MiR-489-3p reduced pancreatic cancer proliferation and metastasis by targeting PKM2 and LDHA involving glycolysis

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Abstract

Background: Malignant proliferation and chemotherapy resistance are some of the causes of high mortality in pancreatic cancer. MicroRNAs have long been a hot spot in cancer research and are involved in tumor formation and metabolic stress responses. miR-489-3p is involved in inhibiting the growth of many tumors, but its relationship with the growth and metabolism of pancreatic cancer is not clear.

Methods：We used RNA in situ hybridization to analyze the differential expression of miR-489-3p in pancreatic cancer tissues and adjacent tissues. The qRT-PCR experiment detected the content of miR-489-3p in pancreatic cancer cell lines and ordinary pancreatic ductal epithelial cells. Then we did experiments in vivo (subcutaneous tumor formation in nude mice) and in vitro (plate cloning, transwell, glycolysis related experiments) experiments to verify that miR-489-3p can continue the invasion and metastasis of pancreatic cancer and glucose metabolism. Furthermore, we confirmed that LDHA and PKM2 are the two targets of miR-489-3p through dual luciferase reporter gene experiments. Finally, several reply experiments were done to verify the regulation mechanism of miR-489-3p.

Results: We determined that miR-489-3p is under-expressed in pancreatic cancer tissues by RNA in situ hybridization, and the function acquisition and deletion experiments and glycolysis experiments confirmed that miR-489-3p can inhibit the proliferation and invasion of Glycolysis. We then analyzed the website to find that miR-489-3p can target LDHA and PKM, and then we verified this finding with a luciferase report experiment. Therefore, we proceeded with recovery experiments on LDHA and PKM2, and concluded that miR-489-3p performs its function by targeting LDHA and PKM2. Finally, in vivo experiments confirmed that highly expressed miR-489-3p inhibited the growth of pancreatic cancer.

Conclusion: In short, we identified miR-489-3p as a novel chemotherapy target for pancreatic cancer, and its diagnostic value deserves further study.

Background

Pancreatic ductal adenocarcinoma (PDAC) is a deadly cancer with a poor treatment methods. Early metastasis and invasion are the main factors leading to its poor prognosis[1, 2]. Only sufficient energy and biosynthetic precursors can sustain this aggressive biology. However, one of the characteristics of pancreatic cancer microenvironment is dense desmoplasia[3, 4]. This dense mass result in the creation of enormous solid stress and fluid pressure in the tumors and compression of the vasculature. Therefore, its hypovascularization decreases the supplement of materials for biosynthesis into cancer cells and generates an energy shortage. Nevertheless, tumor cells can adapt to this change by metabolic reprogramming; the most typical example of this is enhanced glycolysis , which was initially named as the “Warburg Effect”[5]. This reprogramming provides energy, macromolecular precursors and reducing equivalents, which is crucial to the abnormal growth and survival of cancer cells[6]. Although aerobic glycolysis has been verified in PDAC, the mechanism driving it remains hardly known. Hence, the elucidation of this mechanism is essential for the research and treatment of pancreatic cancer.

MicroRNAs, a noncoding RNAs group of 18–23 nucleotide, are high-profile molecular family participated in mediating metabolic stress response in cancer[7-9]. For example, they are widely involved in the regulation of signal pathways such as p53, LKB1/AMPK, c-Myc and others that regulate metabolic response[10-12]. It has been reported that micro RNAs can promote glycolysis of a variety of tumors directly and indirectly, such as pancreatic cancer[13], gastric cancer[14], bladder cancer[15], etc. Meanwhile, literatures also have found multiple micro RNAs like miR-135[13], miR-124 [16]and others have been implicated in metabolic reprogramming of pancreatic cancer. And miR-489-3p has been reported to inhibit tumor progression[17, 18]. Therefore, we hypothesized that it can also inhibit the progression of pancreatic cancer, and it is inversely related to the glycolysis that can promote tumor progression.

In this study, we find the MiR-489-3p involved in glucose metabolism reprogramming and malignancy in PC. Here, we found that MiR-489-3p was negative associated with expression of tumor tissue in PC patients and cell lines in PC. To further explore its function, we conducted loss- and gain-of-function assays to observe proliferation and metastasis in vivo and vitro , and metabolism experiments in cells. These all proved MiR-489-3p inhibits malignancy and glucose metabolism in PC. We further demonstrate LDHA and PKM2, critical enzyme for glycolytic flux, are miR-489-3p target genes, thus controlling glycolysis and PC progression. This study provides important evidence that miRNA is actively involved in pancreatic cancer cell adaptation to the nutrient-poor microenvironment.

Materials And Methods

human pancreatic tumor samples.

A total of 90 cases of pancreatic cancer tissues and adjacent tissues from the pancreatic surgery department of Wuhan University People's Hospital from 2009 to 2019 were collected for RNA in situ hybridization. All patients were diagnosed with prostate cancer according to the World Health Organization's diagnostic criteria. All samples were approved by the ethics review committee and the patient's informed consent was obtained.

Cell culture

Two human pancreatic cancer cell lines PANC-1 and MIA-PACA2 were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and both grown in high-glucose DMEM medium (Hyclone, Logan, Utah, USA) containing 10% fetal bovine serum (Gibco, NY, USA). Both cell lines cultured at 37 °C in a humidified 5% CO2 incubator according to ATCC protocols.

RNA in situ hybridization

Indirect labeling method using digoxin was used to detect miR-489-3p. The RNA probe labeling method was used to construct plasmids and then synthesized by transfection. Sample processing, probe

preparation, in situ hybridization, washing, blocking, and finally enzyme reaction detection. Finally, observe and take pictures under the microscope.

Cell viability analysis

Cell Counting Kit-8 (beyotime, China) was used to detect the proliferation of PC cells. According to the manufacturer's instructions, 100ul of 2x10^3 cells were seeded into a 96-well plate, and 10ul of cck8 reagent was added to each well. Absorbance values were measured every 0, 24, 48, 72 and 96 hours and recorded for statistical analysis.

Colony formation assay

Two types of pancreatic cancer cells were inoculated into six-well plates at a rate of 500 cells per well and cultured in incubators at 37°C and 5% CO2 for two weeks. The medium was then poured out, fixed with 4% paraformaldehyde for 20 minutes, and stained with 0.2% crystal violet for 30 minutes. The number of clones was observed under an optical microscope and analyzed with statistical software.

