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Phosphoglycerate Mutase 1 (PGAM1) Promotes Pancreatic Ductal Adenocarcinoma (PDAC) Metastasis by Acting as a Novel Downstream Target of the PI3K/Akt/mTOR Pathway

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive tumors known, with an overall 5-year survival rate of less than 6% due to early local invasion and distant metastasis. Exploring suitable therapeutic targets associated with invasion and metastasis is required for improving the prognosis of PDAC. In this study, we investigated the role of the glycolytic enzyme phosphoglycerate mutase 1 (PGAM1) in PDAC. PGAM1 expression was examined in tissue samples of 54 PDAC patients using immunohistochemistry, and the correlation between clinicopathological expression and PGAM1 expression was determined. A survival curve was generated using the Kaplan–Meier method. After silencing PGAM1 by siRNA in pancreatic cancer cell lines Aspc-1 and Panc-1, the changes in proliferation, migration, and invasion, and signal pathways were determined. In this study, the expression of PGAM1 was found positively related to poor differentiation, metastasis, advanced clinical stage, and poor survival rate. Silencing PGAM1 decreased the proliferation of Aspc-1 and Panc-1 cells with an S phase arrest, but without influencing cell apoptosis. Migration and invasion also decreased significantly, independent of proliferation. PGAM1 was also found to promote EMT of PDAC cell lines by regulating the Wnt/β-catenin pathway. PGAM1 itself was modulated by the PI3K/Akt/mTOR pathway as a novel downstream target and has a positive mutual regulation with HIF-1α. This study indicates that PGAM1 is closely associated with clinical metastasis and poor prognosis of PDAC. PGAM1 is considered as a potential therapeutic target in PDAC metastasis.

Key words: Pancreatic ductal adenocarcinoma (PDAC); Phosphoglycerate mutase 1 (PGAM1); Metastasis; Wnt/β-catenin; PI3K/Akt/mTOR; Hypoxia-inducible factor 1α (HIF-1α)

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant tumors known and is characterized by aggressive growth and a poor prognosis[1]. The overall 5-year survival rate of patients who are diagnosed with pancreatic cancer remains at less than 6%[2], without significant progress made in the last few decades. The disappointing prognosis is mainly due to poor diagnosis at an early stage, uncontrolled early invasion and metastasis, and resistance to most chemical therapies. For patients diagnosed with PDAC, only 5%–10% of cases with localized and resectable tumors are curable by surgery. However, because of early local invasion and distant metastases, either before or after surgery, about 80%–90% of such cases do not survive longer than 5 years[3]. Therefore, early invasion and metastasis are the main obstacles to a satisfactory treatment outcome. Identifying the key modulating proteins involved in PDAC is very important in finding effective therapies.

Phosphoglycerate mutase 1 (PGAM1) is a crucial glycolytic protein that catalyzes the reversible reaction of 3-phosphoglycerate (3-PG) to 2-phosphoglycerate (2-PG)[4]. Thus far, many studies have shown that PGAM1 is highly expressed in various tumors such as lung cancer[5], hepatocellular carcinoma[6], colon cancer[7], and oral squamous cell carcinoma[8]. The promoting roles of PGAM1 in aggressive tumors have received increasing attention in recent years. Several studies have suggested that PGAM1 promoted tumor growth by upregulating the glycolysis process[9,10]. In a research from Hitosugi et al.[11], the phosphorylation of PGAM1 (Y26) induced by oncogenic tyrosine kinases has been shown to activate its

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activity and promote tumor growth. Up to now, limited studies have focused on the role of PGAM1 in PDAC, and whether PGAM1 plays a critical role in PDAC progression is also unclear. In this study, by analyzing the expression of PGAM1 in clinical samples and performing the correlation analysis of PGAM1 expression to clinicopathological features, PGAM1 was found closely correlated with poor differentiation, metastatic tendency, and poor prognosis of PDAC. In vitro, we found that silencing PGAM1 can markedly decrease the migration and invasion of pancreatic cancer cell lines independent of proliferation. PGAM1 was also found to enhance the epithelial–mesenchymal transition (EMT) of pancreatic cancer cell lines by activating the Wnt/β-catenin pathway, and was modulated by the PI3K/Akt/mTOR pathway, which seems to be important for the high metastatic potential of PDAC. PGAM1 and hypoxia-inducible factor 1α (HIF-1α) also showed a positive mutual regulation. This study provides new understanding of the functions of PGAM1 and suggests PGAM1 as a potential therapeutic for PDAC metastasis.

MATERIALS AND METHODS

Patients and Specimens

Fifty-four human paraffin-embedded tissue samples with paired paracancerous tissues were retrospectively selected from the pathological database of China Medical University Affiliated Shengjing Hospital, China, between 2010 and 2016, for immunohistochemistry (IHC) analysis. This study was approved by China Medical University Affiliated Shengjing Hospital Medical Research and New Technology Ethics Committee. All patients received routine preoperative preparation and surgical therapy without any previous radiation or chemotherapy. The tumor grade was classified as well, moderately, or poorly differentiated. The pathological stage was defined according to the American Joint Committee on Cancer (AJCC) TNM staging system[12]. Both the tumor grade and pathological stage were evaluated by two pathologists independently.

