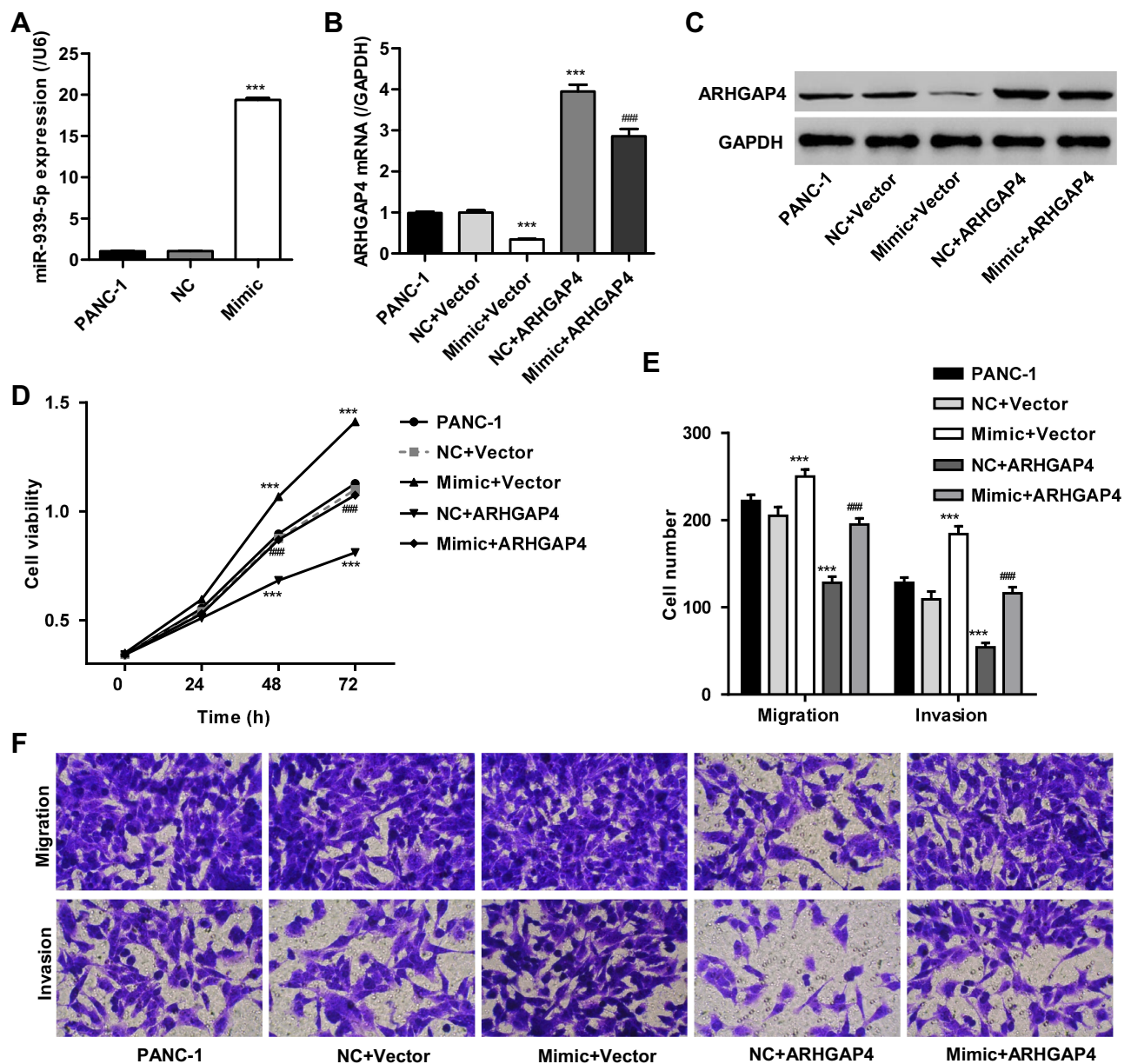
Recently, increasing evidences have demonstrated an important role of miRNAs in the development and progression of pancreatic cancer. For example, miR-196a-5p promoted the invasion, migration and EMT markers in MIA PaCa-2 cells.<sup>27</sup> Knockdown of miR-21 inhibited the malignant phenotype of PANC-1 cells, at least partially by regulating the VHL/HIF-1 $\alpha$ /VEGF pathway and the expression of MMP-2 and MMP-9, and tumor growth in vivo.<sup>28</sup> miR-129-5p suppressed the progression of pancreatic cancer by targeting PBX3, leading to the



**Figure 4** miR-939-5p down-regulation inhibited pancreatic cancer cell invasion and migration. **(A)** ARHGAP4 mRNA expression in five pancreatic cancer cell lines, including BxPC-3, Capan-1, PANC-1, MIA PaCa-2 and SW1990. After BxPC-3 **(B, D)** and SW1990 **(C, E)** cells were transfected with miR-939-5p inhibitor, the cell viability, invasion and migration were decreased than those transfected with negative control (NC). \*\*\* $P < 0.001$  compared with NC.