Transwell assays

Panc-1 and mia-paca2 cells of different treatment groups were seeded into the upper chamber of Tanswell in an amount of 1x10 ^ 5 per well. 200 ul of a medium containing 10% serum was added to the upper chamber, and 700 ul of serum-free medium was added to the lower chamber. Placed in the incubator for 24 hours, discard the culture medium, washed with PBS(Hyclone, USA), and fixed with 4% paraformaldehyde(Biosharp, China) for 30 minutes. Then dyed it with 0.5% crystal violet(Solarbio,Beijing,China) solution for 30 minutes, rinse it with pure water, observe and take pictures under a microscope. Finally, the software counts and analyzes.

Quantitative real-time PCR

The cells of each group were seeded in a six-well plate, and the total RNA of each group was extracted using Trizol reagent (Invitrogen, CA, USA) when the fusion degree reached 80% -90%. CDNA was then synthesized using a PrimeScript RT reagent kit (Takara) according to the manufacturer's instructions. Real-time quantitative PCR was performed by Powerup SYBR Green PCR Master Mix (Life Technologies).

Luciferase reporter assay

Seed the cells in a good state into a six-well plate. When the six-well plate reaches 50% confluence, use lipofectamine reagent (Invitrogen) with miR-489-3p or TALD promoter-containing luciferin Enzyme reporter genes were transfected for 4-6 hours. High glucose DMEM was used to change the medium, and then cultured in an incubator for 24 hours. Then disposed according to the product instructions for the double luciferase reporter gene detection kit (Beyotime Biotechnology, China). In short, 500 microliters of reporter gene cell lysate was discarded after the medium was discarded, and the supernatant was taken for determination after sufficient lysis.

Western blot

The cells of each group were seeded in a six-well plate, and when the band fusion reached 90%, the protein was obtained by lysis with RIPA lysate (beytime, China). BCA reagent (beytime, China) was used to quantify the protein, and then the protein was diluted to the same concentration, and then boiled in a refrigerator at -20 ° C until use. Electrophoresis was performed using Solarbio reagents and 10% separation gel and 5% concentrated gel were prepared according to the manufacturer's instructions. After electrophoresis and transfer, exposure is performed.

Glucose uptake andlactate production measurements

Glucose uptake was performed using the D-2-deoxyglucose method. D-2-deoxyglucose comes from Beyotime, China, and the lactic acid detection kit comes from Leagene Biological,Beijing. Analysis of glucose intake and lactic acid production was performed according to product instructions.

Cellular ATP level

Experiments were performed using an ATP detection kit (Beyotime Biotechnology, China). Add 200 μl of lysate to each well of the six-well plate according to the product instructions, and centrifuge the supernatant after full lysis. Prepare ATP working solution and add it to the detection tube to measure the luminescence value.

Extracellular acidification rate (ECAR)

Hippocampal experiments were performed with Agilent equipment: Hippocampus XFe24 Micro Edition and XFe24 cartridge. Cells of each group were incubated at 500 ° C for 7.5 × 104 cell seeds at 37 ° C for 1 hour at the hippocampal preparation station.

56 μl glucose (100 mm) (G8270σ), 62 μl oligomycin (10 μM) (σ) 75351 and 69 μl 2 dg (1 meter) (D6134, Sigma) were added to the cartridge wells. Then read the ECAR value.

In vivo assay

10 ^ 7panc-1 cells (miiR-NC group and miR-489-3p up-regulation group) were injected to 4 weeks of age female NCr nude mice were injected subcutaneously (Hua Fukang Biotechnology, Beijing). Tumor size was measured with calipers every 7 days. Over time, the size of the tumor is measured. At the end of the experiment, the mice were euthanized and subcutaneous tumors were collected. Perform other analyses.

IHC analysis

Immunohistochemical staining was used to detect the expression of proliferation and metabolic indicators. In short, the subcutaneous tumor tissue was cut into 3um and then dewaxed. The sections were then incubated with rabbit anti-monoclonal and refrigerated at 4 ° C overnight. After washing three

times with PBS, each piece was incubated with goat anti-rabbit IgG for 30 minutes and then developed with 3,3 'diaminobenzidine (DAB). All antibodies were from Proteintech, USA.

Statistical analyses

The results are calculated and analyzed using the mean ± standard deviation. GraphPad Prism 7.0 (San Diego, California, USA) was used for mapping and statistical analysis. Chi-square test was used to analyze the relationship between miR-489-3p expression level and clinicopathological characteristics in PC. The Kaplan-Meier curve method was used to analyze the overall survival rate. Student's t test was used for statistical comparison between the groups. * P <0.05, * * P <0.05.

Results

The expression of miR-489-3p and its relationship with clinical prognosis of patients.

In order to verify the expression of miR-489-3p in pancreatic cancer, we performed RNA in situ hybridization (ISH) experiments on the tumor tissues of 90 patients with pancreatic cancer and found that miR-589-3p expression in tumor tissues was much lower adjacent tissues(Fig1a-b). We further verified that the expression of miR-489-3p in tumor tissues was lower than in adjacent tissues by quantitative RT-PCR (qRT-PCR)(Fig1c).Also miR-489-3p showed lower expression in PC cell lines(Fig1d). Based on the pathological characteristics of miR-489-3p expression and clinical relevance of pancreatic cancer (Table 1), we found that miR-489-3p expression was significantly related to tumor size (P <0.01) and distant metastatic ability (P <0.05). In addition, we performed survival analysis on patients who provided tissue samples and found that patients with low expression of miR-489-3p had a worse prognosis（P=0.0323,HR=1.843）(Fig1e).

MiR-489-3p inhibits proliferation and invasion of pancreatic cancer.

Associated with miR-489-3p is underexpressed in PC, we speculated that miR-489-3p can inhibit the progression of pancreatic cancer. To verify this assumption, panc-1 cells were transfected with miR-NC, miR-489-3p mimics and mia-paca-2 were transfected with anti-miR-NC and anti-miR-489-3p inhibitors(Fig2a). Several proliferation experiments have shown that overexpression of miR-489-3p inhibits PC cells proliferation, and inhibition of miR-489-3p increases PC cells proliferation(Fig2b-e). Invasion experiment show that overexpression of miR-489-3p can inhibit the invasion ability of PC cells(fig2f)

MiR-489-3p regulates PC glycolysis.

Since miR-489-3p can target LDHA and PKM2 which were glycolytic-related enzymes, we hypothesized that miR-489-3p can regulate glycolysis. We performed glycolysis-related experiments to observe the metabolic parameters of PC tumor cells after overexpression or inhibition of miR-489-3p. The measurement of metabolic parameters showed that lactate production, glucose production, and ATP production of pancreatic cancer cells were reduced after miR-489-3p was overexpressed. Conversely, after

miR-489-3p was down-regulated, these parameters all increased(Fig3a-c). Seahorse assays showed that reducing the expression of miR-489-3p inhibited the ECAR(extracellular acidification rate) of PC cells(Fig3d-e).