Immunohistochemistry (IHC)

After deparaffinization with xylene and dehydration with a serial ethanol gradient, antigens were retrieved by heating slides in citrate buffer (pH 6.0) for 7 min on high heat. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide. Samples were incubated overnight at 4°C with anti-PGAM1 (1:100; Abcam, Cambridge, UK). Slides were stained with 3,3′-diaminobenzidine and hematoxylin, followed by dehydration through an ethanol gradient, and the slides were mounted. The percentage area of protein expression was evaluated by ImageJ software (1.48v; Wayne Rasband National Institutes of Health, Bethesda, MD, USA) (area% ≥50%: high expression; area% <50%: low expression).

Cell Lines and Cell Culture

The highly invasive and metastatic human pancreatic cancer cell lines Aspc-1 and Panc-1 were used. Cells were incubated in RPMI-1640 (Gibco, ThermoFisher Scientific, Waltham, MA, USA), supplemented with 10% fetal bovine serum (Certified, US origin), 10,000 U/ml penicillin G, and 10,000 μg/ml streptomycin (Gibco, ThermoFisher Scientific) at 37°C and 5% CO_2/95% air.

Cell Transfection

Small interfering RNAs (siRNAs) were purchased from GenePharma (GenePharma Co. Ltd., Shanghai, P.R. China). The PGAM1-siRNA sequence of the selected region to be targeted was 5′-CCA CAU CUG UAG ACA UCU UTT-3′. The HIF-1α-siRNA sequence of the selected region to be targeted was 5′-AAA ACU UCA GAU UCU UUA CUU-3′. Cells were transfected with siRNA using Lipofectamine iMAX (Thermo Fisher Scientific; Invitrogen, Carlsbad, CA, USA) in medium without antibiotics according to the manufacturer's instructions. Silencing efficiencies were verified by Western blot.

Cell Counting Kit-8 (CCK-8) Proliferation Assay

For the CCK-8 assay, 1×10^3 cells were seeded onto 96-well plates. Twenty-four hours after transfection, 10 μl of CCK-8 (Dojindo, Kumamoto, Japan) was added to each well at 24, 48, and 72 h, respectively. After an additional 3 h of incubation, the absorbance was measured at 450 nm using a multifunctional microplate reader (Synergy H1; BioTek, Winooski, VT, USA).

Cell Cycle Analysis

After transfection for 48–72 h, cells were harvested and fixed in 70% ethanol overnight at 4°C. Cells were then incubated in 100 μl of RNase A (KeyGen BioTech, Nanjing, China) in a 37°C water bath for 30 min. Cells were stained with 400 μl of propidium iodide (PI) at 4°C for 30 min (avoiding light) and analyzed for their distribution in different phases of the cell cycle on a FACScalibur flow cytometer using CellQuestPro software (Becton Dickinson, Franklin Lakes, NJ, USA).

Cell Apoptosis Analysis

After transfection for 48–72 h, cells were harvested and incubated with 1× annexin V binding solution (Dojindo). According to the manufacturer's instructions, cells were stained with PI or annexin V and then analyzed with a FACScalibur flow cytometer using CellQuestPro software (Becton Dickinson).

Cell Migration and Invasion Assays

After transfection for 24 h, Aspc-1 and Panc-1 cells were seeded onto filters of a 24-well Transwell chamber (Millipore, Burlington, MA, USA). For the migration

assay, 50,000 cells in 400 µl of serum-free RPMI-1640 medium were loaded into the upper chambers, while the lower chambers were supplemented with 0.6 ml of RPMI-1640 medium with 10% FBS. For the invasion assay, 50,000 cells were incubated in the upper chambers with membranes coated with Matrigel (1:6 dilution; BD Biosciences, San Jose CA, USA). After 24 h of incubation, the insert plates were rinsed with 1× PBS, and the upper surfaces of the membranes were scraped to remove cells. After fixation by methyl alcohol, cells on the underside of the membrane were stained with crystal violet at room temperature and counted under a microscope.

Western Blot Analysis

Protein extracts (40 µg) were resolved on 10% SDS-PAGE and detected by Western blot using the following antibodies: anti-PGAM1 (1:1,000; Abcam), anti-HIF-1α (1:500; Proteintech, Rosemont, IL, USA), anti-vimentin (1:500; Proteintech), anti-N-cadherin (1:500; Proteintech), anti-E-cadherin (1:500; Proteintech), anti-catenin-β (1:500; Proteintech), anti-pho-β-catenin (Y489) (1:500; Proteintech), anti-mTOR (1:500; Proteintech), anti-pho-mTOR (Ser2448) (1:500; Proteintech), anti-Akt (1:500; Proteintech), anti-pho-Akt (Ser473) (1:500; Proteintech), and anti-β-actin (1:2,000; Proteintech). Proteins were detected using ECL reagent (Santa Cruz Biotechnology, Dallas, TX, USA). Western blot signals were quantified using an Amersham Imager 600 (GE Healthcare, Little Chalfont UK), and band signals were expressed as relative protein amounts compared to β-actin.

