Circular RNA Circ_0001017 Acts as ceRNA Adsorbing miR-197-3p to Regulate PNLIP Signaling and Affect the Proliferation, Apoptosis and Glycolysis of Pancreatic Ductal Adenocarcinoma Cells

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Abstract: To investigate the effects of circ_0001017 as a molecular sponge adsorbing miR-197-3p to regulate PNLIP signaling on the proliferation, apoptosis and glycolysis of pancreatic ductal adenocarcinoma (PDAC) cells, the expressions of circ_0001017, miR-197-3p and PNLIP in PDAC tissues and cells were detected. The dual-luciferase reporter assay and RNA-binding protein immunoprecipitation assay were used to verify the targeting relationship between miR-197-3p and circ_0001017 or PNLIP. The expressions of circ_0001017/miR-197-3p/PNLIP in cells were interfered and they were grouped. The proliferation activity and apoptosis of the cells were measured by methyl thiazolyl tetrazolium (MTT) assay and flow cytometry, respectively. Glucose, lactate, and ATP detection kits were used to assess glucose consumption, lactate production, and ATP levels. Compared with paracancerous tissues, miR-197-3p expression in PDAC tissues was significantly up-regulated, while circ_0001017 and PNLIP expressions were decreased (all P<0.05). Compared with negative control group, cell proliferation and glycolysis were inhibited, and apoptosis was induced after circ_0001017 overexpression (P<0.05). Circ_0001017 knockdown promoted PDAC cell proliferation and aerobic glycolysis, and inhibited cell apoptosis, which was partially rescued by miR-197-3p inhibitor and PNLIP knockdown (all P<0.05). miR-197-3p overexpression could promote proliferation and glycolysis of PDAC cells and inhibit cell apoptosis, which could be partially rescued by PNLIP overexpression (P<0.05). Circ_0001017 regulated the miR-197-3p/PNLIP axis and played a protective role in PDAC progression. It could inhibit the proliferation of PDAC cells, induce the apoptosis of tumor cells, and reduce the aerobic glycolysis capacity of the cells.

Key words: Circ_0001017, Pancreatic ductal adenocarcinoma, Proliferation, Apoptosis, Aerobic glycolysis

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a kind of digestive system malignant tumor with strong invasiveness, high incidence and fatal mortality, and its characteristics include difficulty in early diagnosis and difficulty in controlling the late pathological development, making it one of the most difficult cured malignant tumors in human beings1. For oncologists, targeted molecular markers with good therapeutic potential, strong specificity and comprehensive control have become an important breakthrough and entry point in the fight against PDAC.

Unlike linear RNA molecules, circRNA has a unique closed loop structure without a 5'-terminated cap structure and a 3'-terminated polyadenylate tail2. Due to its special circular structure, it can avoid the degradation of RNA enzyme3. Therefore, circRNA exists abundantly and stably in mammals. In recent years, more and more experiments have shown that circRNA is closely related to the diagnosis and prognosis of PDAC4. Circ_0050102 is abnormally highly expressed in PDAC tissues, which can target miR-1182/NPSR1 to promote the proliferation, migration and invasion of PDAC cells5. In addition, blocking circ_0013912 can partially inhibit the growth, migration and invasion of PDAC cells by sponging miR-7-5p, thus playing a good protective role in PDAC patients6. Circ_0001017 has abnormal expression in gastrointestinal tumors, and circ_0001017 is suspected to play a certain role in PDAC development7.

CircRNA can act as a molecular sponge to isolate miRNA by binding to microRNAs (miRNAs)8. miRNA is a kind of non-coding RNA with a length of 18-22 nucleotides. It can degrade mRNA to perform its function and help suppress gene expression at the post-transcriptional level9. MiR-197 can induce the transformation of PDAC epithelial cells into mesenchymal cells by targeting p120 catenin, which has a detrimental effect on the prognosis of PDAC10. PNLIP is a gene encoding pancrelipase, a key enzyme in the process of fat digestion, and is only expressed in the pancreas11. Compared with paracancerous tissues, PNLIP expression in PDAC was significantly reduced, leading to a decrease in free fatty acids. More importantly, PNLIP gene can significantly induce the expression of TRAIL in PDAC cells, trigger the apoptosis
of PDAC cells, and inhibit cell proliferation. In addition, PNLIP gene is also a risk gene for the development of chronic pancreatitis\(^{13, 14}\). However, there have been no studies on the application of PNLIP regulation of miRNA in PDAC, and whether ceRNA regulates PNLIP at the post-transcriptional level.

