Original Paper

PGK1 is a Potential Survival Biomarker and Invasion Promoter by Regulating the HIF-1 α -Mediated Epithelial-Mesenchymal Transition Process in Breast Cancer

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Key Words

Breast cancer • PGK1 • HIF-1 α • Epithelial-mesenchymal transition

Abstract

Background/Aims: Glycolysis, a multi-step enzymatic reaction, is considered to be the root of cancer development and progression. The aim of this study is to figure out which glycolysis enzyme participates in the progression of breast cancer and its possible mechanisms. **Materials:** We firstly screened out PGK1 by performing an RT-PCR array of glycolysis-related genes in three paired breast cancer samples, and further investigated PGK1 using TCGA and our own database. The effect and mechanism of PGK1 on cell invasion was further explored both *in vitro* and using patient samples. **Results:** PGK1 was most upregulated in T3N0 with distant metastases compared to those with no metastases. In the TCGA database, high PGK1 expression predicted poor overall survival (OS) in breast cancer and some other cancers ($P < 0.001$). In the validation cohort, high PGK1 expression was significantly correlated with larger tumor size ($P = 0.011$) and advanced TNM stage ($P = 0.033$), and PGK1 expression was an independent prognostic factor for OS and disease free survival (DFS) in both univariate and multivariate regression analyses ($P < 0.05$). Functional studies indicated that knockdown of PGK1 expression significantly inhibited invasion and reversed the epithelial-mesenchymal transition process in breast cancer cells ($P < 0.05$). Mechanistically, PGK1 increased HRE luciferase activity in a dose-dependent manner, while silencing PGK1 expression decreased HRE activity. **Conclusion:** High PGK1 expression was associated with poor prognosis in breast cancer, because PGK1 and HIF-1 α formed a positive feed-forward loop and thus stimulated breast cancer progression and metastases. Based on these results, PGK1 may serve as a promising biomarker and target therapy for breast cancer.

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Introduction

Cancer cells exhibit a series of common features, including uncontrolled proliferation, resistance to programmed cell death, and enhanced metastasis ability [1, 2]. Of these, alteration of metabolic pathways is regarded as a driving force of tumor initiation and progression [2, 3]. Tumor cells always have a hypoxic microenvironment, and to overcome this harsh environment, cancer cells develop adaptive responses to hypoxia by activating hypoxia-inducible genes [4-6]. Hypoxia is a major characteristic of cancer, and in hypoxic conditions, metabolism in cancer cells is reprogrammed from oxidative phosphorylation to glycolysis [7, 8]. A feature of this phenomenon, called the Warburg effect or aerobic glycolysis, is increased lactate production even at normal oxygen concentrations. Many glucose metabolic enzymes are unregulated or downregulated in cancer tissues, and have been validated as multifaceted enzymes that are involved in various processes in cancer metastases and progression [9-12].

Phosphoglycerate kinase 1 (PGK1), a rate-limiting enzyme in the glycolysis process, catalyzes the conversion of 1, 3-diphosphoglycerate to 3-phosphoglycerate and generates a molecule of ATP [3]. PGK1 and pyruvate kinase M2 (PKM2) are the only enzymes that control production of ATP during aerobic glycolysis in cancer cells [1, 3]. In addition to the important role of PGK1 in glycolysis, there are reports of its role in cancer. High PGK1 expression predicts poor survival in endometrial cancer [13] and gastric cancer [14]. However, Lu *et al.* demonstrated that low expression of PGK1 is associated with progression and correlated with poor prognosis in patients with gallbladder cancer [15].

Considering the conflicting results of PGK1 in different cancers and that the clinical significance of PGK1 in breast cancer has not been fully investigated, we focused on PGK1 in breast cancer progression and metastases. We found that high PGK1 expression predicted shorter survival in breast cancer and other cancers. An *in vitro* study found that silencing PGK1 impaired cell migration and the reverse epithelial-mesenchymal transition (EMT) process. Mechanistically, PGK1 and hypoxia-inducible factor 1- α (HIF1 α) formed a positive loop that stimulated breast cancer progression.

Materials and Methods

Patient samples

Patients in TCGA. The TCGA gene expression database was used to study PGK1 in various cancers. TCGA is a project to catalogue gene expression or mutations responsible for cancer using bioinformatics methods and genome sequencing. To explore TCGA datasets, we used cBioPortal (<http://www.cbioportal.org/index.do>) to find pathways of interest in one or more cancer types [16-18]. Sequencing of the dataset was based on the Illumina HiSeq platform [19]. Inclusion criteria were as follows: patients diagnosed by pathological examination, with no pretreatment, with fully characterized tumors and intact overall survival (OS) information. Expression data of each PGK1 family were standardized, and the standardization between different samples was performed using the Limma package in R software [20].