Immunofluorescence Staining

Cells were grown on coverslips and then after fixing in 4% paraformaldehyde at 37°C for 30 min the cells were blocked with 5% BSA at room temperature for 2 h. After incubation with primary antibody overnight at 4°C and washing by PBS, cells were incubated with a fluorescence-conjugated secondary antibody [anti-PGAM1, 1:40, Cy3-conjugated Affinipure goat anti-mouse IgG (H+L); Proteintech] and [anti-HIF-1α, 1:40, Alexa Fluor 488-conjugated Affinipure goat anti-rabbit IgG (H+L); Proteintech] for 1 h. Nuclei were counterstained with 4′-6-diamidino-2-phenylindole (DAPI; Life Technologies, Carlsbad, CA, USA) for 5 min and observed and imaged using a fluorescence microscope (Eclipse Ci; Nikon, Tokyo, Japan).

Statistical Analysis

Statistical analyses and graphics were conducted using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). Results are presented as the mean ± standard error of mean (SEM) from three independent experiments. Comparisons of quantitative data were analyzed by Student's *t*-test (two-tailed; $p<0.05$ was considered statistically significant). The relationship between PGAM1 and clinicopathological features was analyzed using chi-square test. The Kaplan–Meier method was used to construct the survival curve.

RESULTS

PGAM1 Is Associated With Metastasis and the Poor Prognosis of PDAC

The expression of PGAM1 in PDAC was examined using IHC from the tissue samples of 54 patients. Of the 54 cases, 30 tissues were collected from male patients (55.6%) and 24 from female patients (44.4%) with an average age of 57.19 ± 10.32 years. Histopathologic subtypes included 21 well-differentiated adenocarcinomas (38.9%), 15 moderately differentiated adenocarcinomas (27.8%), and 18 poorly differentiated adenocarcinomas (33.3%); 11 cases (20.3%) showed regional lymph node metastasis, and 13 cases (24.1%) had invasion to surrounding organs and tissues. According to the percentage area of protein expression, the samples were classified into the PGAM1 high-expression group or the PGAM1 low-expression group (area% ≥ 50%: high expression, area% < 50%: low expression), and PGAM1 was predominantly expressed in the cytoplasm and cell membrane. By IHC, PGAM1 was detected significantly overexpressed in 54 primary cancer tissues compared to paracancerous tissues (Fig. 1A), significantly overexpressed in poorly differentiated pancreatic cancer tissues compared to moderately and well-differentiated tissues (Fig. 1A and B), and significantly overexpressed in metastatic lesions compared to in primary sites (Fig. 1A). The correlation between PGAM1 expression and several clinicopathological features of PDAC progression was also summarized. There was no apparent relevance between the expression of PGAM1 and patients' age, gender, tumor size, and CA199 levels. PGAM1 expression was significantly correlated with differentiation degree ($p=0.01$) (Fig. 1A and B), metastasis situation ($p=0.02$) (Fig. 1A and B), and clinical stage (stage III/IV vs. stage I/II, $p=0.033$). Kaplan–Meier survival analysis was conducted to determine the prognostic significance of PGAM1 expression. The results suggested that PDAC patients with higher levels of PGAM1 had a worse overall survival (OS) ($p < 0.05$) (Fig. 1C).

Silencing PGAM1 Decreased the Proliferation of Aspc-1 and Panc-1 Cell Lines Without Influencing Cell Apoptosis

To investigate the influence of PGAM1 on the proliferation of Aspc-1 and Panc-1 cells, we performed CCK-8, cell cycle, and cell apoptosis analyses. At 24, 48, and 72 h after silencing PGAM1 by siRNA, CCK-8 assay was performed. The results demonstrated that silencing PGAM1 decreased the proliferation of pancreatic cancer cells with an obvious effect at 72 h (Fig. 2A). Cell cycle analysis showed a significant S phase arrest at 72 h after