The aim of this study was to explore the role of circ_0001017 in PDAC and its mechanism. The results of this study revealed the new mechanism of circ_0001017/miR-197-3p/PNLIP axis in PDAC development, providing a theoretical basis for new targeted molecular therapy.

Materials and Methods

**Ethics statement**

This study was approved by the Ethics Committee of West China Hospital, Sichuan University (approval number: 2020 (652)) and was performed in accordance with the Declaration of Helsinki. All patients received written informed consent, and they or their families signed the informed consent. All samples collected were numbered and treated with confidentiality.

**PDAC tissues collection**

A total of 35 patients with PDAC admitted to our hospital from January 2018 to December 2019 were randomly selected as study subjects. All patients were diagnosed with PDAC by imaging examination. The 35 cases of PDAC tissues and corresponding paracancerous tissue samples were surgically resected. The edge of paracancerous tissue was more than 3 cm away from the tumor tissue. Among the selected PDAC cases, 25 were male and 10 were female, aged from 25 to 65 years old, with an average age of (47.5±15.2) years old.

**Cell culture and transfection**

The human PDAC cells and normal human pancreatic ductal epithelial (HPDE) cells verified by STR were purchased from American Type Culture Collection, ATCC, Manassas, VA, USA. The cells were frozen in RPMI 1640 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) medium containing 10% fetal bovine serum (FBS) and 5% dimethyl sulfoxide (DMSO) according to the instructions, and stored in liquid nitrogen. They were cultured in an incubator (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C and 5% CO\(_2\). The cells in the logarithmic growth phase were digested with trypsin and inoculated into a 24-well plate (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at a volume ratio of 1:1, and then fixed at 4°C for 5-10 min. The excess paraformaldehyde was removed by centrifugation at 12,000 r/min for 3 min, and 1 ml 0.01 mol/l phosphate buffered saline (PBS) was added to wash twice and the supernatant was discarded. The solution was smeared on the slide and then heat fixed and gradient dehydration was used to prepare the slide. Then in situ hybridization of probe was carried out, firstly, the sealed hybridization box was wetted with hybridization solution, 24 μl of hybridization solution and 1 μl of probe were added to the sample, and the hybridization reaction was carried out at 46°C for 5 h. After taking out, the slide was immediately put into the preheated container filled with PBS, DAPI (4'-6-diamidino-2-phenylindole) dyeing solution was added in darkness for 10 min, the slide was washed again with PBS solution for 15-20 min, the excess PBS was removed with clean water, and then the slide was dried at room temperature. Next the slide was sealed in the darkness. Finally, the cells were observed and photographed under a fluorescence microscope (IXplore; Olympus Corporation, Tokyo, Japan).

**RNase R digestion experiment**

Total RNA (2 μg) extracted from tissues and cells was incubated at 37°C for 20 min, and 3 U/μg RNase R was added. RNA was purified by RNeasy MinElute purification kit (Qiagen Inc., Valencia, CA, USA) and detected by quantitative real-time polymerase chain reaction (qRT-PCR).