MiR-489-3p targets LDHA and PKM2.

Furthermore, we explored the mechanisms of miR-489-3p regulating PC growth . We found LDHA and PKM2 were RNA binding protein (RBP) of miR-489-3p by STARBASE3.0(http://starbase.sysu.edu.cn/). Therefore we speculated that miR-489-3p performs its biological function by targeting them. To verify this hypothesis, we overexpressed and inhibited miR-489-3p in PC cells, and found that LDHA and PKM2 decreased and increased, respectively (Fig4a-c). And the tissue co-expression correlation map showed that miR-489-3p was negatively correlated with LDHA and PKM2(Fig4d-e). The binding sequences and mutation sites of LDHA and PKM2 on miR-489-3p are shown in Figures 4f. Overexpression of miR-489-3p significantly reduced the luciferase activity of LDHA and PKM2 WT, but did not reduce the luciferase activity of LDHA and PKM2 Mut (Fig4g). In contrast, down-regulating miR-489-3p significantly increased the luciferase activity of LDHA and PKM2 WT, but did not increase the luciferase activity of LDHA and PKM2 Mut (Fig4h). These results indicate that miR-489-3p can target LDHA and PKM2.

LDHA and PKM2 restore miRNA-mediated proliferation and invasion

We have previously demonstrated that miR-489-3p can regulate the progression of pancreatic cancer, and that miR-489-3p can target LDHA and PKM2. Therefore we speculated that miR-489-3p performs its function by targeting LDHA and PKM2. QRT-PCR and western blot(WB) assays showed that overexpression of miR-489-3p can down-regulate LDHA and PKM2, and overexpression of LDHA and PKM2 can restore this change(Fig5a-b). In contrast, inhibiting miR-489-3p can up-regulate LDHA and PKM2, and down-regulating LDHA and PKM2 can restore this phenomenon (Fig5c-d). Next, we conducted several response assays of the functional experiment. Proliferation assays showed that miR-489-3p can target LDHA and PKM2 to regulate the proliferation of pancreatic cancer cells (Fig5e-h). Invasion assays showed that miR-489-3p can regulate the invasion ability of PC cells through LDHA and PKM2(Fig5i).

MiR-489-3p supresses glycolysis through LDHA and PKM2.

To confirm whether miR-489-3p also inhibited glycolysis of PC cells by targeting LDHA and PKM2, we verified this conjecture by overexpression and knockdown of LDHA and PKM2. Up-regulation of miR-489-3p significantly reduced glucose consumption, lactic acid production, and ATP production, while simultaneously increasing LDHA or PKM2 increased their production. In contrast, down-regulating miR-489-3p significantly increased glucose consumption, lactic acid production, and ATP production levels, while simultaneously lowering LDHA or PKM2 could reduce their production(Fig6a-f). In addition, the hippocampal XF extracellular flux analyzer showed that LDHA and PKM2 can restore the extracellular acidification rate(ECAR) caused by miR-89-3p (Fig6g-h).

Effects of overexpression of miR-489-3P on PC proliferation and metabolism in vivo.

To investigate the effect of miR-489-3p on tumor progression in vivo, we used a xenograft tumor model. The picture (Fig7a) was taken after 30 days of subcutaneous tumor formation in nude mice. Changes in tumor weight and volume indicate that overexpression of mir-489-3p can inhibit tumor growth (Fig7b-c). The expression of miR-489-3p was significantly lower in the control group than in the samples transfected with miR-489-3p mimics(Fig7d). Then, IHC-stained images of cell proliferation factors show that overexpression of miR-489-3p can inhibit tumor proliferation (Fig7e). Last, In order to investigate whether miR-489-3p regulates glycolysis, we used IHC to stain glycolysis-related indicators. Apparently, the staining results showed that after miR-489-3p was overexpressed, tumor glycolysis was also inhibited(Fig7f).

Discussion

Pancreatic cancer is a highly malignant pancreatic cancer of the digestive tract, which is extremely difficult to diagnose and treat. It accounts for 8% of cancer-related mortality in men and women.[19]. The highly invasive nature of pancreatic cancer is one of the causes of lethality of pancreatic cancer, and it is well known that the occurrence and development of tumors are related to the abnormal expression of specific genes[20]. Therefore, finding genes related to the growth of pancreatic cancer is very important for studying pancreatic cancer. At the same time, we know that pancreatic cancer is highly resistant to radiotherapy and chemotherapy[21-23], and inhibiting glycolysis to control the energy supply of cancer cells has become an emerging chemotherapy approach[24-26]. Interestingly, scientists have long found that glycolysis intensity is positively correlated with tumor invasion and metastasis[27]. For example, lactic acid, the product of glycolysis, forms an acidic microenvironment that is essential for the transformation of lung metastases to malignant metastases[27]. The role of MiRNAs in tumors is extensive. The miR-489-3p found in this study is known to inhibit the growth of a variety of tumors, such as bladder cancer[28], renal cell carcinoma[29], and osteosarcoma[17]. However, its relationship with pancreatic cancer is unclear. We found by microarray analysis that the expression of miR-489-3p in pancreatic cancer is lower than that in adjacent tissues, and it is closely related to the prognosis of clinical patients. Functional experiments have also demonstrated that it can inhibit the invasion and metastasis of pancreatic cancer cells, and glucose metabolism experiments have demonstrated that the highly expressed miR-489-3p inhibits glycolysis. However, the mechanism by which overexpression of miR-489-3p inhibits pancreatic cancer growth and glucose metabolism is not clear.

Furthermore, a luciferase reporter experiment showed that miR-489-3p can target the expression of LDHA and PKM. LDHA catalyzes pyruvate to lactic acid during glycolysis. Previous studies have shown that LDHA is inseparable from the aerobic glycolysis of tumors[30]. PKM2 is a rate-limiting enzyme for glycolysis. There are two isomers of M-type pyruvate kinase: PKM1 and PKM2. PKM2 is the only form of pyruvate kinase found in cancerous tissues[31, 32]. A transcription factor HIF (Hypoxia Inducible Factor) is highly expressed in tumors, and one of its subtypes, HIF-1, promotes the expression of the promoter region of the MYC gene. Myc is a transcription factor with a wide range of biological functions, including cellular energy metabolism[33]. Myc can stimulate expression of many genes, including LDHA and PKM2[34, 35]. We therefore determined that LDHA and PKM2 are relevant to our study.