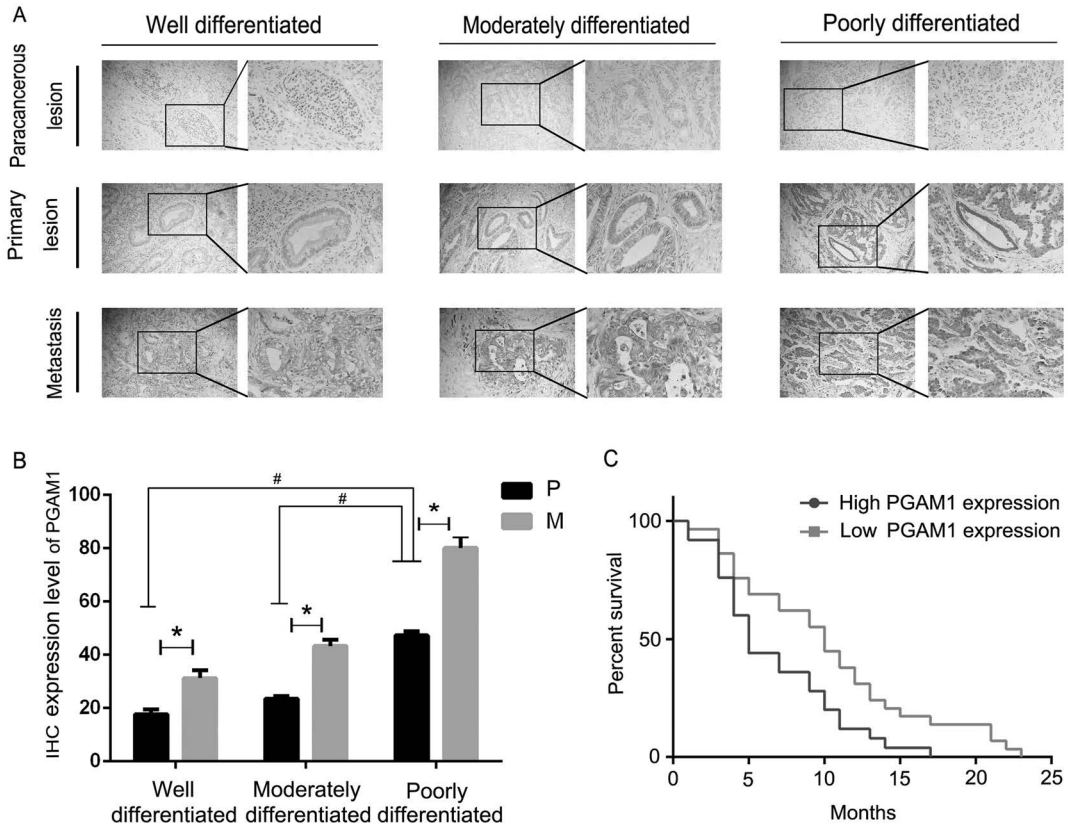

Figure 1. Phosphoglycerate mutase 1 (PGAM1) expression in pancreatic cancer tissues and indications of a poorer prognosis for patients with pancreatic cancer. (A) The expressions of PGAM1 in pancreatic ductal adenocarcinoma (PDAC) tissues versus paracancerous tissues, various differentiated PDAC tissues, and PDAC primary tissues versus PDAC metastatic tissues using immunohistochemistry. (B) The expression level of PGAM1 is higher in PDAC metastatic sites compared to primary lesion of PDAC (P, primary lesion of PDAC; M, metastasis lesion of PDAC). The expression level of PGAM1 is higher in poorly differentiated PDAC tissue compared to moderately and well-differentiated PDAC tissue (*$p<0.05$ or #$p<0.05$). (C) Kaplan–Meier analysis of the correlation between the PGAM1 level and overall survival of pancreatic cancer patients (*$p<0.05$).

transfection (Fig. 2C), but no significant changes in cell apoptosis were found (Fig. 2B).

Silencing PGAM1 Decreased the Migration and Invasion of Aspc-1 and Panc-1 Cell Lines

To verify the role of PGAM1 on the migration and invasion abilities of Aspc-1 and Panc-1 cells, we performed Transwell analysis. The analysis was performed 48 h after silencing PGAM1. The results demonstrated that silencing PGAM1 decreased pancreatic cancer cell migration and invasion in a marked manner (>60%) (Fig. 3A). In addition, at 48 h after transfection, there was no significant difference in cell proliferation (Fig. 2A). This indicates that the inhibition of PGAM1 on migration and invasion is independent of proliferation.

PGAM1 Promotes EMT Through Activating the Wnt/β-Catenin Pathway

Because PGAM1 affects the migration and invasion of pancreatic cancer cell lines, it might influence the EMT of pancreatic cancer cell lines. To verify this hypothesis, we first performed immunofluorescence staining to investigate the expression sites of PGAM1 in pancreatic cancer cells and the influence of PGAM1 on cell morphology. The results showed that PGAM1 was mainly expressed on cytoplasm and cell membrane. After silencing PGAM1, the scatter patterns of Aspc-1 and Panc-1 cell lines reduced with the contraction of pseudopodium, and the decreased intracellular space made them more aggregated (Fig. 4D).

To verify the influence of PGAM1 on several EMT indicators, we performed Western blot. The results showed that silencing PGAM1 increased the E-cadherin expression level and decreased the expression levels of N-cadherin and vimentin (Fig. 3B). Because the Wnt/β-catenin pathway is a crucial pathway to modulate EMT, we further performed Western blot to investigate the relationship between PGAM1 and the Wnt/β-catenin pathway. The result showed that silencing PGAM1 increased the phosphorylation level of β-catenin (Fig. 3C), which