Patient samples for primary screening. To find the glycolytic enzymes that may be involved in breast cancer progression, six breast primary tumor tissues were used in a PCR array for glycolytic enzymes. All primary tumors were pathologically diagnosed with T3N0 and were categorized as Luminal A. Three patients diagnosed with distant metastases were regarded as having high metastasis abilities, while three patients with no distant metastases were used as controls. Primary tumors were immediately put in RNA later and stored at -20°C for further study.

Patients in the validation cohort. The patient samples used for external validation have been described previously [21]. All underwent intentionally curative surgical resection and received standard adjuvant therapy and follow up according to the National Comprehensive Cancer Network (NCCN) guidelines for breast cancer. All patients were restaged according to the 8th edition of the TNM-UICC/AJCC classification.

The OS was defined as the time from treatment to death from any cause, and the DFS was defined as the time from treatment to the first recurrence or death. Patients who were alive at last follow-up were censored for analysis.

Cell culture

Human breast cancer cell lines (MDA-MB-231 and MCF-7) were originally obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA) and were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco-Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco-Invitrogen), 100 U/ml penicillin, and 100 µg/ml streptomycin. Cells were incubated at 37°C in a humidified incubator containing 5% CO₂.

Lentivirus production and stable cell line selection

To generate shRNA expression constructs against PGK1, a pLKO.1 TRC cloning vector (Plasmid10878, Addgene, Cambridge, MA, USA) was used. The 21 bp shRNA target against PGK1 was 5'-GCAAGGATGTTCTGTTCTTGA-3'. pLKO.1-scramble shRNA (Plasmid1864, Addgene) with limited homology with any known sequences in humans was used as a negative control. Lentiviral particles were produced by co-transfection of pLKO.1-shPGK1 constructs with psPAX2 and pMD2.G into HEK-293T cells at a ratio of 4:3:1. Cell lines were obtained by infection of MDA-MB-231 and MCF-7 cells with lentiviral particles followed by puromycin selection.

Total RNA extraction and real-time polymerase chain reaction (real-time PCR)

Total RNA was extracted from breast cancer tissues and cells with the SV Total RNA Isolation system (Promega, Madison, WI, USA) according to the manufacturer's protocol. A PrimeScript™ RT Master Mix (Perfect Real Time) kit (RR036A, Takara) was used to synthesize first-strand cDNA from total RNA. mRNA expression was determined by real-time PCR using SYBR Green in an ABI 7900HT (Applied Biosystems, USA).

Sequences of PCR primer pairs were: human β-actin, forward 5'-CTACGTCGCCCTGGACTTCGAGC-3' and reverse 5'-GATGAGCCGCCGATCCACACGG-3'; and human PGK1, forward 5'-ATGCTGAGGCTGTCACTCGG-3' and reverse 5'-CACAGCAAGTGGCAGTGTCTCC-3'. Relative gene expression values were determined using the 2^{-ΔΔCT} method as previously described [21]. The human β-actin gene was used as an endogenous control for sample normalization. Results were presented as fold increases relative to the expression of human β-actin.

Western blot analysis

Adherent cells were rinsed with deionized water and lysed with lysis buffer (RIPA Lysis Buffer; Thermo Scientific Pierce Protein Biology). Western blots were carried out as previously described in detail [21]. The membrane was either incubated overnight at 4°C in Tween-TBS-milk 5% with primary antibodies PGK1 (1:1000; Abcam, Cambridge, UK), anti-E-cadherin antibody (ab40772, Abcam, 1:1000), anti-N-cadherin antibody (ab18203, Abcam, 1:1000), anti-vimentin (ab92547, Abcam, 1:1000), or mouse anti-human β-actin monoclonal antibody (ab133626, Abcam, 1:1000). After three washes with Tween-TBS and one with TBS, immunoreactivity was detected using enhanced chemiluminescence (Pierce, Thermo Scientific, USA).