reduction of proliferation, migration and invasion, and induction of apoptosis of AsPC-1 and BxPC-3 cells.<sup>29</sup> Knockdown of miR-155 increased apoptosis, inhibited proliferation and JAK2/STAT3 pathway in SW1990 cells by targeting SOCS3.<sup>30</sup> Moreover, many ARHGAP family genes have also been found to play important role in the involvement of miRNAs in tumorigenesis. miR-200b suppressed the metastasis in triple negative breast cancer by targeting ARHGAP18.<sup>31</sup> miR-3174 inhibited mitochondria-dependent apoptosis and autophagic cell death in gastric cancer by targeting

ARHGAP10.<sup>32</sup> miR-744 promoted the metastasis and progression of nasopharyngeal carcinoma by targeting ARHGAP5.<sup>33</sup> These data suggest that the regulation of ARHGAP4 by miRNAs may also exist in pancreatic cancer. Indeed, miR-939-5p directly targeted ARHGAP4 in pancreatic cancer cells and was negatively correlated with ARHGAP4 in pancreatic cancer tissues and cell lines. miR-939 expression in ovarian and triple-negative breast cancers was increased and associated with worse disease-free survival of patients with triple-negative breast cancer,<sup>34,35</sup> which were in line with our



**Figure 5** miR-939-5p up-regulation promoted pancreatic cancer cell invasion and migration through targeting ARHGAP4. After PANC-1 cells were transfected with miR-939-5p mimic and/or transduced with lentivirus expressing ARHGAP4, miR-939-5p levels (**A**), ARHGAP4 mRNA levels (**B, C**) and cell viability (**D**), migration, and invasion (**E, F**) were measured. \*\*\* $P < 0.001$  compared with NC+blank lentivirus vector (Vector). #### $P < 0.001$  compared with mimic+Vector.

findings. In addition to survival, pathological stages were also correlated with miR-939-5p expression in pancreatic cancer patients. Similarly, miR-939-3p expression was up-regulated in lung cancer and associated with TNM stage, metastasis, and poor prognosis.<sup>22</sup> However, miR-939-5p expression in gastric and bladder cancer was decreased.<sup>21,36</sup> These data suggest that the expression and clinical correlation of miR-939 vary in different cancers.

miR-939-5p promoted cell proliferation and repressed cell apoptosis of tongue squamous cell carcinoma,<sup>20</sup> and miR-939 also regulated epithelial-mesenchymal transition

in epithelial ovarian cancer, implicating in tumorigenesis, tumor progression, metastasis and relapse.<sup>24</sup> miR-939-3p promoted cell invasion, migration and proliferation of lung cancer.<sup>22</sup> However, miR-939-5p suppressed invasion and migration of colorectal cancer cells through directly targeting LIMK2,<sup>23</sup> and miR-939 overexpression inhibited gastric cancer cell metastasis, proliferation and chemosensitivity through directly targeting SLC34A2.<sup>21</sup> Considering the expression of miR-939-5p is increased in pancreatic cancer tissues, we investigated whether miR-939-5p acts as an oncogene role in cell proliferation, migration, and invasion

of pancreatic cancer. The results demonstrated that miR-939-5p down-regulation inhibited cell viability, migration and invasion in pancreatic cancer, while its up-regulation showed an inverse effect by targeting ARHGAP4. Previous studies showed that ARHGAP4 inhibited invasion and migration in pancreatic cancer cells through inhibiting  $\beta$ -catenin activation<sup>12</sup> and regulated Warburg effect through HIF-1 $\alpha$  and mTOR signaling pathways.<sup>13</sup>  $\beta$ -catenin as well as its target C-myc expression was increased in ovarian cancer cells with miR-939 up-regulation, but decreased with miR-939 down-regulation.<sup>34</sup> C-myc has also been reported to regulate aerobic glycolysis and promote tumor progression in pancreatic cancer.<sup>37</sup> These results suggest that miR-939-5p may regulate Warburg effect and tumor progression in pancreatic cancer via the ARHGAP4/ $\beta$ -catenin/C-myc axis. HIF-1 $\alpha$  and mTOR not only involved in the aerobic glycolysis but also regulated tumor growth and metastasis.<sup>38</sup> However, further investigations on the functional role and mechanism by which miR-939-5p/ARHGAP4 regulates aerobic glycolysis and tumorigenesis are urgently needed, and whether miR-939-5p/ARHGAP4 regulates tumorigenicity through control of glucose metabolism also need further investigation.

## Conclusion

In conclusion, our study illustrated that miR-939-5p was increased in pancreatic cancer tissues and associated with pathological stages and prognosis. miR-939-5p overexpression promoted pancreatic cancer cell invasion and migration by down-regulating ARHGAP4. miR-939-5p/ARHGAP4 axis might thus serve as a novel biomarker to predict the progression and prognosis of pancreatic cancer, as well as a potent therapeutic target for pancreatic cancer treatment.

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## Disclosure

The authors declare that they have no competing interests.

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