**Dual luciferase reporter analysis**

First, according to the bioinformatics online prediction website, it was found that circ_0001017/PNLIP and miR-197-3p had targeted binding sites, and the corresponding binding site sequences were amplified respectively and constructed on the reporter gene vector (Promega Inc., Madison, WI, USA), denoted as pmirGLO-PNLIP-3’UTR-WT and pmirGLO-circ_0001017-WT luciferase reporter plasmid. At the same time, the disordered binding site sequences were constructed on the reporter gene vector, denoted as pmirGLO-PNLIP-3’UTR-MUT and pmirGLO-circ_0001017-MUT reporter plasmid. The 1*10\(^5\) PDAC cells were inoculated into a 24-well plate (Thermo Fisher Scientific, Inc., Waltham, MA, USA) to ensure that the cell confluence reached 70%-80% during transfection. The targeting relationship between miR-197-3p and PNLIP was verified by co-transfection of pmirGLO-PNLIP-3’UTR-WT / pmirGLO-PNLIP-3’UTR-MUT, miR-197-3p mimics and control into PDAC cells respectively by Lip3000 liposome transfection method. The pmirGLO-circ_0001017-WT / pmirGLO-circ_0001017-MUT, miR-197-3p mimics and control were co-transfected into PDAC cells to verify the targeting relationship between circ_0001017 and miR-197-3p. After continuous transfection for 8h, the culture was continued in an incubator with a volume fraction of 5% CO\(_2\) at 37°C, and the complete medium was changed 6 h later. The samples were collected 48 h later. The activity of firefly luciferase in transfected cells was detected by microplate...
Quantitative real-time polymerase chain reaction (qRT-PCR)

The tissue and cell samples were ground into powder with liquid nitrogen, and the total DNA was extracted by adding the TRIZol Reagent RNA (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) extraction reagent. The concentration and purity of RNA were detected by ultraviolet absorption. Reverse transcription kit (Takara Biotechnology, Inc., Shiga, Japan) was used to obtain cDNA by reverse transcription using total RNA as template according to the instructions. The SYBR Premix EX Taq kit (Takara Biotechnology, Inc., Shiga, Japan) was used to complete the real-time fluorescence quantitative PCR test. Multiple reference genes were selected in this study, including GAPDH, ACTB and TFRC. The target gene and reference gene were both amplified for subsequent experiments. Circ_0001017 and PNLIP were standardized with GAPDH, ACTB and TFRC. The target gene and reference gene were both amplified for subsequent experiments. Circ_0001017 and PNLIP were standardized with GAPDH, ACTB and TFRC as internal references, U6 as the control of miR-197-3p, and 2^(-ΔΔCt) was used to calculate the relative expression. All the experiments were repeated three times. The primer sequences were shown in Table 1.

Western blot

Western blot was used to detect the expression level of PNLIP in the cells. The protein was first lysed with RIPA lysis buffer (Solarbio Science & Technology Co., Ltd., Beijing, China) and centrifuged at 10,000 g (Centrifuge 5702R; Eppendorf, Hamburg, Germany) at 4°C for 10 min. The supernatant was collected, and the protein concentration was determined with BCA kit (Sigma-Aldrich, Inc., St. Louis, MO, USA). The sample proteins were separated and transferred to PVDF membrane (Millipore; Thermo Fisher Scientific Inc., Cambridge, MA, USA) by 8%-10% sodium dodecyl sulphate-polyacrylamidegel electrophoresis. The membrane was sealed in TBS containing 5% skimmed milk powder for 1 h at room temperature. A primary antibody, PNLIP (1/20,000, Abcam Biotechnology Inc., Cambridge, UK), diluted with TBST (TBST containing 0.1% Tween-20), was added and incubated overnight in a refrigerator at 4°C. On the second day, the cells were washed with TBST and then incubated with the corresponding second antibody (Abcam Biotechnology Inc., Cambridge, UK) at room temperature for 1 h. Then, the color developing solution was prepared according to the instructions of enhanced chemiluminescence (ECL, Millipore; Thermo Fisher Scientific Inc., Waltham, MA, USA) at the ratio of 1:1. The color developing solution was added to the membrane uniformly, and the extracted protein was photographed under gel imager (E-Gel Imager, Thermo Fisher Scientific, Inc., Carlsbad, CA, USA). Finally, the images were analyzed and processed with Image J software (1.39f, NIH-Bethesda Corporation, MD, USA), and GAPDH was used as the control.

