

## Review Article

# PGK1-mediated cancer progression and drug resistance

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**Abstract:** Phosphoglycerate kinase 1 (PGK1) is an essential enzyme in the aerobic glycolysis pathway. PGK1 catalyzes the reversible transfer of a phosphate group from 1,3-bisphosphoglycerate to ADP and produces 3-phosphoglycerate and ATP. In addition to cell metabolism regulation, PGK1 is involved in multiple biological activities, including angiogenesis, autophagy and DNA repair. Because of its multi-faceted functions, PGK1's involvement in cancer development is complicated. High intracellular expression of PGK1 leads to tumor cell proliferation. However, high extracellular expression of PGK1 suppresses cancer malignancy through a suppression of angiogenesis. PGK1 is also associated with chemoradiotherapy resistance and poor prognosis of cancer patients. In this manuscript, we summarize the influence of PGK1 and its post-translational modifications on cancer initiation and progression. PGK1-mediated drug resistance and potential small molecule inhibitors targeting PGK1 are discussed for their future clinical applications.

**Keywords:** PGK1, cancer, post-translational modification, drug resistance, small molecule inhibitors

## Introduction

Phosphoglycerate kinase (PGK) is a major enzyme which catalyzes the formation of ATP in the aerobic glycolysis pathway. PGK exists in all organisms with high sequence conservation throughout evolution [1]. The two isoforms of PGK, PGK1 and PGK2, have similar functions and structures in human beings, encompassing 417 amino acids with 87-88% sequence identity and near a 45-kDa molecular mass [2]. However, PGK1 and PGK2 have different expression distribution. PGK2 encoded by an autosomal gene is only expressed during spermatogenesis, while PGK1 located on the X-chromosome is ubiquitously expressed in all cells [3].

PGK1 is the only enzyme encoded by an X-linked gene and involved in the first ATP-generating step of the glycolytic pathway [4]. PGK1 and pyruvate kinase M2 (PKM2) are the only two enzymes controlling ATP production during aerobic glycolysis in cancer cells [5]. PGK1 catalyzes a reversible transfer of a phos-

phate group from 1,3-bisphosphoglycerate (1,3-BPG) to ADP, producing 3-phosphoglycerate (3-PG) and ATP:  $1,3\text{-BPG} + \text{ADP} \rightleftharpoons 3\text{-PG} + \text{ATP}$ . PGK1 plays a rate-limiting role in coordinating energy production with biosynthesis and redox balance by controlling ATP and 3-PG levels [6].

The protein structure of PGK1 has been well understood [7]. It is a monomeric protein containing two similar sized Rossmann fold domains, corresponding to the N- and C-terminal respectively, which are connected by a hinge region through hydrophobic interactions and hydrogen bonds [8]. The N-terminal domain of PGK1 allows 1,3-BPG or 3-PG to bind, while the C-domain binds to the nucleotide substrate ADP. Once the two substrates are bounded, a hinge-bending motion occurs to bring domains and their bound substrates into a closed conformation for substrate contact [9].

PGK1 has other functions besides regulating glycolytic metabolism, including mediating autophagy initiation [10-13], DNA replication and repair in mammal cell nuclei [14]. An abnormal

expression level of PGK1 is related to disease occurrence. PGK1 deficiency has linkage with parkinsonism, hereditary non-spherocytic haemolytic anaemia, neurological impairment and myopathy [15-18]. At present, it is no definite treatment for PGK deficiency. On the other hand, PGK1 is overexpressed in synovial tissues and blood of rheumatoid arthritis, suggesting it participates in pro-inflammation and synovial hyperplasia of the disease [19].

More noticeably, PGK1 mediates glycolysis that generates ATP for tumor cells especially under hypoxic conditions, which is related to development and progress of various cancers [20]. Recently PGK1 has gradually become a focused target in cancer research. However, up to now no review has systematically summarized the important functions and mechanisms of PGK1 in tumorigenesis. This paper reviews the role of PGK1 and its post-translational modifications (PTMs) in cancer initiation and progression, and prospects the application of PGK1 as a new diagnostic biomarker and therapeutic target in cancer and drug resistance.

### Bioinformatics analyses of PGK1 expression with cancer prognosis

To understand associations of PGK1 expression level and prognostic value in human cancers, we have analyzed datasheet from several online databases, including Oncomine microarray database (<http://www.oncomine.org>) containing PGK1 mRNA level between tumors and normal tissues in multiple cancers. The parameter thresholds are set as follows according to the previous research: *p*-value: 1E-4; fold change: 2; gene rank: top 10%; analysis type: cancer vs. normal analysis; data type: mRNA [21]. The database contains a total of 353 unique analyses for PGK1. In 36 studies, PGK1 is ranked within the top 10% of all genes showing significant statistical differences, from which 33 cases of research data have confirmed a higher expression level of PGK1 in tumor than normal tissues. Although the mRNA expression level of PGK1 varies with the individual type of tumor, PGK1 is usually overexpressed in most cancers (Figure 1A).