Next, we performed functional experiments and glycolysis-related experiments by overexpression or knockdown of LDHA or PKM2. Apparently, overexpression of LDHA or PKM2 restored the inhibition of pancreatic cancer cell growth and glycolysis caused by overexpression of miR-489-3p. In contrast, knocking down LDHA or PKM2 alleviated the fast growth and hypermetabolism caused by inhibition of miR-489-3p. Finally, we performed in vivo experiments. The xenograft tumors in the overexpressing miR-489-3p group were significantly smaller than the NC group, and the immunohistochemistry of the xenograft tumors showed that the proliferative and metabolic markers of the overexpressing miR-489-3p group were lower than the NC group.

Conclusion

In summary, in vivo and in vitro experiments have demonstrated that miR-489-3p inhibits the growth of pancreatic cancer and inhibits glycolysis of pancreatic cancer. And provide new ideas for molecular targeted therapy of pancreatic cancer.

Declarations

Ethics approval and consent to participate

Human samples were obtained from Renmin Hospital of Wuhan University. All patients included in the study provided written informed consent, and the study was approved by the Institutional Ethics Committee of Renmin Hospital of Wuhan University. Patients did not receive financial compensation. All methods were performed in accordance with relevant guidelines and local regulations.

Consent for Publication

All authors have agreed to publish this manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors have no conflicts of interest to disclose

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Authors' contributions

JX Jiang and ZW He contributed to the experiment design, and data analysis. D Zhang, YY Shen and J Wang contributed to the experiment implementation, Tao Liu and JX Jiang contributed to manuscript draft and data analysis. All authors read and approved the final manuscript.

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Table

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

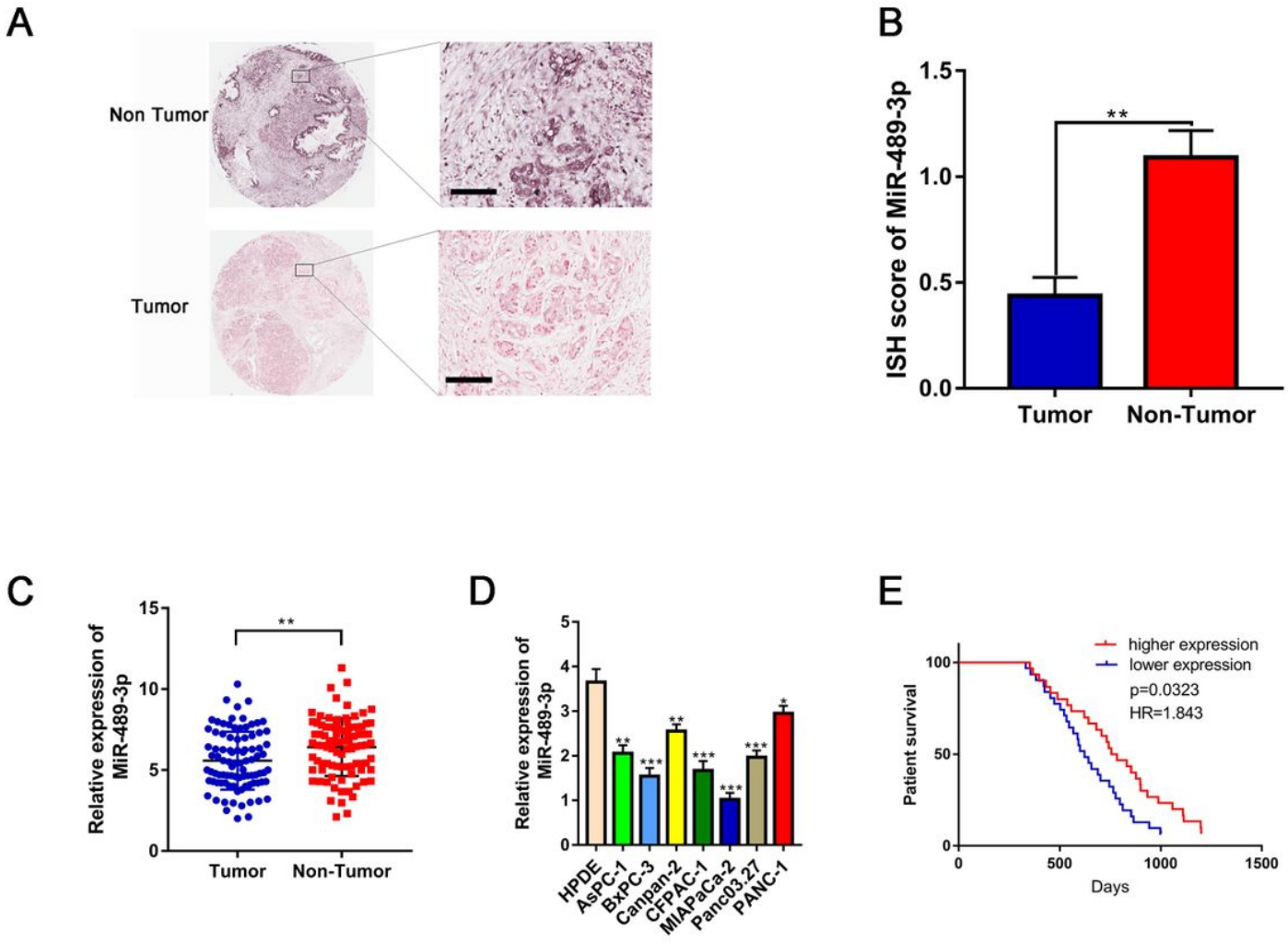

Figure 1

The expression of miR-489-3p and its relationship with clinical prognosis of patients. (a-b) RNA in situ hybridization experiments showed the expression of miR-489-3p in paracancerous and cancerous tissues. The bar stands for 50 microns (c) qRT-PCR analysis of the relative expression of miR-489-3p in adjacent tissues and PC tissues. (d) qRT-PCR showed the relative expression of miR-489-3p in PC cell lines and pancreatic normal duct epithelial cells (HPDE). (e)Kaplan-Meier curve was divided into survival periods by miR-489-3p expression. Among them, patients were divided into high expression group (red) and low expression group (blue) by median expression of miR-489-3p. (*P < 0.05, ** P < 0.01, *** P < 0.001)