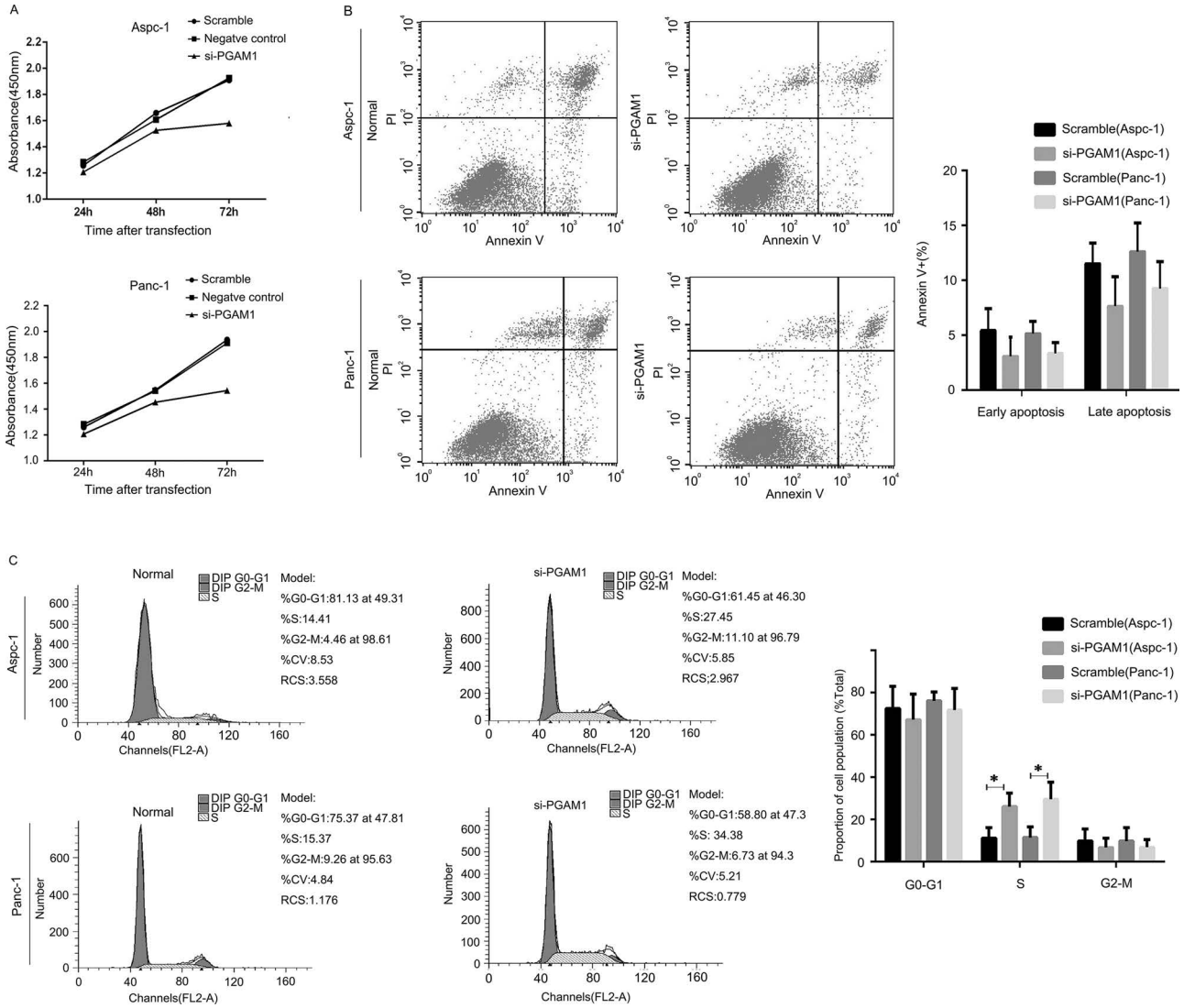

Figure 2. Silencing PGAM1 decreased proliferation with S phase arrest of Aspc-1 and Panc-1 cells, but without influence on cell apoptosis. (A) Silencing PGAM1 with small interfering RNA (siRNA) decreased the proliferation of Aspc-1 and Panc-1 cells as measured by a cell counting kit-8 (CCK-8) proliferation assay (invalid siRNA was used as a negative control). (B) Flow cytometry revealed that silencing PGAM1 did not influence apoptosis of Aspc-1 and Panc-1 cells. (C) Flow cytometry revealed that silencing PGAM1 induced S phase arrest of Aspc-1 and Panc-1 cells without significantly affecting apoptosis (*$p<0.05$).

indicated inhibition of the Wnt/β-catenin pathway. In turn, after we treated Aspc-1 and Panc-1 cells with the Wnt/β-catenin pathway inhibitor XAV-939, no change in the expression level of PGAM1 with statistical significance was found. These data indicated that PGAM1 may promote the EMT of Aspc-1 and Panc-1 cells by activating the Wnt/β-catenin pathway.

PGAM1 Acts as a Downstream Target of the PI3K/Akt/mTOR Pathway

In our previous proteomics analysis (unpublished data), PGAM1 was the top upregulated protein in highly invasive and metastatic pancreatic cancer cell lines. Several pathways, such as PI3K/Akt and mTOR, were enriched with higher activity in highly aggressive pancreatic cancer cell lines. Moreover, the PI3K/Akt/mTOR pathway was indicated to play essential roles in tumor aggression. Therefore, in this research, we investigate the relationship between PGAM1 and the PI3K/Akt/mTOR pathway. Western blot showed that the expression level of PGAM1 decreased when treated by the PI3K/Akt pathway inhibitor, wortmannin, and the mTOR pathway inhibitor, rapamycin (Fig. 4A and B). However, when silencing PGAM1 by siRNA, the phosphorylation levels of Akt (ser473) and mTOR (ser2448) remained relatively unchanged (Fig. 4A and B).