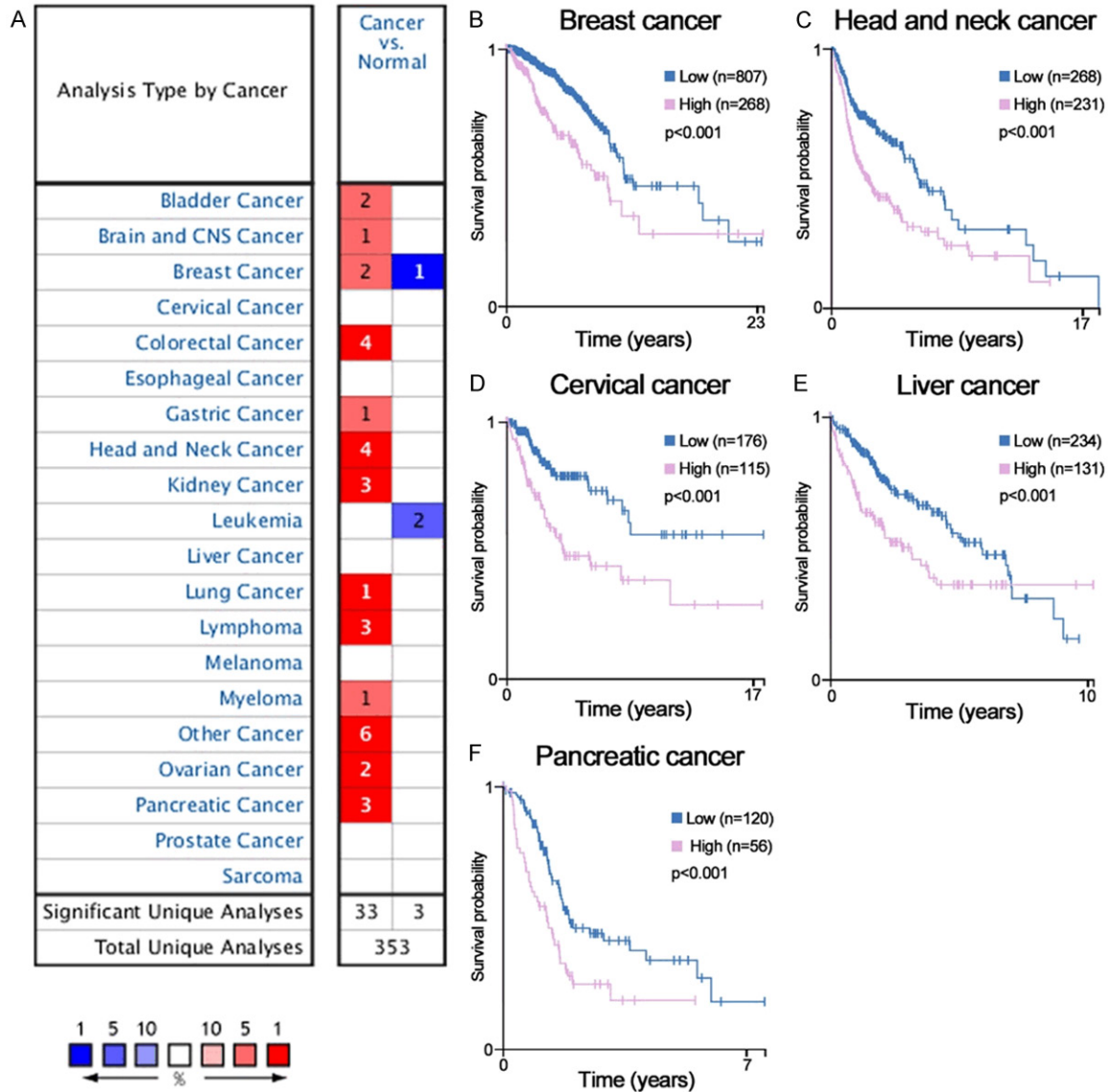
Next, we assess the correlation between PGK1 expression and clinical outcomes of cancer patients by bioinformatics analysis on the Human Protein Atlas (HPA) database ([\[www.proteinatlas.org/\]\(http://www.proteinatlas.org/\)\). It is an important tool for biomarker discovery \[22\]. The mRNA expression level of PGK1 is significantly associated with prognosis of patients in five different types of cancer. High PGK1 predicts poor survival in breast cancer, head and neck cancer, cervical cancer, liver cancer and pancreatic cancer \( \$P < 0.001\$ , Figure 1B-F\).](https://</a></p></div><div data-bbox=)

### PGK1-mediated tumor suppression mechanisms

Through database analysis, we find that PGK1 is overexpressed in most cancers and its overexpression indicates a poor prognosis in some specific cancer types. However, PGK1 is originally thought to be a tumor suppressor. It seems to play contradictory even opposite functions of the extracellular PGK1 compared with the intracellular PGK1 for cancer occurrence. Extracellular PGK1 can function as a disulfide reductase, which generates angiostatin by increasing the reduction of disulfide bonds in plasmin [23]. The formation of angiostatin from plasmin restricts tumor growth by suppressing angiogenesis [24]. Therefore, the extracellular PGK1 plays an inhibitory role in tumor growth and metastasis under certain conditions. For example, intraperitoneal administration of recombinant human PGK into fibrosarcoma-bearing mice and pancreatic tumor-bearing mice both cause an increase in plasma levels of angiostatin and a decrease in tumor angiogenesis and tumor growth [23]. Furthermore, PGK1 secreted into cellular matrix reduces VEGF level and enhances generation of angiostatin to inhibit angiogenesis in prostate cancer (PCa) [25]. Another recent study demonstrates that PGK1 expression is repressed by MVIH, a long noncoding RNA (lncRNA), which activates the tumor-induced angiogenesis in hepatocellular carcinoma (HCC) [26].

In addition to being a tumor suppressor by generating the angiogenesis inhibitor angiostatin, PGK1 can reduce the tumor growth of Lewis lung carcinoma by downregulating the expression of COX-2 and promoting anti-tumor immunity *in vivo* [27]. PGK1 comprises a nucleotide-binding domain (NBD) and two catalytic domains (CDI and CDII). Similar to full-length PGK1, both CDI and NBD domains of PGK1 can inhibit the growth of lung cancer cells *in vivo*. Regarding the molecular mechanism responsi-

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**Figure 1.** Bioinformatic analyses of the expression and prognosis of PGK1 in cancer. (A) The mRNA expression levels of PGK1 in various cancers are analyzed via the Oncomine database. The number in the colored cell represents the amount of cases that satisfy the thresholds. Cell coloring is determined by gene ranking. The more intense red (overexpression) or blue (underexpression) suggests a more highly significant upregulated or downregulated gene. (B-F) The relationship between the mRNA expression of PGK1 and the survival time of cancer patients is assessed using the HPA database online. High PGK1 mRNA expression predicts poor survival in breast cancer (B), head and neck cancer (C), cervical cancer (D), liver cancer (E), and pancreatic cancer (F).

ble for this, the NBD domain of PGK1 decreases COX-2 mRNA stability to inhibit COX-2 expression level and its metabolites PGE2 and TGF- $\beta$ 1 in lung cancer cells [28]. Glycolytic proteomic analysis of lung cancer tissues shows that poorly differentiated tumors have significantly lower PGK1 expression levels compared with moderate or highly-differentiated ones, suggesting PGK1 expression is downregulated during the development of lung cancer [29].