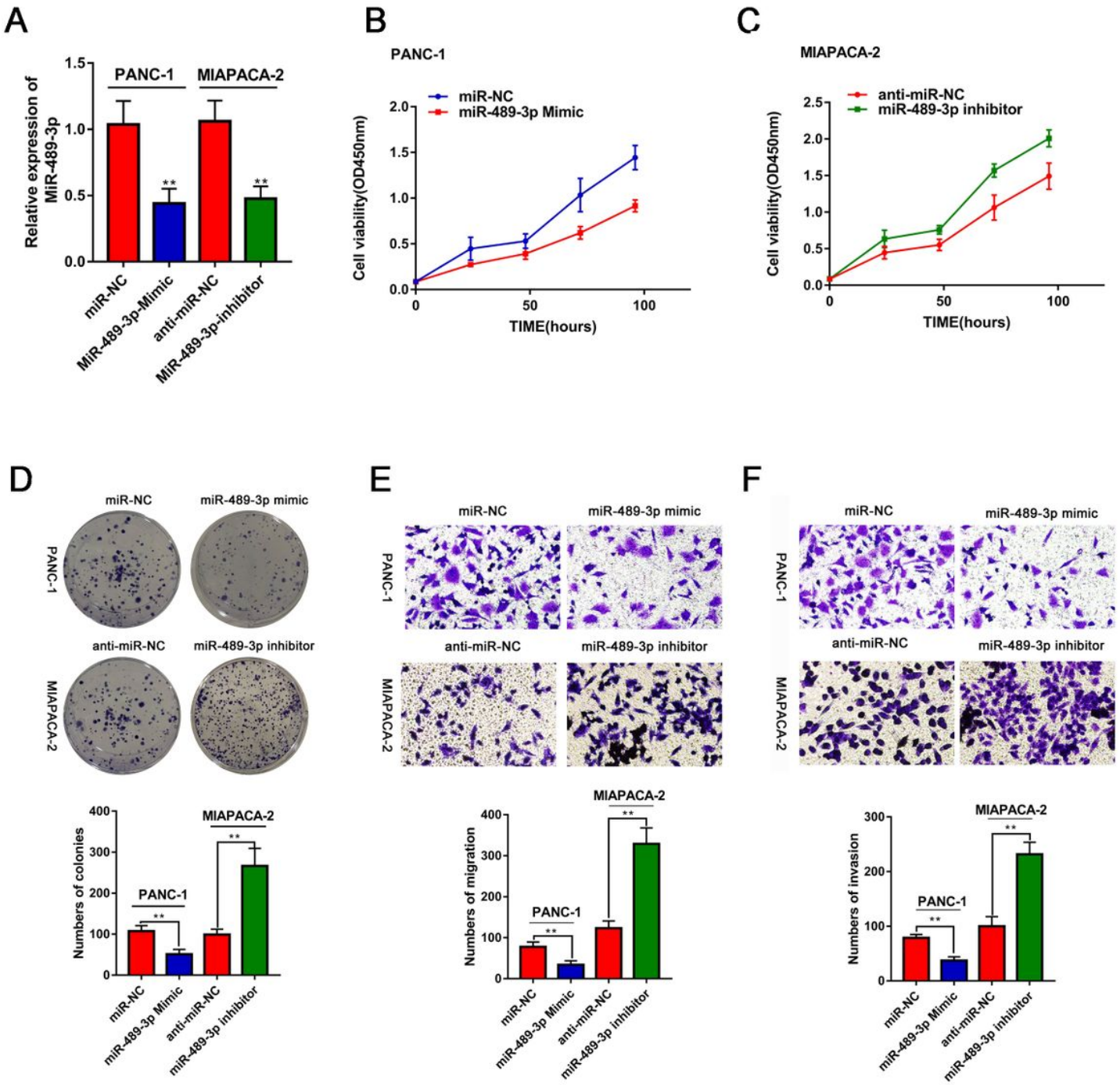

Figure 2

MiR-489-3p inhibits proliferation and invasion of pancreatic cancer. (a-b) qRT-PCR showed the relative expression of miR-489-3p after transfection of miR-489-3p mimic in panc-1 cells and addition of miR-489-3p inhibitor in mia-paca2 cells. (b-e) CCK8 and plate cloning andtranswell proliferation assays showed the cell proliferation ability of panc-1 cells transfected with miR-489-3p mimics and mia-paca2 cells with miR-489-3p inhibitors. (f) Transwell invasion assays showed the cell invasion ability of panc-1 cells transfected with miR-489-3p mimics and mia-paca2 cells with miR-489-3p inhibitors. (** $P < 0.01$)