Immunohistochemical staining (IHC)

Immunohistochemistry for PGK1 was performed as previously described [21]. Briefly, paraffin sections were baked for 60 min at 70°C, de-paraffinized in xylene, rehydrated in an ethanol gradient (75%, 95%, 100%), and then the sections were placed in 3% H₂O₂ to neutralize endogenous peroxidase for 30 min. Antigen retrieval was processed with citrate buffer (pH=6.0) in a pressure cooker. After antigen retrieval, the sections were incubated with PGK1 primary antibody (1:200; Abcam, Cambridge, UK) and secondary antibody. The sections were then stained with DAB (3, 3-diaminobenzidine) terminated in PBS, and then counterstained with hematoxylin. Based on the staining intensity of PGK1 in each case, the standards for scoring were as follows: 0, negative; 1, weak; 2, moderate; and 3, strong. Zero and 1 were regarded as low expression and 2 and 3 were regarded as high expression. Two observers scored staining intensity independently.

CCK8 assay

Cells were plated in 96-well plates at a density of 2×10^3 cells/well and cultured for 24, 48, 72, or 96 h. Ten μ L of CCK8 solution (Dojindo Laboratories, Kumamoto, Japan) was then added to each well, plates were incubated for 2 h at 37°C and read at 450 nm. All experiments were performed three times.

Colony formation assay

Cells were seeded in six-well plates at a density of 200 per well and cultured at 37°C for 14 days. Cells were washed twice with PBS, fixed with 4% paraformaldehyde, stained with Giemsa for 10 minutes, and washed three times with double distilled H₂O. Colonies containing more than 50 cells were counted using light microscopy. Colony-forming efficiency (CFE %) was defined as the ratio of the number of colonies formed in culture to the number of cells inoculated.

In vitro cell invasion assays

Invasion assays were conducted in a 24-well Transwell chamber (Costar, Cambridge, MA) with uncoated membranes or membranes coated with Matrigel (BD Biosciences, San Jose, CA, USA). Cells (4×10^4 per well) were resuspended in serum-free medium and seeded into the upper chamber. Culture medium containing 10% fetal bovine serum was added to the lower chamber as the chemoattractant. The cells were incubated in a humidified incubator at 37°C for 48 hours. Non-invading cells in the upper chambers were removed with cotton swabs. The cells attached to the lower surface were fixed and stained with crystal violet. The number of cells that attached to the lower surface was counted in five random fields under a microscope ($\times 200$).

Ethics statement

This study was approved by the Northern Jiangsu People's Hospital Research Ethics Committee. The methods were carried out in accordance with the approved guidelines. Informed consent was obtained from each patient according to the committee's regulations.

Statistical analysis

The statistical associations between clinical parameters and IHC staining were tested using a χ^2 test to compare the expected frequencies and the observed frequencies in two groups. The five-year OS and DFS were calculated with the Kaplan-Meier method to estimate the survival and hazard functions of censored data. The difference in survival between the groups at each observed event time was compared using the logrank test. Variables that seemed to be significantly associated with survival in univariate analysis were included in multivariate analysis, which was performed with the Cox proportional hazard model to relate survival to a collection of other risk factors. All *in vitro* experiments were performed independently with three replicates. A *P* value of <0.05 was considered statistically significant. All statistical analyses were carried out using SPSS for Windows, version 21.0 (SPSS, Chicago, IL).

Results

PGK1 might promote tumor metastasis in breast cancer

Glycolysis is catalyzed by a series of glycolytic enzymes. To screen the candidate enzymes that may be involved in breast cancer progression and metastasis, an RT-PCR array was used to determine the mRNA levels of various glycolytic enzymes in three paired primary breast cancer tissues with or without distant metastases as mentioned above. The results indicated that the up-regulated expression of PGK1 and down-regulated expression of FBP1 were most significant in patients with distant metastases compared with those without distant metastases (Fig. 1A and 1B). As FBP1 has been thoroughly classified [10], we chose PGK1 for further study.

High PGK1 expression correlated with shorter OS in breast cancer and other tumors

As PGK1 may promote breast cancer metastases, we then asked whether PGK1 could predict survival in breast cancer. We first investigated PGK1 expression in the TCGA database

Fig. 1. PGK1 is upregulated in patients with high invasive ability. (A) Heatmap of glycolytic genes in three paired breast cancer samples using RT-PCR with results normalized by Z-score. Each column represents a specimen denoted below and each row represents a gene denoted on the right. Red indicates genes that were upregulated and green indicates genes that were downregulated. P1-3: patients diagnosed with T3N0M0; P1'-3': patients diagnosed with T3N0M1. (B) PGK1 was most upregulated, while FBP1 was significantly downregulated in T3N0M1 patients compared to those diagnosed with T3N0M1.