Moreover, PGK1 upregulation reduces the expression of cell surface urokinase-type plasminogen activator receptor (uPAR) and inhibits the uPAR-mediated cell proliferation and migration in H157 lung carcinoma cells [30]. PGK1 can also play a tumor suppressor role by enhancing the specific cytotoxic effects of immune cells on tumors. For instance, in HLA-A2<sup>+</sup> colon cancer, PGK1 can be recognized as a tumor-associated antigen specifically by cytotoxic T

lymphocyte to increase the production of IFN- $\gamma$  in tumor-infiltrating T lymphocyte and enhance the killing effect of T lymphocyte on tumor [31].

On the other hand, PGK1 is notably downregulated in the highly metastatic gallbladder carcinoma (GBC) cell line GBC-SD18H compared to the poorly metastatic GBC-SD18L cells [32]. Decreased PGK1 expression is associated with poor prognosis in patients with GBC, which indicates PGK1 is a useful potential prognostic biomarker for GBC [33]. However, the specific molecular mechanism of how PGK1 exerts its anti-cancer function in GBC is not yet clear. Overall, PGK1 can serve as a tumor suppressor in some specific cancers, and PGK1-mediated tumor suppression mechanisms are summarized detailedly in **Table 1**.

### **PGK1-involved oncogenic signal pathways**

As our previous bioinformatics analysis shows, PGK1 functions as an oncogene in most cases. We summarize several key PGK1-involved oncogenic signal pathways as follows (**Table 2**).

#### *HIF-1 $\alpha$ -PGK1 axis-induced glycolysis enhancement*

Even under oxygen-sufficient conditions, cancer cells prefer glycolysis rather than mitochondrial oxidative phosphorylation for glucose metabolism during proliferation and metastasis, which is called Warburg effect. Hypoxia inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) serves as a transcriptional factor to promote cell glycolysis for Warburg effect [34, 35]. As an important ATP-generating enzyme in the glycolytic pathway, PGK1 is directly regulated by HIF-1 $\alpha$  in many cancers. Earlier studies have shown that PGK1 is transcriptionally activated by HIF-1 $\alpha$  in colorectal cancer cells and hepatoma cells under hypoxia stress [36]. In human colon carcinoma cells, PGK1 is identified as a potential biomarker for reflecting intracellular oxidative stress status, and antioxidants can inhibit the expression of HIF-1 $\alpha$  and its downstream target protein PGK1 [37].

Moreover, PGK1 is relative with cancer cell metastatic ability due to HIF-1 $\alpha$ /PGK1 mediated epithelial-mesenchymal transition (EMT) process. Higher expression levels of PGK1 and HIF-1 $\alpha$  are detectable in high metastatic HCC cells HCCLM9 compared with MHCC97L cells with low metastasis [38]. Similarly, the upregu-

lated HIF-1 $\alpha$  and PGK1 activate the Notch pathway to promote progression of human glioblastoma (GBM) [39]. A high PGK1 expression indicates poor prognosis in patients with breast cancer, so PGK1 is a promising invasion promoter and survival biomarker for breast cancer [40]. In addition, PGK1 is significantly increased in HIF-1 $\alpha$ -overexpressed leukemic cell K562. Overexpression of HIF-1 $\alpha$  protects K562 cells from 1,4-benzoquinone-induced toxicity by inhibiting reactive oxygen species (ROS), apoptosis and enhancing glycolysis [41]. Generally, intracellular PGK1 production by tumor cells is enhanced under hypoxia, but its extracellular secretion is inhibited. This is responsible for the different functions of intracellular and extracellular PGK1 [20].

#### *MYC-PGK1 pathway-mediated cell metabolism*

Similar to HIF-1 $\alpha$ , MYC is one of main regulators of the glycolytic switch observed in cancer cells [42, 43]. PGK1 plays an important role in MYC-induced metabolic reprogramming, which leads to enhanced Warburg effect in a variety of cancers. MYC-dependent PGK1 induces the overexpression of key glycolysis enzymes GLUT4, HK2 and LDHA to accelerate glycolysis rate and produce large amounts of ATP and lactate, which promotes HCC proliferation and metastasis [44]. On the contrary, knockdown of endogenous MYC significantly reduces PGK1 levels, suggesting MYC is a major regulator in controlling PGK1 enzyme activity in HCC cells [5]. In ovarian cancer, Pim1-MYC-PGK1 pathway is activated to change cell metabolic process [45]. MYC is also recruited to activate PGK1 in the development of human breast cancer [46] and clear cell renal cell carcinoma (ccRCC) [47].

#### *PGK1-CXCR4/CXCL12 pathway*

A tight relationship between PGK1 and the CXCR4/CXCL12 (C-X-C chemokine receptor 4/C-X-C chemokine ligand 12) axis has been verified in several cancers. CXCR4 is overexpressed in many solid and hematologic cancers, and activates the downstream oncogenic signaling pathway by binding its ligand, CXCL12 [48]. In gastric cancer, tumor samples with peritoneal carcinomatosis have a higher mRNA expression of PGK1, CXCR4, CXCL12 and  $\beta$ -catenin compared with tissues without peritoneal carcinomatosis [49]. Moreover, PGK1 and CXCR4 are able to regulate each other via a co-stimulating way in gastric cancer cells, and overexpression

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**Table 1.** PGK1-mediated tumor suppression mechanisms

Cancer types	Molecular mechanisms	Function/clinical association	References
Fibrosarcoma	Promote the angiostatin formation	Inhibit tumor angiogenesis and tumor growth	[23]
Pancreatic cancer			[23]
Hepatocellular carcinoma	-	Inhibit tumor angiogenesis	[26]
Prostate cancer	Reduce VEGF secretion; Promote the angiostatin formation	Inhibit tumor angiogenesis	[25]
Lung cancer	Inhibit COX-2 expression; Promote anti-tumor immunity <i>in vivo</i>	Inhibit tumor growth	[27]
	Inhibit COX-2 expression	Inhibit tumor growth	[28]
	-	Associated with prognosis of patients	[29]
	Reduce expression of uPAR	Inhibit cell proliferation and migration	[30]
HLA-A2* colon cancer	Recognized as a tumor-associated antigen specifically by cytotoxic T lymphocytes	Enhance the killing effect of T lymphocyte on tumor	[31]
Gallbladder carcinoma	-	Associated with prognosis of patients	[32]
		Associated with prognosis of patients	[33]

Note: '-' means no detailed information.