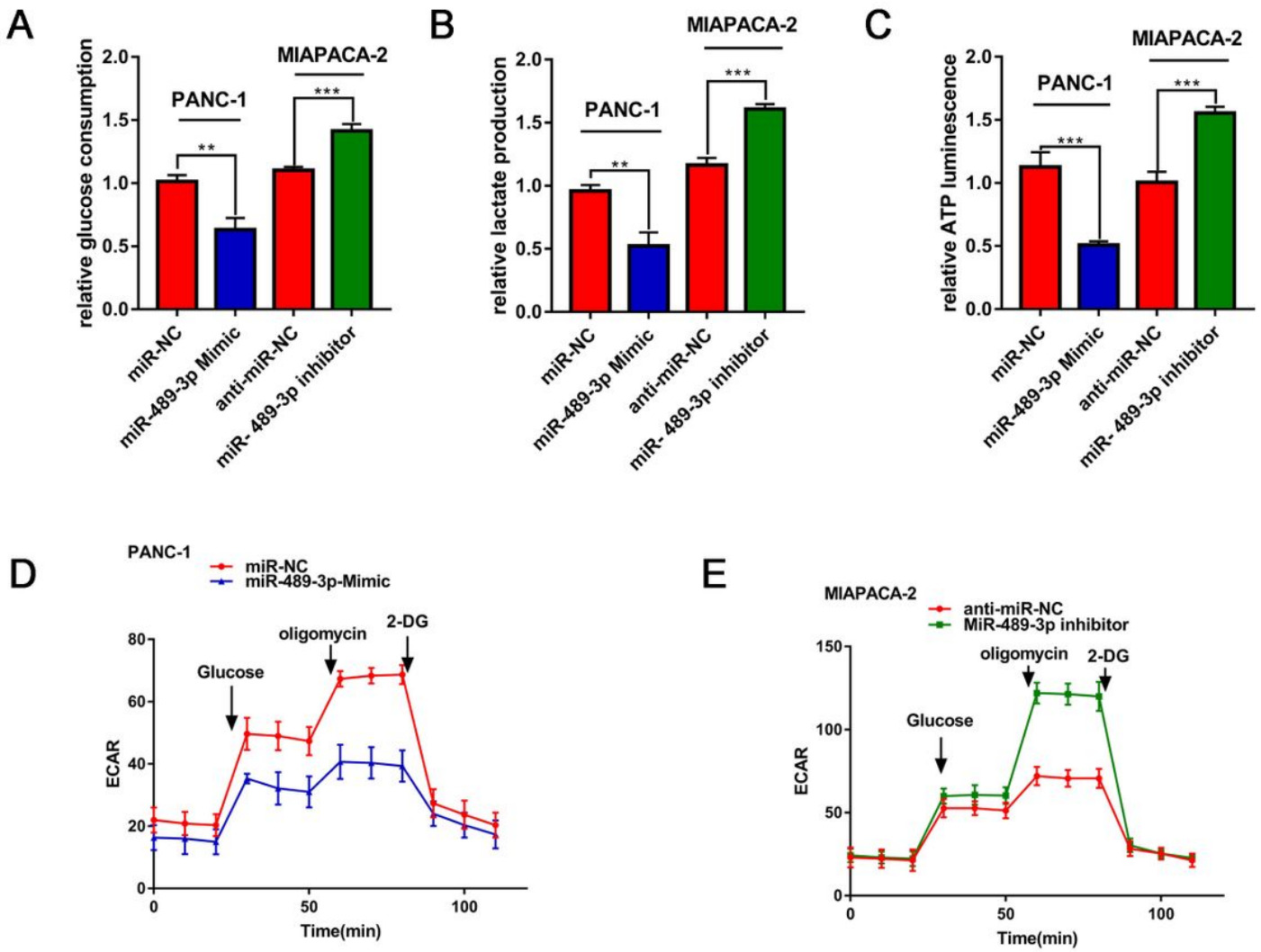

Figure 3

MiR-489-3p regulates PC glycolysis. (a-c) Cell metabolism experiments showed glucose uptake, lactic acid production, and ATP production of miR-489-3p mimics in panc-1 cells and miR-489-3p inhibitors in mia-paca2 cells. (d-e) The hippocampal XF extracellular flux analyzer showed that ECAR was added to miR-489-3p mimics in panc-1 cells and miR-489-3p inhibitors in mia-paca2 cells. (** P < 0.01, *** P < 0.001)

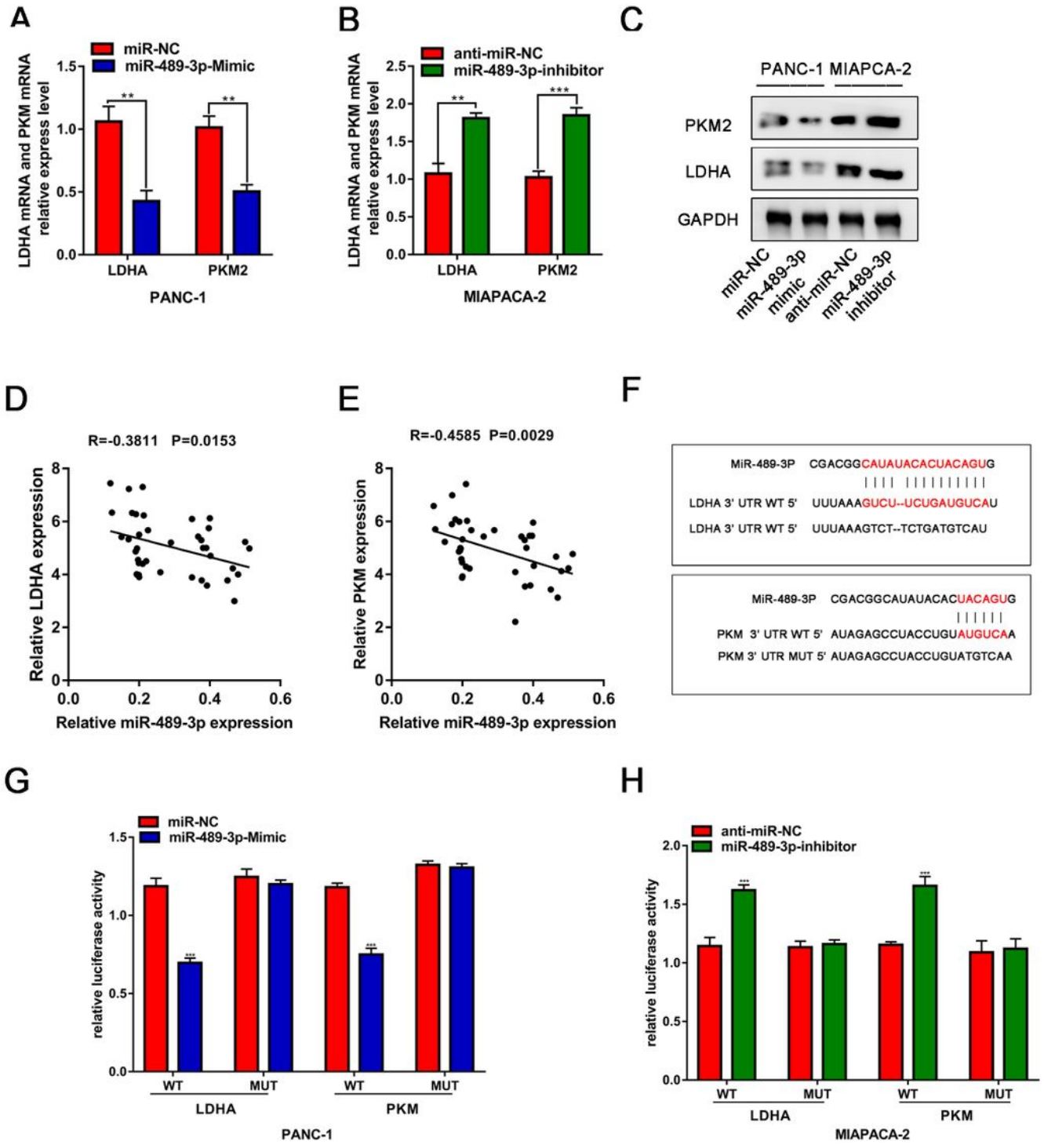

Figure 4

MiR-489-3p targets LDHA and PKM2. (a,c) qRT-PCR and western blot assays showed relative expression of LDHA and PKM2 after panc-1 cells were transfected with miR-489-3p mimics. (b,c) qRT-PCR and western blot assays showed relative expression of LDHA and PKM2 after miR-489-3p inhibitor was added to mia-paca2. (d-e) Spearman rank correlation analysis showed a statistical relationship between miR-489-3p and LDHA and PKM. (f)The predicted binding site of miR-489-3p in human LDHA and PKM gene 3

UTR, and the corresponding sequence in the mutated version. (g-h) Analysis of the luciferase reporter gene indicated a statistical relationship between miR-489-3p and LDHA and PKM. (** P < 0.01, *** P < 0.001)