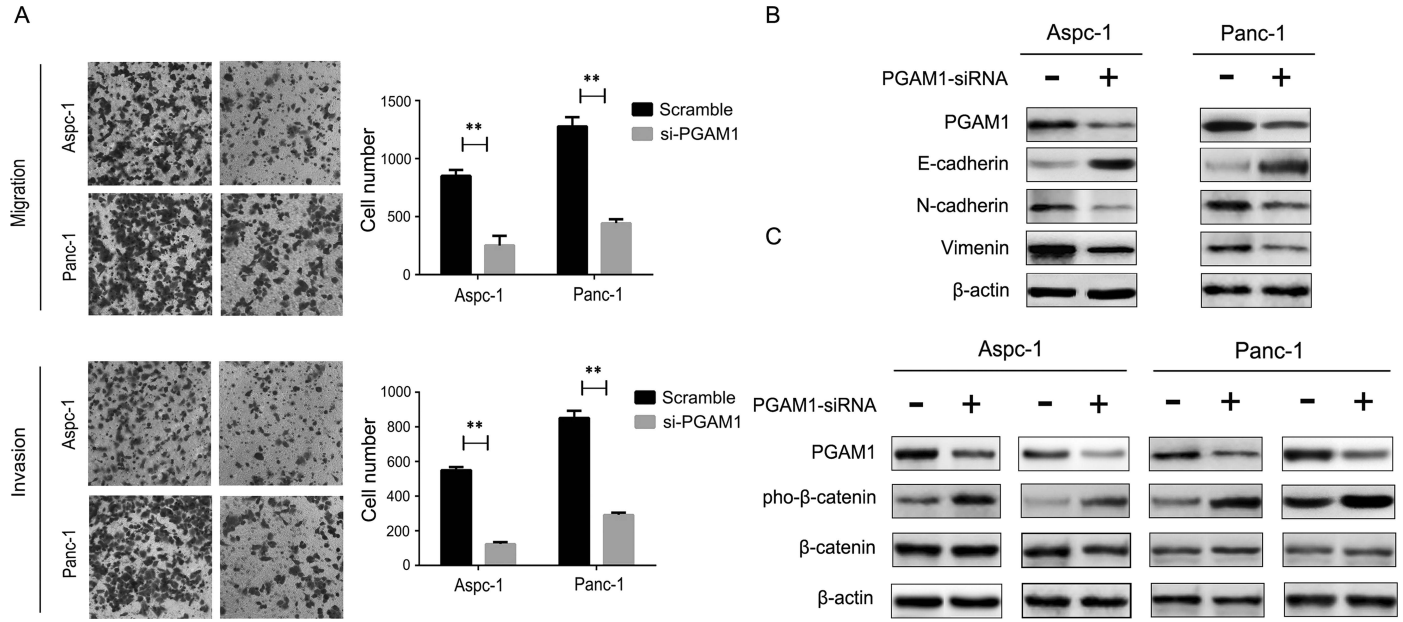

Figure 3. Silencing PGAM1 decreased the migration and invasion of Aspc-1 and Panc-1 cells, and inhibited cell epithelial–mesenchymal transition (EMT) through the Wnt/β-catenin pathway. (A) Silencing PGAM1 inhibited the migration and invasion of Aspc-1 and Panc-1 cells as measured by Transwell assay (**$p<0.01$). (B) Silencing PGAM1 increased the expression of E-cadherin and decreased the expression of N-cadherin and vimentin in Aspc-1 and Panc-1 cells as shown by Western blot. (C) The interaction between PGAM1 and the Wnt/β-catenin pathway. Cells were treated with siRNA against PGAM1 (PGAM1-siRNA) or XAV-939 or untreated (Scramble).