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**Table 2.** PGK1-involved oncogenic signal pathways

Cancer types	Signaling pathway/mechanisms	Function/clinical association	References
Hepatocellular carcinoma	HIF-1 $\alpha$ /PGK1 pathway	Promote cell metastasis	[38]
	MYC/PKG1 pathway	Induce glycolysis	[44]
	PGK1/AKT/mTOR pathway	Inhibit cell growth	[57]
	-	Associated with a poor outcome of patients	[70]
Ovarian cancer	MYC/PKG1 pathway	Induce glycolysis	[45]
Brain cancer	HIF-1 $\alpha$ /PGK1-induced activation of Notch pathway	Promote the tumorigenesis of GBM	[39]
	PGK1/CXCR4 pathway	Promote tumor metastasis into the bone; Associated with a poor outcome of patients	[52]
	PGK1/CXCR4/ $\beta$ -catenin pathway	Promote the migration and invasion of glioma cells	[53]
Breast cancer	E2F1 binds to and activates the promoter of the PGK1	Induce glycolysis; Promote tumor progression	[67]
	HIF-1 $\alpha$ /PGK1 pathway	Promote EMT process	[40]
	MYC/PKG1 pathway	Promote cancer development	[46]
	miR-548a-3p represses the transcription factor SIX1-dependent transcription of PGK1	Promote the Warburg effect	[59]
	PPAR $\gamma$ inhibits PGK1 gene transcription activity by targeting the consensus PPRE motif of PGK1 promoter region	Induce glycolysis	[68]
	Treatment with a specific HER-2 inhibitor Herceptin decreases the expression of PGK1 obviously	Inhibit cell growth	[69]
Renal cancer	MYC/PKG1 pathway	Upregulated in RCC compared with adjacent normal kidney	[47]
Gastric cancer	PGK1/CXCR4/ $\beta$ -catenin pathway	Associated with a poor outcome of patients	[49]
	PGK1/CXCR4/ $\beta$ -catenin pathway	Enhance the peritoneal dissemination and metastasis of gastric cancer	[50]
	PGK1/AKT/mTOR pathway	Promote the oncogenic properties of gastric cancer cell	[62]
Prostate cancer	-	Enhance bone formation at the metastatic site <i>in vivo</i>	[54]
	-	Induce glycolysis and promote the metastatic ability of prostate CTCs	[55]
Colorectal cancer	miR-548c-5p recognizes the 3'-UTR of PGK1 and decreases the expression of PGK1	Suppress cell proliferation	[58]
	Upregulate EGR1 and CYR61	Promote colon cancer metastasis	[65]
Lung cancer	MetaLnc9-mediated PGK1/AKT/mTOR pathway	Promote cancer metastasis <i>in vitro</i> and <i>in vivo</i>	[61]
	OTUB2-mediated PGK1/AKT/mTOR pathway	Promote the Warburg effect and tumorigenesis	[63]
	Rab11FIP2-mediated PGK1/AKT/mTOR pathway	Suppress tumor growth	[64]
	-	Associated with a poor outcome of patients	[73]
Papillary thyroid cancer cell	SIRT6 increases the expression of PGK1	Promote the Warburg effect and enhance tumor aggressiveness	[66]
Endometrial cancer	-	Associated with a poor outcome of patients	[74]
	-	Associated with a poor outcome of patients	[75]
Pancreatic cancer	-	Associated with a poor outcome of patients	[71]
	-	Associated with a poor outcome of patients	[72]
Oral squamous cell carcinoma	-	Associated with a poor outcome of patients	[76]

Note: '-' means there are no detailed information.

of PGK1 increases the peritoneal dissemination and metastasis of gastric cancer by activating the CXCR4/CXCL12/ $\beta$ -catenin signaling pathway [50]. The ability of PGK1 to promote the progression and metastasis of gastric cancer is further demonstrated in a tumor mouse model [51]. PGK1, CXCR4 and  $\beta$ -catenin are all overexpressed in high metastatic HCC cells [38].

PGK1 is a biomarker for predicting glioma metastatic capacity and a promising therapy target for metastatic glioma. The upregulated PGK1 in neuroblastoma cells is positively correlated with tumor metastasis to bone marrow, and poor prognosis of patient [52]. PGK1 knock-down suppresses migration and invasion of glioma cells by decreasing CXCR4 and  $\beta$ -catenin [53]. Most studies have shown that intracellular PGK1 actually plays an oncogenic role in PCa. PGK1 improves bone formation at the metastatic site of PCa *in vivo* [54]. Circulating tumor cells (CTCs) are cancer cells derived from tumor origin or metastasis that release into the peripheral blood. High expression of PGK1 in prostate CTCs accelerates glycolysis process and increases the supply of energy to promote cell metastatic ability [55]. This indicates PGK1 seems to be a biomarker for prostate cancer metastasis. In addition, PGK1 induces fibroblasts towards the cancer-associated fibroblasts phenotype in tumor microenvironment of PCa. PGK1 upregulation in PCa-associated stromal cells stimulates CXCR4/CXCL12 signaling pathway to produce cytokines released into tumor microenvironment, thereby it contributes migration and invasion of PCa cells [56].