Fig. 2. PGK1 is a novel biomarker for survival in various cancers. High PGK1 implies poor survival in breast cancer (A), cervical cancer (B), head and neck cancer (C), liver cancer (D), and pancreatic cancer (E). Patients were divided into PGK1 high or low expression subgroups with optimal cutoff (left panel) or median value (right value). All $P < 0.001$.

and found that high PGK1 expression predicts poor survival in breast cancer (HR: 1.984, 95% CI 1.481-2.656, $P < 0.001$). PGK1 mRNA expression levels had a nearly normal distribution, so we then divided the patients into high and low PGK1 expression subgroups based on the median level. The five year OS was 88.2% and 73.1% for patients in the high and low PGK1 expression groups, respectively ($\chi^2 = 8.751$, $P = 0.003$) (Fig. 2A). Furthermore, we found that high PGK1 expression also implied short OS in head and neck cancer, cervical cancer, liver cancer, and pancreatic cancer using the THPA database (Fig. 2B-2E).

PGK1 was an independent prognostic factor for survival in the validation cohort

A total of 185 patients were used for external validation of clinical significance of PGK1 in breast cancer. Patient characteristics are shown in Table 1. High PGK1 expression was found in 124 (67.0%) patients. Representative figures are in Fig. 3A. Chi-square analysis demonstrated that high PGK1 expression was significantly correlated with larger tumor size ($P = 0.011$) and advanced TNM stage ($P = 0.033$) (Fig. 3B and 3C) (Table 1).

Table 1. Comparison of baseline clinical characteristics based on PGK1 expression level

Variable	n	PGK1 Low (%)	PGK1 High (%)	χ^2 value	P value
Age				0.013	0.911
	≤55	86 (28)	58 (46.8)		
	55	99 (33)	66 (53.2)		
Tumor Diameter				6.438	0.011
	<2cm	73 (32)	41 (33.1)		
	≥2cm	112 (29)	83 (66.9)		
Grade				2.016	0.365
	I	96 (35)	61 (49.2)		
	II	70 (20)	50 (40.3)		
	III	19 (6)	13 (10.5)		
Stage				6.801	0.033
	I	26 (13)	13 (10.5)		
	II	75 (34)	41 (33.1)		
	III	84 (14)	70 (56.5)		
Subtypes				2.794	0.424
	Luminal A	88 (34)	54 (43.5)		
	Luminal B	42 (12)	21 (16.9)		
	Basal-like	33 (8)	25 (20.2)		
	Her-2+	22 (7)	15 (12.1)		
ER Status				2.966	0.085
	Negative	48 (11)	37 (29.8)		
	Positive	137 (50)	87 (70.2)		
PR Status				0.773	0.379
	Negative	72 (21)	51 (41.1)		
	Positive	113 (40)	73 (58.9)		
HER-2/neu Status				0.209	0.648
	Negative	152 (49)	103 (83.1)		
	Positive	33 (12)	21 (16.9)		

Fig. 3. High PGK1 expression predicts poor survival in validation cohorts. (A) Representative images of high (upper panel) and low (lower panel) PGK1 expression in breast cancer. The original figure was 100X in the left panel, amplified to 200X in the right panel. (B, C) High PGK1 expression was significantly correlated with larger tumor size (B) and advanced tumor stage (C). Kaplan-Meier analyses were performed to evaluate the correlation of PGK1 expression and patients' survival. Overexpression of PGK1 was correlated with worse OS (D) and DFS (E) ($P < 0.001$).

Five-year OS in the high PGK1 expression subgroup was 67.9% compared with 90.0% in the low expression subgroup. The log-rank analysis showed that the differences were significant ($\chi^2 = 14.694$, $P < 0.001$, Fig. 3D). Similarly, patients with low PGK1 expression levels showed better five-year DFS than those with high expression (83.4% vs. 54.5%, $\chi^2 = 13.984$, $P < 0.001$) (Fig. 3E). Furthermore, multivariate Cox regression analysis showed that PGK1 expression was an independent factor for predicting both OS (HR: 3.155, 95% CI: 1.316 -7.569, $P = 0.010$) (Table 2) and DFS (HR: 2.229, 95% CI: 1.105 -4.499, $P = 0.025$) (Table 3). These data further confirmed that PGK1 is a potential prognostic marker in clinical practice.