MTT assay

Cell proliferation activity was determined by MTT assay. The PDAC cells in the logarithmic growth phase of each group after transfection were collected. After Trypsin (Sigma-Aldrich, Inc., St. Louis, MO, USA) digestion, cell concentration was adjusted to 1×10^6 cells/well and inoculated into 96-well plates. The cells were cultured in 5% CO₂ incubator at 37°C. MTT assay was carried out at 5 time points of 0 h, 24 h, 48 h, 72 h and 92 h, respectively. 20 μl MTT (5 mg/ml, prepared by PBS, Sigma-Aldrich, Inc., St. Louis, MO, USA) was added into each well, and then cultured in the incubator for 4 h. The supernatant was absorbed and discarded, and 150 μl DMSO (Sigma-Aldrich, Inc., St. Louis, MO, USA) was added to each well to dissolve the crystals. After 10

Figure 1. Expression of circ_0001017 was reduced in PDAC and could be used as an effective diagnostic indicator for PDAC. A: qRT-PCR detection of circ_0001017 expression in PDAC tissues (n=35) and paracancerous tissues (n=35); B: diagnostic value curve (AUC=0.8784, P<0.0001); C: qRT-PCR detection of circ_0001017 expression in PANC-1 cell line and normal HPDE cell line. Compared with Normal, ****P<0.0001; compared with HPDE cells, **P<0.01. PDAC: pancreatic ductal adenocarcinoma; qRT-PCR: quantitative real-time polymerase chain reaction; AUC: area under the curve; HPDE: human pancreatic ductal epithelial.

| Name sequences (5’-3’) | Name sequences (5’-3’) |
|------------------------|------------------------|
| forward                | reverse                |
| circ_0001017           | GTTCTGAATTGTAGTCT      |
| miR-197-3p             | TTGGCTCAATCCGGAATTG   |
| PNLIP                  | CTTAAACCATTGACGTAG    |
| GAPDH                  | AGCTCTCCAGTAGACTAC    |
| ACTB                   | GAGCTGCAACATTGAGCA    |
| TFRC                   | ATTCCTCGATCCATGCACA   |
| U6                     | GCCATGCGCATAAGCACAT   |
|                        | Reverse ACUGAGAUGUCAUGCUCGGAU |

Note: qRT-PCR: quantitative real-time polymerase chain reaction.
min of incubation, the solution was shaken at room temperature for 5 min. The optical density (OD) value at the wavelength of 490 nm was measured by a microplate reader.

Flow cytometry analysis

The PDAC cells of different groups were digested with 0.25% pancreatic enzyme (Solarbio Science & Technology Co., Ltd., Beijing, China) without EDTA to produce single-cell suspension, centrifuged at 1,000 r/min for 5 min, and then the supernatant was absorbed and discarded. Then the suspension was washed with PBS. Double staining was performed by Annexin V/PI double-staining cell apoptosis detection kit (BestBio Science Co., Ltd., Shanghai, China). Within 1 h, the prepared samples were placed in flow cytometry (Epics-XL, Beckman Coulter, Chaska, MN, USA) to detect apoptosis. Each experiment was carried out three times.

Determination of glucose, lactate and ATP

To estimate glucose utilization rate, lactate concentration and ATP production in PDAC cells, glucose colorimetric assay kit (Cayman chemicals Inc., Ann Arbor, MI, USA), ATP content determination kit (Sigma-Aldrich, Inc., St. Louis, MO, USA) and lactate assay kit (Sigma-Aldrich, Inc., St. Louis, MO, USA) were used according to the manufacturer’s instructions. The ATP assay kit used creatine kinase, which catalyzed the reaction between creatine and ATP to produce creatine phosphate. Phosphomolybdic acid colorimetric method could be used to detect the content of phosphocreatine at 700 nm, so as to reflect the con-
tent of ATP. Under the action of lactate dehydrogenase, lactate generated pyruvate, while reducing NAD+ to produce NADH and H+, and H+ was passed to PMS to generate PMSH2 to reduce MTT to produce a purple substance with a characteristic absorption peak at 570 nm, so as to calculate the lactate content. The kit was stored at -20°C for subsequent experiment.