### *PGK1 functions regulated by non-coding RNA*

In recent two years, the regulation of PGK1 roles by non-coding RNA has become a research hotspot. It seems PGK1 expression is regulated by several microRNAs (miRNAs). A negative correlation between miR-450b-3p and PGK1 protein is observed in HCC specimens using Pearson's correlation analysis. The 3'-UTR of PGK1 contains a complementary sequence of miR-450b-3p, therefore PGK1 probably is a direct target of miR-450b-3p. Moreover, miR-450b-3p inhibits cell growth by downregulating PGK1 and PGK1-mediated AKT phosphorylation in HCC cells [57]. The miR-548c-5p suppresses colorectal cancer cell proliferation by recognizing the 3'-UTR of PGK1 to decrease the expression of PGK1 [58]. In breast cancer, transcrip-

tion factor SIX1 promotes the Warburg effect and tumor growth *in vitro* and *in vivo* by binding to the promoter region of PGK1 and recruiting histone acetyltransferase HBO1 to induce the transcription of PGK1. While SIX1 is directly inhibited by miR-548a-3p, which leads to the decrease of PGK1 expression and aerobic glycolysis [59]. These evidences indicate miR-548a-3p downregulates PGK1 to suppress glycolysis.

In non-small cell lung cancer (NSCLC), the lncRNA LINC00963 (MetaLnc9) physically interacts with PGK1 to prevent PGK1 degradation by ubiquitination, which stimulates PGK1-activated oncogenic AKT/mTOR signaling pathway to promote cancer metastasis *in vitro* and *in vivo* [60, 61]. Similarly, another a long non-coding RNA GBCDRlnc1 directly binds to PGK1 and inhibits PGK1 ubiquitination, and then the overexpression of PGK1 induces chemoresistance of GBC cancer cells by activating autophagy [13].

### *PGK1/AKT/mTOR pathway*

PGK1 mediated AKT/mTOR pathway is commonly activated in cancers. In addition to the two examples mentioned above [57, 60, 61], PGK1 interacts with other targets to involve in AKT/mTOR pathway to promote tumorigenesis. Overexpression of gankyrin alleviates cellular oxidative stress and increases the oncogenic properties of gastric cancer cell by activating PGK1/AKT/mTOR pathway [62]. In NSCLC cells, OTUB2 directly binds to U2AF2 and reduces its ubiquitination, thus stabilizing the protein expression of U2AF2 [63]. Then, U2AF2 enhances the Warburg effect and tumorigenesis via the PGK1/AKT/mTOR signaling pathway. Moreover, the combined evaluation of OTUB2, U2AF2 and PGK1 is more accurate to predict prognosis for patients. A high expression of OTUB2, U2AF2 and PGK1 is significantly correlated with worse prognosis in NSCLC patients [63]. Furthermore, Rab11FIP2 can interact with PGK1 and improve its degradation in NSCLC cells, resulting in inactivation of the oncogenic PGK1/AKT/mTOR signaling pathway to suppress tumor growth [64].

### *Other PGK1-mediated signaling pathways*

PGK1 also promotes tumorigenesis through other specific signal transduction pathways, which are not common and occur only in some spe-

cific tumors. For instance, PGK1 upregulates the expression of EGR1, a metastasis-related factor, to enhance colon cancer metastasis. On the other hand, PGK1 positively regulates CYR61 and its downstream transcription factors FOS and JUN to promote the metastasis of colon cancer [65]. In papillary thyroid cancer cell, SIRT6 enhances tumor aggressiveness by upregulating the expression of key Warburg effect genes including PGK1, PKM2, GLUT1, HK2, LDHA, ENO1 and GAPDH [66]. Moreover, E2F1 directly binds to the promoter of PGK1, which results in the promotion of aerobic glycolysis and tumor progression of neuroblastoma [67]. In addition, PPAR $\gamma$  inhibits PGK1 gene transcription activity by targeting the consensus PPRE motif of PGK1 promoter region, leading to the reduced glycolysis of breast cancer cells [68]. Besides, the overall expression of PGK1 is much higher in HER-2/neu-positive breast cancer compared with that in HER-2/neu-negative breast cancer, and treatment with a specific HER-2 inhibitor Herceptin decreases the expression of PGK1 obviously in breast cancer cells [69].

### **Clinical significance of PGK1 expression in cancer**

As the incidence of cancer rises continuously, the need to develop highly sensitive and specific biomarkers for early stage diagnosis and treatment of cancer has become an important priority. It is a trend that liquid biopsy will replace traditional tumor tissue biopsy in the future. PGK1 is a secretory protein, therefore the abnormal expression of PGK1 can be detected conveniently not only in tumor tissues, but also in peripheral blood and saliva of patients.

Several studies have demonstrated that PGK1 has potential to be used independently as a diagnostic, prognostic, and treatment biomarker in various cancer. For example, it is confirmed that the high serum level of PGK1 is tightly associated with HCC early relapse and worse prognosis, and the serum level of PGK1 is complementary with alpha-fetoprotein (AFP) to further enhance the sensitivity and specificity for predicting the recurrence of HCC [70]. On the other hand, the serum levels of PGK1 are significantly higher in pancreatic cancer patients than those in healthy controls [71], and high PGK1 level predicts poor prognosis in patients with pancreatic cancer [72]. Similarly,

elevated levels of PGK1 in serum and tumor tissue are also significantly linked with poor outcome in patients with lung adenocarcinoma [73]. In addition, the expression of PGK1 in endometrial cancer is higher compared to normal samples, and it is significantly correlated with tumor grade [74]. Another research also indicates that high PGK1 expression correlates positively with FIGO stage, histological grade, lymph node status, and survival time of patients with endometrial cancer [75]. Moreover, the prognosis of oral squamous cell carcinoma (OSCC) patients can be predicted by detecting the expression of PGK1 in tumor tissues and saliva samples. Patients with upregulated PGK1 in the invasive tumor front (ITF) of OSCC have a higher risk of local relapse and worse survival, and the low expression of PGK1 in saliva samples from OSCC patients is associated with lymph node metastasis and advanced clinical stage [76]. In conclusion, PGK1 exhibits a good ability to predict diagnosis and prognosis in many cancers, but the underlying molecular mechanisms need to be further clarified in the future.