Table 2. Univariate and multivariate survival analyses of PGK1 expression and overall survival for patients with breast cancer. NI: not included in multivariate survival analysis

Variable	Univariate analysis HR (95%CI)	P	Multivariate analysis HR (95%CI)	P
Age	1.022 (0.997-1.048)	0.090		NI
Tumor Diameter	3.748 (1.829-7.681)	<0.001	2.763 (1.337-5.710)	0.006
Grade	1.955 (1.346-2.840)	<0.001	1.777 (1.192-2.649)	0.005
Stage	2.991 (1.790-4.999)	<0.001	1.637 (1.123-2.749)	0.018
Subtypes	1.206 (0.952-1.528)	0.121		NI
ER Status	0.532 (0.305-0.927)	0.026	0.845 (0.478-1.493)	0.561
PR Status	1.003 (0.578-1.740)	0.992		NI
HER-2/neu Status	1.055 (0.530-2.102)	0.879		NI
PGK1	4.528 (1.934-10.598)	0.001	3.155 (1.316-7.569)	0.010

Table 3. Univariate and multivariate survival analyses of PGK1 expression and disease free survival for patients with breast cancer. NI: not included in multivariate survival analysis

Variable	Univariate analysis HR (95%CI)	P	Multivariate analysis HR (95%CI)	P
Age	1.027 (1.004-1.050)	0.020	1.699 (1.023-2.820)	0.040
Tumor Diameter	2.722 (1.534-4.832)	0.001	2.055 (1.148-3.677)	0.015
Grade	1.899 (1.359-2.654)	<0.001	1.572 (1.093-2.259)	0.015
Stage	2.705 (1.762-4.152)	<0.001	1.689 (1.082-2.636)	0.021
Subtypes	1.279 (1.014-1.573)	0.019	1.139 (0.912-1.432)	0.250
ER Status	0.595 (0.360-0.985)	0.043	0.918 (0.534-1.578)	0.756
PR Status	0.856 (0.530-1.385)	0.527		NI
HER-2/neu Status	0.808 (0.413-1.580)	0.533		NI
PGK1	3.323 (1.697-6.504)	<0.001	2.229 (1.105-4.499)	0.025

Fig. 4. Silencing PGK1 expression inhibits invasion and reverses the EMT process in breast cancer. PGK1 expression was silenced by shRNA in breast cancer cells, and the knockdown efficacy was determined by Western blotting (A) and RT-PCR (B). Knockdown PGK1 expression significantly inhibited cell invasive abilities (C). Western blot (D) and RT-PCR (E) analyses indicated that knockdown of PGK1 expression significantly increased the expression level of E-cadherin and decreased the levels of vimentin and N-cadherin. * $P < 0.05$.

Silencing PGK1 expression impaired breast cancer cell invasion and the reverse EMT process

To assess the role of PGK1 in regulating breast cell invasion, PGK1 expression was silenced by lentivirus-mediated transfection. Western blot and RT-PCR analysis demonstrated that PGK1 expression was significantly decreased at both the transcription level and the protein level in MDA-MB-231 and MCF-7 cells (Fig. 4A and 4B). To assess the role of PGK1 in cell viability, transwell analysis was performed, and our results demonstrated that knockdown of PGK1 expression significantly attenuated cell invasion abilities (Fig. 4C). To determine whether it was EMT that mediated the effect of PGK1 on invasion, we examined the expression of several EMT markers in PGK1 knockdown cells. As expected, silencing PGK1 expression increased the expression level of E-cadherin and decreased the levels of vimentin and N-cadherin (Fig. 4D). In the TCGA database, there was also a significant relationship among PGK1 and EMT-related markers (Fig. 4E).

PGK1 and HIF-1 α formed a positive feedback loop to promote metastases in breast cancer

PGK1 is a transcriptional target of HIF-1 α , and glycolysis is necessary for maintaining HIF-1 α activity. So, we hypothesized that PGK1 can affect HIF-1 α activity in breast cancer. HIF-1 α acts by binding to HIF-1 α response element (HRE) upon hypoxia; we thereby utilized the HRE-luciferase reporter to examine whether PGK1 affects HRE luciferase activities. As expected, PGK1 increased HRE luciferase activity in a dose-dependent manner (Fig. 5A), while silencing PGK1 expression decreased HRE activity (Fig. 5B). Moreover, we observed a close relationship between PGK1 and HIF-1 α expression in 185 breast cancer samples (Fig. 5C and Table 4). In addition, the PGK1 mRNA expression level was significantly correlated with the HIF-1 α mRNA level in the TCGA database (Fig. 5D), implying that HIF-1 α and PGK1 could form a positive feedback loop to promote metastases in breast cancer.