Statistical analysis

SPSS 22.0 statistical software (IBM Corporation, Armonk, NY, USA) was used for statistical analyses of the data in this study. All measurement data with normal distribution and homogeneous variance were expressed in the form of mean ± standard deviation (x̅ ± sd). Independent sample t test was used for comparison between two groups, One-Way ANOVA was used for comparison among multiple groups, and post-hoc test was used for pairwise comparison within groups. The enumeration data were expressed as percentage, using Pearson chi-square test. The diagnostic value of circ_0001017 for PDAC was evaluated by receiver operating characteristic (ROC) curve. P<0.05 was considered statistically significant.

Results

**Circ_0001017 expression was reduced in PDAC tissues and cells**

The qRT-PCR method was used to detect the expression of circ_0001017 in PDAC tissues. Compared with paracancerous tissues (n=35), expression level of circ_0001017 was lower in the tumor tissues (Fig. 1A, P<0.0001). Besides, circ_0001017 was significantly down-regulated in PANC-1 cell line compared with that in HPDE cell line (Fig. 1C, both P<0.01). The results showed that circ_0001017 was low expressed in PDAC tissues and cells. Meanwhile, ROC curve analysis found that the area under the ROC curve of PDAC was 0.8784.
when the expression of circ_0001017 in the paracancerous tissues was used as a control (Fig. 1B, P<0.0001). Henceforth, circ_0001017 could be used as a useful molecular diagnostic biomarker for PDAC.

**Circ_0001017 inhibited the proliferation and aerobic glycolysis of PDAC cells and induced apoptosis**

The expression of circ_0001017 in each group was detected by qRT-PCR to verify the transfection efficiency, and the results showed that circ_0001017 transfection was successful (Fig. 2A, P<0.05). The MTT assay results showed that compared with control group, circ_0001017 could inhibit the proliferation activity of PDAC cells (Fig. 2B, P<0.05), while there was no significant difference in the proliferation activity between NC group and blank group (Fig. 2B, both P>0.05). The results of flow cytometry showed that the apoptosis rate of PDAC cells was significantly increased after circ_0001017 overexpression (Fig. 2C, P<0.05). Glucose, lactate and ATP detection kits were used to detect the metabolism of glycolysis. The results showed that, compared with control group, circ_0001017 overexpression had significant inhibitory effects on glucose uptake, lactate concentration and ATP production (Fig. 2D, P<0.05).

**Circ_0001017 was mainly located in cytoplasm and could not be degraded by RNase R enzyme**

Circ_0001017 was non-degradable by RNase R, and circ_0001017 expression was not significantly decreased, indicating circ_0001017 has a circular structure (Fig. 3A, P<0.05). In situ hybridization detection, DAPI was used to label the nucleus and fluorescent in situ was used to label circ_0001017 molecules. After image combination, circ_0001017 was clearly shown to be expressed in both cytoplasm and nucleus, but was mainly located in the cytoplasm (Fig. 3B).
MiR-197-3p was up-regulated in PDAC tissues and cells and had a targeted binding relationship with circ_0001017

Compared with paracancerous tissues (n=35), expression level of miR-197-3p was higher in tumor tissues (Fig. 4A, P<0.05). In addition, compared with HPDE cell line, miR-197-3p was significantly up-regulated in PANC-1 cell line (Fig. 4B, P<0.05). Correlation analysis showed that circ_0001017 level in PDAC tissue was negatively correlated with miR-197-3p level (Fig. 4C, r=-0.4136, P=0.0135). The bioinformatics tool circinteractome (https://circinteractome.nia.nih.gov) showed that there was a specific binding site between circ_0001017 and miR-197-3p (Fig. 4D). In order to confirm the relationship between them, the dual luciferase reporter assay confirmed that transfection of miR-197-3p significantly reduced the luciferase activity of circ_0001017-WT (Fig. 4E, P<0.05), but did not change the luciferase activity of circ_0001017-MUT (Fig. 4F).
activity of transfection with circ_0001017-MUT (P>0.05).