### **PTMs of PGK1 in cancer**

Several somatic variants of PGK1 have been identified in cancer cells, being summarized on the database COSMIC (<http://cancer.sanger.ac.uk>) [77]. The nucleotide mutations in the 3-PG binding region, hinge region and ADP binding region of PGK1 have important effects on its own catalytic activity and conformational stability [7]. Besides, nucleotide mutations probably have influences on protein PTMs. Therefore, PTMs of PGK1 have related to occurrence and development of tumors. Previous studies have confirmed several PTMs of PGK1, including acetylation, phosphorylation, ubiquitination and succinylation, play an important role in regulating tumor metabolism and growth (Table 3).

#### *Acetylation of PGK1*

PGK1 is acetylated at K323 in liver cancer, which is correlated with poor survival of HCC patients [5]. Acetylation at the K323 site of PGK1 is considered as a significant regulatory mechanism for promoting PGK1 enzymatic activity, cell metabolism, glucose uptake and tumorigenesis. K323 acetylation is modulated by the upstream regulators PCAF and SIRT7 [5]. PCAF is a major acetyltransferase that inter-



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**Table 3.** Post-translational modifications of PGK1

PTMs	PTM sites	Function	Cancer types	Possible pathways and mechanisms	Regulators	References
Acetylation	K323	Oncogenic role	HCC	Enhance PGK1 enzymatic activity for glycolytic metabolism.	PCAF: interact with and acetylate PGK1; SIRT7: bind to PGK1 and reduce its acetylation	[5]
	K220	Decrease glycolytic ATP production and cellular redox state	-	Inhibit PGK1 enzyme activity by blocking its binding with substrate ADP.	KAT9: acetylate and inactivate PGK1; HDAC3: deacetylate and activate PGK1	[6]
	K388	Oncogenic role	GBM	Lead to the activation of VPS34-Beclin1-ATG14L complex required for autophagosome formation to promote tumor growth.	Glutamine deprivation and hypoxia result in ARD1-dependent PGK1 K388 acetylation	[10]
Phosphorylation	S203	Oncogenic role	GBM	Phosphorylate and activate PDHK1, leading to decrease of mitochondrial pyruvate metabolism and increase of glycolysis.	Hypoxia, activation of EGFR, and K-Ras/B-Raf mutation all induce ERK-dependent phosphorylation of PGK1 at S203	[79]
	T243	Oncogenic role	GBM	Reduce the 3-PG affinity of PGK1 to facilitate the equilibrium of the PGK1-catalyzed reaction toward glycolysis.	M2 macrophages can secrete IL-6 to increase PDPK1-dependent PGK1 T243 phosphorylation	[81]
Ubiquitination	Lysine	Tumor suppressor role	GBC	Enhance the ubiquitin degradation of PGK1.	GBCDRlnc1	[13]
			NSCLC		MetaLnc9	[60]
			NSCLC		Rab11FIP2	[64]
Succinylation	Lysine	Oncogenic role	RCC	PGK1 lysine succinylation is upregulated in RCC tissues compared with adjacent normal tissues.	-	[82]

Note: '-' means there are no detailed information.

acts with and acetylates PGK1, while SIRT7 is a deacetylase which binds to PGK1 and reduces its acetylation. In conclusion, acetylation of PGK1 at K323 increases PGK1 enzyme activity and promotes liver cancer cell proliferation and tumorigenesis.

Acetylation of specific residues has different biochemical roles. For example, another research reports that PGK1 is acetylated at K220, and this acetylation inhibits PGK1 enzyme activity by blocking its binding with substrate ADP [6]. KAT9 is a potential acetyltransferase of PGK1, which improves the PGK1 K220 acetylation level and decreases PGK1 activity. On the other hand, HDAC3 is identified as the deacetylase of PGK1 that deacetylates and activates PGK1. Moreover, insulin-induced PI3K/AKT/mTOR pathway increases HDAC3 S424 phosphorylation, which promotes PGK1-HDAC3 interaction and PGK1 K220 deacetylation to stimulate PGK1 activity. In general, insulin treatment can promote glucose metabolism, glycolytic ATP production and redox state of cells via HDAC3-mediated deacetylation and activation of PGK1 (**Figure 2A**). By now, it has not yet been explored whether K220 acetylation of PGK1 plays an essential role in tumorigenesis.

Autophagy is essential for maintaining cell homeostasis. Many cancer cells upregulate autophagy to promote survival under stress stimulation, including hypoxia, starvation and metabolic pressure [78]. Recent studies have found that acetylation at the K388 site of PGK1 promotes the initial stage of autophagy to promote tumor growth in U87 GBM cells [10-12]. More importantly, GBM patients with high PGK1 K388 acetylation expression have a significantly shorter survival compared with those with low expression of PGK1 K388 acetylation. However, the involvement of PGK1 acetylation in the regulation of autophagy is very complex. First, hypoxia and glutamine deprivation inhibit mTOR and mTOR-dependent S228 phosphorylation of ARD1, which enables ARD1 to interact with PGK1 for PGK1 K388 acetylation [10]. Next, the acetylated PGK1 binds to and phosphorylates Beclin1 at S30, which leads to activation of the VPS34-Beclin1-ATG14L complex required for autophagosome formation. In a word, PGK1 functions as a protein kinase and phosphorylates Beclin1 to induce autophagy and tumorigenesis in GBM (**Figure 3B**).