Fig. 5. PGK1 and HIF-1 α formed a positive feedback loop to promote metastases in breast cancer. (A) PGK1 increased HRE luciferase activity in a dose-dependent manner ($P<0.05$). (B) Silencing PGK1 expression decreased HRE activity ($P<0.05$). (C) Representative images of PGK1 and HIF-1 α co-expressed in breast cancer. (D) PGK1 mRNA expression level is significantly correlated with HIF-1 α mRNA level in the TCGA database ($P<0.001$).

Table 4. Positive correlation of PGK1 expression with HIF-1 α expression in patients with breast cancer

Parameter	PGK1		P value
	Low	High	
HIF-1 α	Low	38	<0.001
	High	23	

Discussion

Glycolytic enzymes have been widely studied as potential therapeutic targets because cancer-specific metabolism depends more on the glycolytic pathway than on aerobic respiration, which has been regarded as one of the ten hallmarks of cancer [2]. In the present study, we first screened out PGK1 by performing RT-PCR arrays of glycolysis-related genes in three different samples with paired metastasis abilities in primary breast cancer tissues. We then explored PGK1 expression using the TCGA database and found that high PGK1 implied poor survival in breast cancer and other cancers, suggesting that unregulated glycolytic enzymes, especially PGK1, is a common phenomenon in cancer.

The TCGA database lacks some important information, such as the quality of surgery and adjuvant therapy strategies, and thus only the transcriptional expression level of PGK1 is available in the TCGA database. We further validated the clinical significance of PGK1 expression with our own database using IHC. Our results demonstrated that high PGK1 expression was significantly correlated with larger tumor size and advanced tumor stage, which suggested that PGK1 may play a critical role in breast cancer progression and metastases. Importantly, our study indicated that PGK1 expression was an independent prognosis biomarker in breast cancer after radical resection.

A functional study indicated that silencing PGK1 expression significantly impaired the ability to invade breast cancer cells. EMT is considered critical for invasive and metastatic progression in cancer. The process of EMT is associated with the downregulation of epithelial markers and aberrant upregulation of mesenchymal markers. As anticipated, knockdown of PGK1 expression significantly upregulated epithelial marker E-cadherin and downregulated mesenchymal markers N-cadherin and vimentin. Some studies have demonstrated that glycolytic enzymes are involved in the EMT process, including FBP1 [10, 11, 22], PKM2 [23], and LDHA. Glycolytic enzymes are also regulated by classic EMT regulators [11, 24-26], so, it is not surprising that PGK1 can regulate the EMT process in breast cancer.

HIF-1 α is central regulator for cancer cell survival and metastases in solid tumors in part by inducing glycolysis and the EMT process [27-29]. HIF-1 α upregulates a series of glycolytic genes during anaerobic glycolysis and has PGK1 as a downstream target. A previous study also suggested that glycolysis is necessary for maintaining HIF-1 α activity [30]. Here, we demonstrated that PGK1 increased HIF-1 α activity. PGK1 and HIF-1 α formed a positive feed-forward loop that stimulates breast cancer progression and metastases. Thus, PGK1 may serve as a promising biomarker and target therapy for breast cancer.

A major strength of this study is that we first screened out PGK1 using a PCR array from breast cancer samples with different invasion abilities and validated clinical significance of PGK1 from two independent populations. PGK1 is not only upregulated in breast cancer, but also in several other tumors. We also used an *in vitro* study and a mechanistic study to explore the underlying mechanism of how PGK1 promotes progression and metastases. However, there are some limitations in our study. First, no *in vivo* experiments using animals were performed to observe the effects of PGK1 on progression and metastases from mouse models. Thus, further *in vivo* animal studies are warranted to confirm our findings. Second, some glycolytic enzymes are involved in drug resistance and radiotherapy resistance, but whether PGK1 has a similar effect requires further study.

Conclusion

High PGK1 expression was associated with poor prognosis in breast cancer. PGK1 and HIF-1 α formed a positive feed-forward loop that stimulated breast cancer progression and metastases. Thus, PGK1 may serve as a promising biomarker and target therapy for breast cancer.

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Disclosure Statement

The authors declare no conflicts of interest.

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