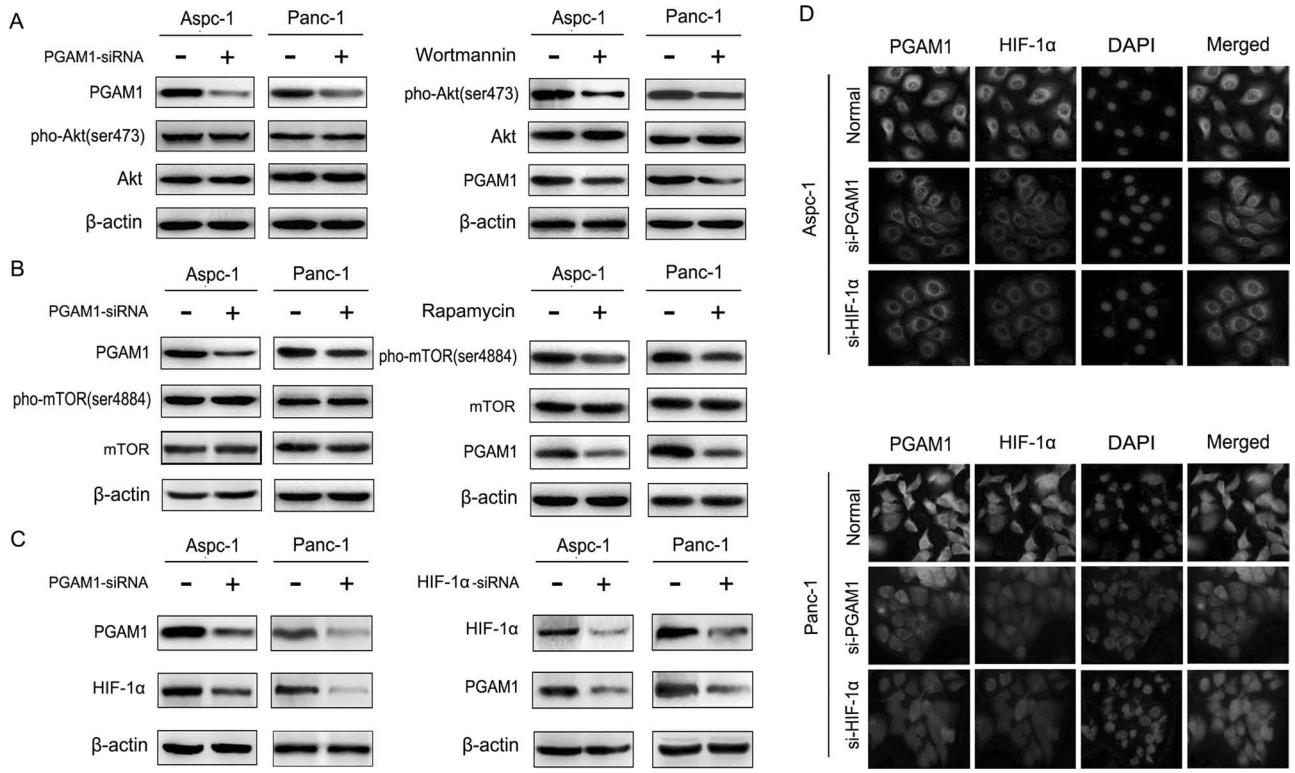

Figure 4. PGAM1 is modulated by the PI3K/Akt/mTOR pathway and has a positive mutual interaction with HIF-1α. (A) The relationship between PGAM1 and the PI3K/Akt pathway. Cells were treated with an siRNA to PGAM1 (si-PGAM1) or wortmannin or untreated (Scramble). (B) The relationship between PGAM1 and the mTOR pathway. Cells were treated with si-PGAM1 or rapamycin or untreated (Scramble). (C) Positive mutual interaction between PGAM1 and HIF-1α as shown by Western blot. Cells were treated with siRNA-PGAM1, siRNA-HIF-1α or untreated (Scramble). (D) The positive mutual interaction and the colocation between PGAM1 and HIF-1α as shown by immunofluorescence analysis. 4′-6-Diamidino-2-phenylindole (DAPI) was used to stain the nucleus. Original magnification: 400×.

PGAM1 and HIF-1α Have a Mutual Positive Regulation

In our previous proteomics analysis (unpublished data), the HIF-1α pathway was also enriched with higher activity in highly aggressive pancreatic cancer cell lines. Because HIF-1α plays an important role in tumor metabolism and aggression, we performed Western blot to investigate the relationship between PGAM1 and HIF-1α. When silencing PGAM1 by siRNA, a significant decrease in both PGAM1 and HIF-1α expression levels can be found. In turn, when silencing HIF-1α by siRNA, a significant decrease in both PGAM1 and HIF-1α expression levels can also be found (Fig. 4C). These data indicated that there was a positive mutual interaction between PGAM1 and HIF-1α. Moreover, to investigate the expression sites of PGAM1 and HIF-1α, we performed immunofluorescence staining. The results showed that PGAM1 and HIF-1α are coexpressed on the cytoplasm and on the cell membrane, especially on the cytoplasm. When silencing PGAM1 or HIF-1α, the expression intensity of both proteins decreased at the same site, mainly in the cytoplasm. The result of immunofluorescence staining indicated that there may be a direct mutual regulation between these two proteins (Fig. 4D).

DISCUSSION

In this study, by analyzing the specimens of 54 pancreatic cancer cases, we found that PGAM1 expression was significantly correlated with poor differentiation, metastasis, advanced clinical stage, and poor prognosis, without correlating with age, gender, CA199, and tumor size. In vitro, the role of PGAM1 in promoting cell proliferation was revealed without significant effect on cell apoptosis. The obvious promoting role of PGAM1 on cell migration and invasion was also detected, independent of influencing proliferation.

PGAM1 is a glycolytic protein that catalyzes the conversion of 3-PG to 2-PG in glycolysis[4]. As an important glycolytic protein, most investigations have centered on its metabolic function in tumor progression. Hitosugi et al.[13] inhibited PGAM1 with a small molecule inhibitor, PGMI-004A, and subsequently found increased 3-PG and

decreased 2-PG levels, leading to significantly decreased glycolysis and ATP production, as well as attenuated cell proliferation in a human non-small cell lung carcinoma cell line, H1299. In this research, we found that silencing PGAM1 decreased the proliferation of both Aspc-1 and Panc-1 cells with S phase arrest, without influencing cell apoptosis. This result means that PGAM1 may promote tumor growth by promoting cell mitosis, which is indeed closely related to its role of boosting energy production.