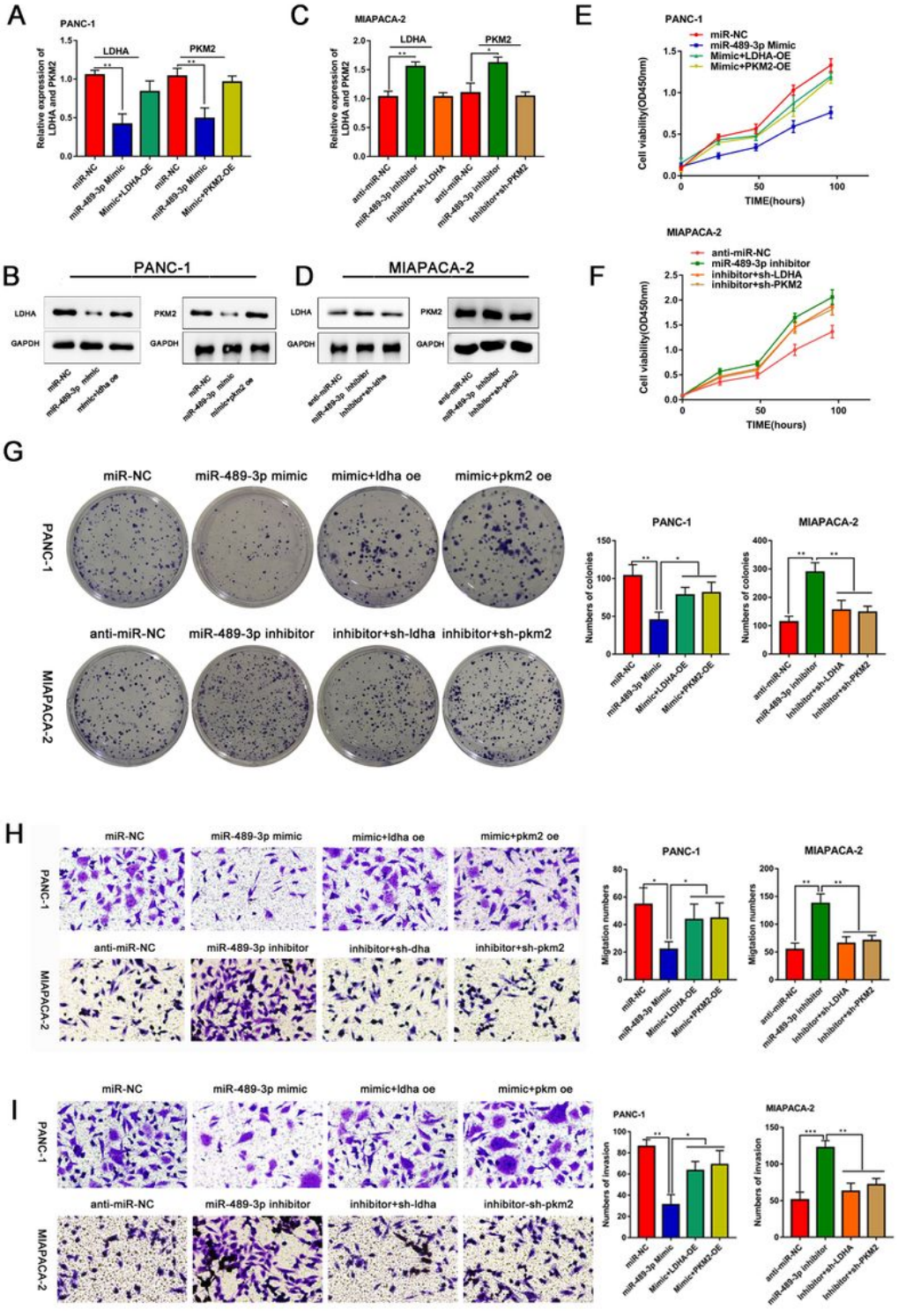

Figure 5

LDHA and PKM2 restore miRNA-mediated proliferation and invasion (a-b) qRT-PCR and western blot experiments showed that the relative expression of LDHA and PKM2 after up-regulating miR-489-3p and

LDHA (PKM2). (c-d) qRT-PCR and western blot experiments showed that the relative expression of LDHA and PKM2 after down-regulating miR-489-3p and LDHA (PKM2) simultaneously. (e-h) CCK8 and plate cloning and transwell migration assays showed that the proliferation ability of PC cells after up-regulating (down-regulating) miR-489-3p and LDHA (PKM2). (i) Transwell invasion assays show that the invasion ability of PC cells after up-regulating (down-regulating) miR-489-3p and LDHA (PKM2) simultaneously. (*P < 0.05, ** P < 0.01, *** P < 0.001)