**Effect of si-circ_0001017 on PDAC cells could be partially rescued by miR-197-3p inhibitor**

In order to further verify our hypothesis, qRT-PCR was used to detect the expression level of miR-197-3p in each group. The results showed that circ_0001017 knock-down significantly up-regulated the expression of miR-197-3p, and its effect on upregulation of miR-197-3p could be partially rescued by miR-197-3p inhibitor (Fig. 5A, all P<0.05). Besides, this study found that si-circ_0001017 promoted the proliferation activity of PANC-1 cells (Fig. 5B, P<0.05), inhibited cell apoptosis (Fig. 5C, P<0.05), and increased glucose uptake, lactate concentration and ATP production (Fig. 5D, P<0.05). Importantly, the promotion of PANC-1 cell growth and metabolism mediated by si-circ_0001017 could be partially rescued by miR-1197-3p inhibitor (Fig. 5B-D, all P<0.05).

**PNLIP was the target of miR-197-3p and was expressed at a low level in PDAC tissues and cells**

GEPIA website was used to predict the expression of PNLIP in pancreatic adenocarcinoma tissues, and the results showed that the expression of PNLIP was significantly decreased in tumor tissues as compared with in nontumor tissues. So, we further measured the expression of PNLIP in tissues and tumor cells in patients. The results showed that in PDAC tissues and cells, the expression of PNLIP was significantly up-regulated compared with that in paracancerous tissues and normal cells (Fig. 6A-C, all P<0.05). Spearman correlation analysis indicated that the mRNA level of PNLIP was negatively correlated with miR-197-3p level in PDAC tissues (Fig. 6D, r=-0.3522, P=0.0380), while positively correlated with circ_0001017 level (r=0.4605, P=0.0054). Targetscan, an online bioinformatics prediction website, proved that there were multiple binding sites between PNLIP and miR-197-3p (Fig. 6E). The dual luciferase reporter assay further verified the targeting relationship between the two (Fig. 6F). The mRNA level of transfected cells was detected by qRT-PCR. After transfection of miR-197-3p mimics, the expression of PNLIP was significantly down-regulated, while overexpression of circ_0001017 could partially reverse this trend (Fig. 6G, P<0.05). Western blot also showed that compared with miR-NC group, the protein content of PNLIP was significantly higher than that of miR-197-3p mimc+pcDNA group (Fig. 6H-I, P<0.05), suggesting that circ_0001017 overexpression could partially rescue miR-197-3p’s inhibitory effect on PNLIP protein.
PNLIP overexpression could partially rescue the effects of miR-197-3p on the proliferation, apoptosis and aerobic glycolysis of PDAC cells

Compared with miR-NC group, overexpression of miR-197-3p could promote the proliferation activity of PANC-1 cells (Fig. 7A, P<0.05), significantly weaken their apoptotic ability (Fig. 7B, P<0.05), and increase glucose uptake, lactate concentration and ATP production (Fig. 7C, P<0.05). In addition, the miR-197-3p-mediated effect on PANC-1 cells could be partially weakened by overexpression of PNLIP (Fig. 7A-C, all P<0.05). So far, this study clarified that miR-197-3p could regulate the expression of PNLIP to regulate the biological behavior of PDAC cells.

PNLIP knockdown could partially rescue the effects of circ_0001017 overexpression on PDAC cell proliferation, apoptosis and glycolysis

Compared with circ-NC group, the cell proliferation activity of circ_0001017 group was induced, cell apoptosis was reduced, and glucose uptake, lactate concentration and ATP production were all increased (all P<0.05). There was no significant difference in indicators between circ_0001017 + si-NC group and circ_0001017 group (all P>0.05). Compared with circ_0001017 + si-NC group, the cell proliferation and aerobic glycolysis were inhibited in circ_0001017 + si-PNLIP group, and apoptosis was induced (both P<0.05). It was suggested that PNLIP knockdown could partially rescue the effects of circ_0001017 overexpression on cell proliferation, apoptosis and aerobic glycolysis. See Fig. 8.