### *Phosphorylation of PGK1*

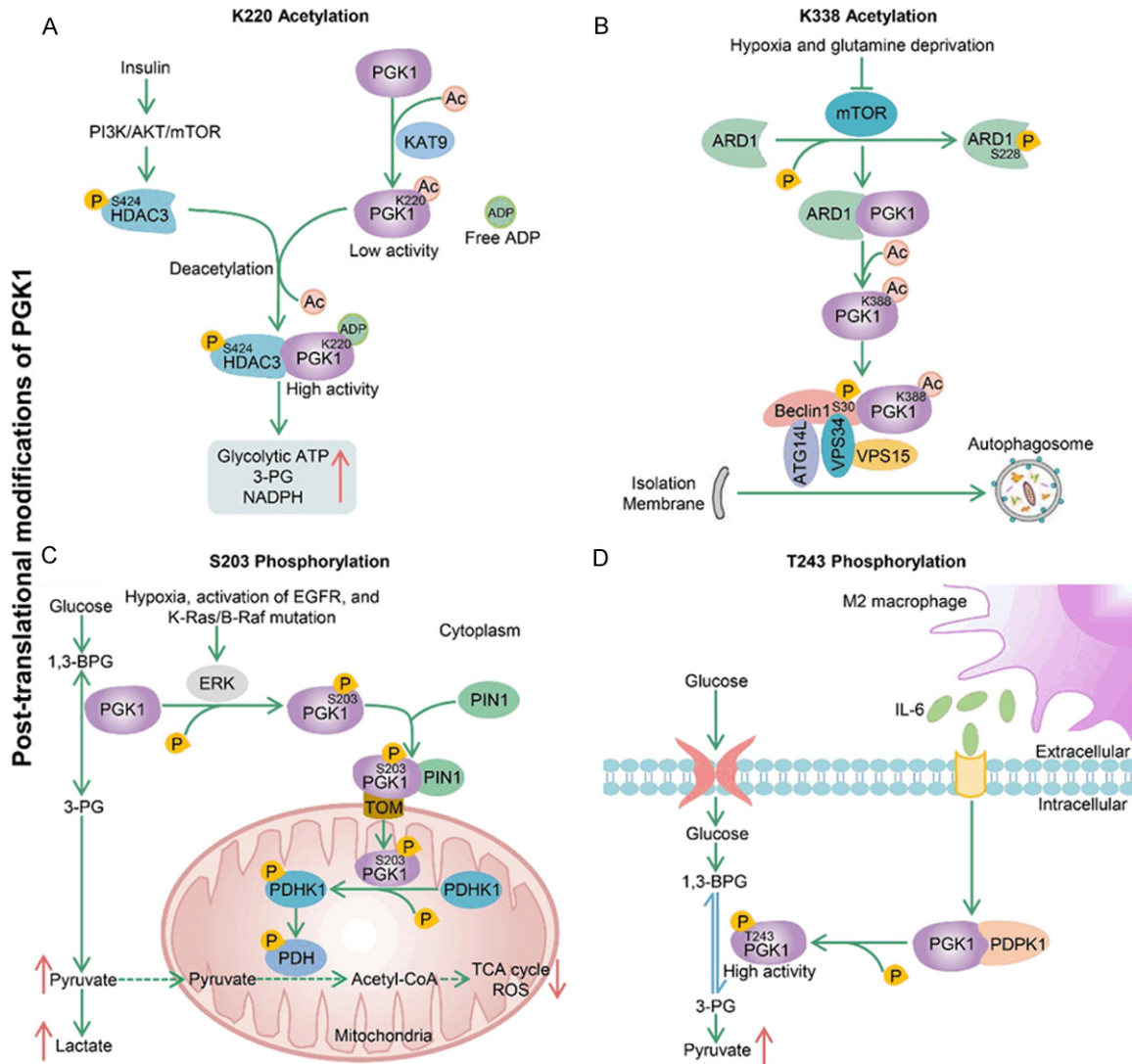
In addition to acetylation, phosphorylation at the S203 site of PGK1 also plays a significant role in promoting cancer cell metabolism and tumorigenesis of GBM cells [79, 80]. High expression of PGK1 S203 phosphorylation independently predicts poor prognosis for GBM patients. A possible underlying mechanism of PGK1 S203 phosphorylation promoting tumor growth is proposed. First of all, multiple factors including hypoxia, EGFR activation, and K-Ras/B-Raf mutation all can induce ERK-dependent phosphorylation of PGK1 at S203 [79]. Secondly, PIN1 binds to and cis-trans isomerizes the pS203P204 motif of phosphorylated PGK1, which makes the 38-QRIKAA-43 residues of PGK1 exposed on protein surface so that they can be recognized by the TOM (translocase of the outer mitochondrial membrane) complex for mitochondrial translocation of PGK1. Following the mitochondrial PGK1 functions as a protein kinase to phosphorylate and activate PDHK1, which further leads to phosphorylation and inactivation of PDH complex. This process results in the decrease of mitochondrial pyruvate metabolism and ROS generation and the enhancement of glycolysis in cytoplasm, and ultimately promotes GBM cell proliferation and tumorigenesis (**Figure 3C**).

Another interesting finding from a recent study suggests that PGK1 T243 phosphorylation is necessary for M2 macrophage-mediated glycolysis and proliferation of GBM cells [81]. M2-polarized macrophages secrete IL-6 to increase PDPK1-dependent PGK1 phosphorylation at T243 in GBM cells. This phosphorylation reduces the affinity of PGK1 with 3-PG, which facilitates the equilibrium of the PGK1-catalyzed reaction toward glycolysis, thereby enhancing cell proliferation (**Figure 3D**). In addition, T243 phosphorylation of PGK1 is associated with macrophage infiltration, grading and prognosis of GBM patients [81]. To summarize, this study provides a new strategy for treatment of GBM, which is to cut off the connection between M2 macrophages and GBM cells by suppressing the T243 phosphorylation of PGK1.

### *Ubiquitination of PGK1*

Two examples mentioned above have indicated that ubiquitination of PGK1 is of great signifi-