In this research, in addition to the impact on cell proliferation, we also found that silencing PGAM1 decreased the migration and invasion of both Aspc-1 and Panc-1 cells. This promoting role was not influenced by cell proliferation, because we examined the migration and invasion abilities of cells at 48 h after transfection, at which time there was no significant change in cell proliferation. The promoting role of PGAM1 in the migration and invasion of PDAC cells has seldom been reported. We think that this finding is different from previous research that centers on the role of PGAM1 on tumor proliferation through metabolic alteration. From a study of Zhang et al.[10], PGAM1 was indicated to interact with a nonglycolytic protein α-smooth muscle actin (ACTA2) to influence cancer cell migration in human embryonic kidney (HEK) 293 cells. Therefore, we think that the role of PGAM1 on the migration and invasion of PDAC may be mostly nonglycolysis associated. To confirm this hypothesis, we further explored the role of PGAM1 on EMT of Aspc-1 and Panc-1 cells. EMT is a biological process in which epithelial cells are transformed into mesenchymal cells. This change is characterized by the downregulation of the epithelial molecular marker E-cadherin, and by the upregulation of mesenchymal molecular markers such as N-cadherin and vimentin[14]. During the EMT process, pancreatic cancer cells gradually gain malignant features, such as invasive and metastatic capabilities, as well as a chemoresistant character[15,16]. The Wnt/β-catenin pathway is a recognized signal pathway to modulate tumor EMT. It is involved in the development of many types of cancers including hepatocellular carcinoma, colorectal carcinoma, lung cancer, and malignant breast tumors[17], and has important roles in tumor initiation and progression. In the Wnt/β-catenin pathway, β-catenin acts as a central mediator, involved in the regulation and coordination of cell–cell adhesion and gene transcription[18]. β-Catenin is thought to induce EMT to mobilize cells and weaken cellular connections. β-Catenin also promotes the production of extracellular matrix (ECM) components, such as type I collagen and fibronectin, to facilitate the falling off of cancer cells. In all, EMT is a crucial step in pancreatic cancer metastasis. The relationship between PGAM1 and EMT of pancreatic cancer cells has never been investigated. In this study, we found that silencing PGAM1 increased E-cadherin expression level and decreased N-cadherin and vimentin expression levels, and reduced the scatter patterns and pseudopodia stretch of Ascp-1 and Panc-1 cell lines. Further pathway analysis indicated that silencing PGAM1 with siRNA elevated the phosphorylation levels of β-catenin. However, after treating Aspc-1 and Panc-1 cell lines with the Wnt/β-catenin pathway inhibitor XAV-939, PGAM1 showed no statistical expression change. All these data indicated that PGAM1 might promote EMT of Aspc-1 and Panc-1 cell lines by activating the Wnt/β-catenin signaling pathway. We think this result may be one of the nonglycolytic mechanisms by which PGAM1 promotes PDAC metastasis.

In our previous proteomics analysis (unpublished data), PGAM1 was the top upregulated protein in highly invasive and metastatic pancreatic cancer cell lines. The PI3K/Akt/mTOR pathway and HIF-1α pathway were enriched with higher activity in highly aggressive pancreatic cancer cell lines. To further clarify the involved correlation, we explored the relationships between PGAM1 and the PI3K/Akt/mTOR pathway and the relationship between PGAM1 and HIF-1α. The PI3K/Akt/mTOR pathway is an intracellular signaling pathway regulating cell growth, proliferation, invasion, survival, energy metabolism, and other important life processes. PI3K activation phosphorylates and activates Akt, localizing it in the plasma membrane[19]. Akt can have several downstream effects, including on mTOR, and can affect HIF-1α. Activation of the PI3K/Akt/mTOR pathway promotes many metabolic pathways, including glycolysis[20]. In many cancers, this pathway is overactive and promotes proliferation and metastasis[21]. HIF-1α is a transcription factor that responds to a hypoxic cellular environment. The expression of HIF-1α is found overexpressed in several human tumors such as bladder, breast, colon, hepatocellular, ovarian, pancreatic, prostate, and renal tumors[22]. It is well recognized that HIF-1α has a critical role in maintaining energy metabolism, angiogenesis, proliferation, and metastasis of tumor cells[23].

In this research, we treated Aspc-1 and Panc-1 cells with the PI3K/Akt pathway inhibitor, wortmannin, and mTOR pathway inhibitor, rapamycin, after which PGAM1 showed significantly decreased expressions. In turn, after silencing PGAM1 with siRNA, the phosphorylation levels of Akt (ser473) and mTOR (ser2448) remained relatively unchanged. These results indicate that PGAM1 is modulated by the PI3K/Akt/mTOR pathway as a downstream target.

By exploring the relationship between PGAM1 and HIF-1α, we found that after silencing PGAM1 or HIF-1α, the cellular expression intensity of both proteins decreased at the same cellular location. PGAM1 and HIF-1α thus were thought to regulate each other in a positive manner and may have a direct interaction. It is clear that HIF-1α can enhance the expression of matrix metalloproteinases,

which can degrade most of the components of the ECM to facilitate the invasion and metastasis of cancer cells[24,25]. PGAM1 may also have roles in promoting the degradation of ECM to facilitate the invasion and metastasis of PDAC by interacting with HIF-1α. These results may explain the role of PGAM1 in the regulation of pancreatic cancer cell invasion and metastasis.

In conclusion, this study demonstrated that the high expression of PGAM1 is closely correlated with metastasis, advanced clinical stage, and poor prognosis of PDAC. PGAM1 was found to promote pancreatic cancer cell proliferation, migration, invasion, and EMT, which might be through interacting with the PI3K/Akt/mTOR pathway, HIF-1α, and the Wnt/β-catenin pathway. PGAM1 is considered as a potential therapeutic target in PDAC.

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