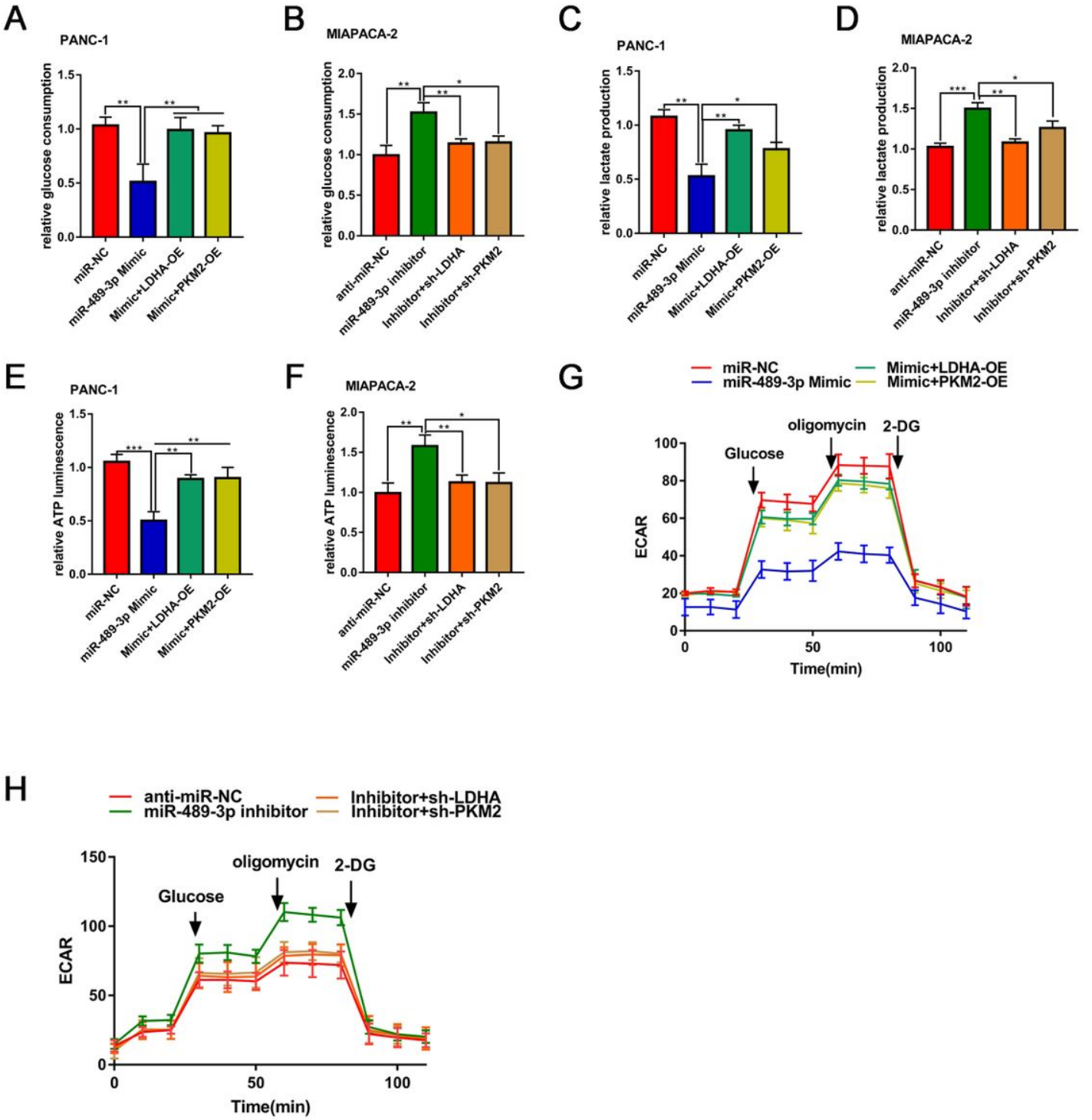

Figure 6

MiR-489-3p supresses glycolysis through LDHA and PKM2. (a,c,e) Cell metabolism experiments showed that glucose uptake, lactic acid production, and ATP production of PC cells after upregulating miR-489-3p and LDHA (PKM2) simultaneously. (b,d,f) Cell metabolism experiments showed glucose uptake, lactic acid production, and ATP production of PC cells after miR-489-3p and LDHA (PKM2) were down-regulated simultaneously. (g) The hippocampal XF extracellular flux analyzer showed ECAR after simultaneously up-regulating miR-489-3p and LDHA (PKM2). (h)The hippocampal XF extracellular flux analyzer showed ECAR after simultaneously down-regulating miR-489-3p and LDHA (PKM2).

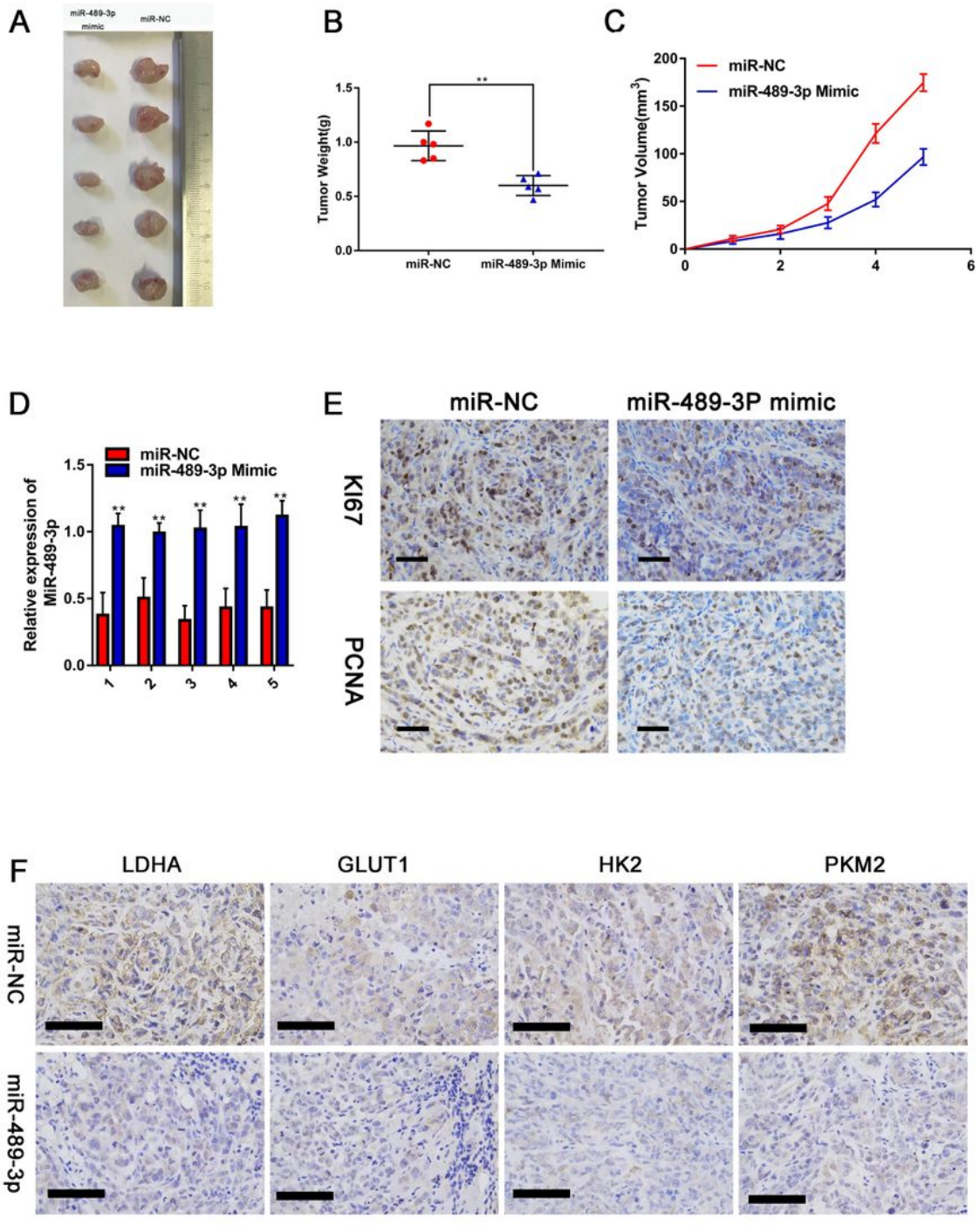

Figure 7

Effects of overexpression of miR-489-3P on PC proliferation and metabolism in vivo. (a)Typical images of nude mice tumors (n = 5), (b) subcutaneous tumor weight, (c) subcutaneous tumor volume, (d) miR-489-3p expression in xenografts by qRT-PCR. (e) Typical IHC staining images of xenografts show Ki-67 and PCNA expression. The bar stands for 50 microns. (f) Typical IHC staining images of xenografts show the expression of metabolic indicators (LDHA, GLUT1, HK2, PKM2). The bar stands for 100 microns.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- renamed4a152.xls