Discussion

CircRNA, as a new RNA, plays an important role in various cancers. It can act as a natural miRNA sponge by creating a post-transcriptional horizontal regulatory network and inhibit the function of other RNA through endogenous competition on miRNA binding sites[15]. CircRNA circ_0001017 can inhibit gastric cancer cells' proliferation and metastasis by adsorbing miR-197, thereby controlling the development of gastric cancer[16]. Circ_0001017 can be used as a potential prognostic tumor biomarker in patients with gastric cancer, and its theory as a tumor suppressor in gastrointestinal malignant tumors is relatively rich[17, 18]. Therefore, we combined the ceRNA network of circ_0001017 with PDAC to make an assumption about the protective effect of circ_0001017 in PDAC. First, we confirmed that circ_0001017 was down-regulated in PDAC tissues through qRT-PCR experiment, and its differential expression in PDAC suggested that it had diagnostic or predictive value. Moreover, its biological function in PDAC cells was verified by MTT assay and apoptosis assay, which could significantly inhibit the proliferation activity of PDAC cells and effectively induce the apoptosis of PDAC cells.

Whether under aerobic or anaerobic conditions, tumor cells mainly rely on glycolysis for metabolism, which is known as Warburg effect, namely aerobic glycolysis[19]. Inhibition of the aerobic glycolysis process of PDAC cells can further inhibit tumor growth and metastasis. Glucose is the main energy source. Tumor cells can convert glucose into lactate, and glycolysis can rapidly produce ATP[20]. However, the increase of lactate content can lead to the acidification of pH value in tumor microen-
vironment, which will promote the proliferation, metastasis and invasion of tumor cells and enable them to resist apoptosis\(^{27}\). We evaluated and calculated the effect of overexpression of circ\_0001017 in PDAC cells on its metabolic level using glucose, lactate and ATP detection kits. The results showed that circ\_0001017 could effectively control the glucose uptake of PDAC cells, reduce the production of lactose and ATP, inhibit the glycosylation of cells, and hinder the metabolism of tumor cells. Therefore, this study found that circ\_0001017 not only inhibited the proliferation activity and promoted the apoptosis of PDAC cells, but also inhibited the glucose uptake, lactate and ATP production in the cells, suggesting that the proliferation potential of PDAC cells may benefit from glucose metabolism, so we speculated that circ\_0001017 might control the malignant phenotype of PDAC cells by regulating the function of aerobic glycosylation. Then, in order to explore the specific molecular mechanism of circ\_0001017 in PDAC, the circular structure of circ\_0001017 that could not be digested by enzymes was determined by means of RNase R experiment. The results of FISH localization that circ\_0001017 was mainly expressed in the cytoplasm again verified that circ\_0001017 could play the role of corresponding isolation miRNA as ceRNA. We found miR-197-3p which had potential binding sites with circ\_0001017 through the RNA22 database. MiR-197-3p can play a role in the progression of a variety of human malignancies through downstream targets, and can inhibit the occurrence of gastric cancer by regulating the expression level of PRKCB gene\(^{22}\). miR-197-5p can also promote the development of gastric cancer cells by up-regulating CHD9\(^{23}\).

Overexpression of miR-197 can promote epithelial-mesenchymal transition of PDAC, suggesting that miR-197-3p can be used as a new therapeutic target for PDAC\(^{24}\). The targeted binding relationship between miR-197-3p and circ\_0001017 was verified through the dual luciferase reporter and RIP assays. Circ\_0001017 can be used as a molecular sponge to adsorb miRNA and negatively regulate the level of miR-197-3p through sponging miR-7-5p. Cancer Manag Res 12: 7291-7303, 2020.

Circular RNAs function as ceRNAs to regulate and control human cancer. Cancer Lett 414: 301-309, 2018.

Overexpression of miR-197-3p can promote epithelial-mesenchymal transition of PDAC, suggesting that miR-197-3p can be used as a new therapeutic target for PDAC\(^{25}\). The targeted binding relationship between miR-197-3p and circ\_0001017 was verified through the dual luciferase reporter and RIP assays. Circ\_0001017 can be used as a molecular sponge to adsorb miRNA and negatively regulate the level of miR-197-3p through sponging miR-7-5p. Cancer Manag Res 12: 7291-7303, 2020.

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In conclusion, this study investigated the biological function of circ\_0001017 in PDAC, and revealed that circ\_0001017 regulated the expression of PNLIP through sponging miR-197-3p, thereby inhibiting the progression of PDAC.

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Conflict of Interest

The authors declare no conflict of interests.

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