## PGK1-mediated cancer progression and drug resistance

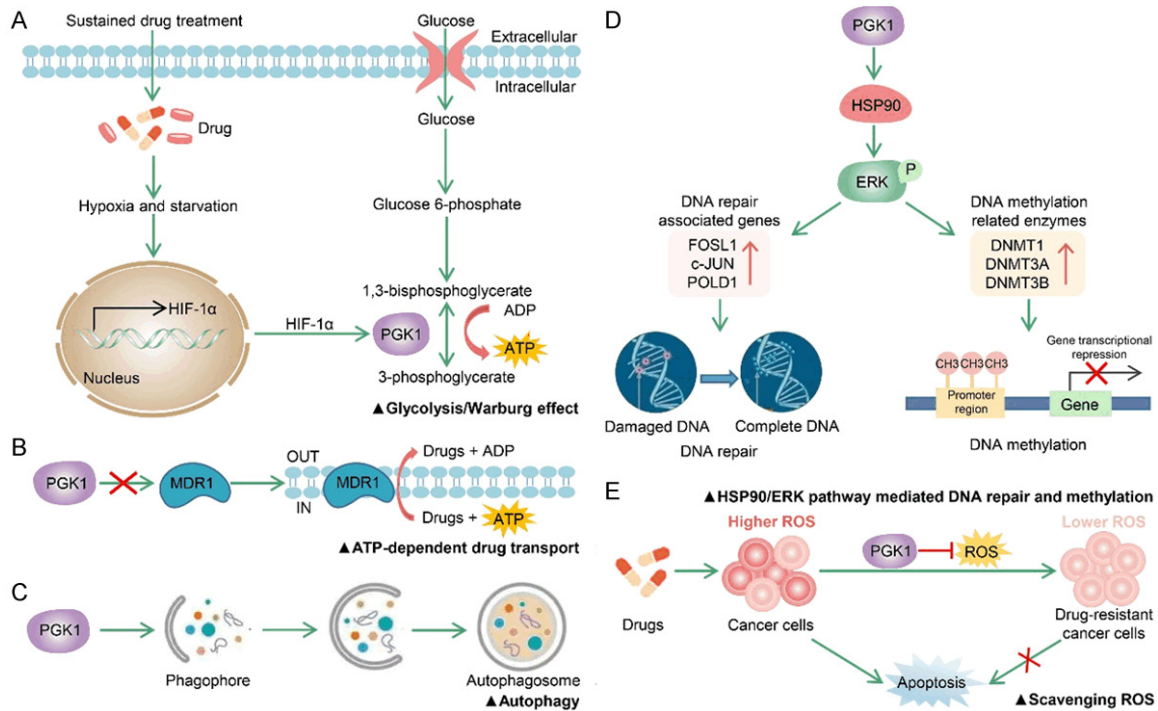


**Figure 2.** Several typical post-translational modifications of PGK1. **A.** KAT9 is a potential acetyltransferase of PGK1, which increases the K220 acetylation level and inhibits PGK1 enzyme activity by blocking its binding with substrate ADP. On the other hand, insulin-induced PI3K/AKT/mTOR pathway increases S424 phosphorylation of HDAC3, which promotes PGK1-HDAC3 interaction and PGK1 deacetylation at K220 to stimulate PGK1 activity. Finally, this HDAC3-dependent PGK1 deacetylation increases the production of glycolytic ATP, 3-PG and NADPH in cells. **B.** Hypoxia and glutamine deprivation inhibit mTOR-dependent ARD1 phosphorylation at S228, which enables ARD1 to interact with PGK1 for PGK1 acetylation. Next, the acetylated PGK1 binds to and phosphorylates Beclin1 at S30, which leads to activation of the VPS34-Beclin1-ATG14L complex for autophagosome formation. **C.** Signals (hypoxia, activation of EGFR, and K-Ras/B-Raf mutation) induce ERK-dependent phosphorylation of PGK1 at S203. Phosphorylated PGK1 is transported to mitochondria under the regulation of TOM complex and PIN1. Mitochondrial PGK1 functions as a protein kinase to phosphorylate and activate PDHK1, which further leads to phosphorylation and inactivation of PDH complex. This process results in the decrease of mitochondrial pyruvate metabolism and ROS generation and the enhancement of glycolysis in cytoplasm, and ultimately promotes GBM cell proliferation and tumorigenesis. **D.** M2-polarized macrophages secrete IL-6 to increase PDK1-dependent PGK1 phosphorylation in GBM cells. This phosphorylation can reduce the 3-PG affinity of PGK1, which facilitates the equilibrium of the PGK1-catalyzed reaction toward glycolysis.

cancer in suppressing the tumor growth and metastasis of NSCLC [60, 61, 64]. In addition, ubiquitin degradation of PGK1 inhibits autophagy-associated chemoresistance of GBC can-

cer cells [13]. However, ubiquitin modification sites of PGK1 are not confirmed in these findings. In conclusion, promoting degradation of PGK1 by increasing its ubiquitination may be

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**Figure 3.** PGK1 involvement in drug resistance. A. Sustained drug treatment leads to tumor starvation and intratumoral hypoxia. B. P-glycoprotein encoded by MDR1 induces multidrug resistance through the ATP-dependent efflux of lipophilic anticancer drugs. C. PGK1 induces chemoresistance of cancer cells by upregulating autophagy. D. PGK1 facilitates chemoresistance by triggering HSP90/ERK pathway mediated DNA repair and methylation. E. PGK1 causes drug resistance by scavenging ROS and inhibiting ROS-stimulated apoptosis.

an effective strategy for PGK1-targeted cancer therapies.

### Succinylation of PGK1

More and more types of PTMs on PGK1 have been discovered recently, and succinylation is one example. PGK1 and its lysine succinylation are both upregulated in RCC tissues compared with matched adjacent normal tissues by quantitative global proteome and lysine succinylome analyses [82]. However, the specific molecular mechanism of PGK1 succinylation in RCC has not been elucidated.

With the development of multiple omics profiling technologies, more PTMs of PGK1 may be uncovered to regulate cell metabolism and growth. However, all previous studies mentioned above only focus one PTM of PGK1. Actually, several PTMs of PGK1 probably interplay together on physiological and pathological conditions. Therefore, considering the cross-talk between different PTM, the combination of individual PTM on PGK1 protein surface can create a PTM code to regulate cell behavior.

This will pave a new way to design drugs for interference tumorigenesis and cancer progression.

### PGK1 with anticancer drug resistance

Drug resistance of cancer cells is one of the main obstacles in clinical cancer treatment, and its mechanism is complex and diverse. Generally, drug resistance of cancer cells is induced by many factors, such as enhancement of cell anti-apoptotic ability, improved DNA damage repair ability and upregulation of ATP-dependent drug transport protein expression. A large amount of clinical and experimental evidence shows that the high intracellular PGK1 plays a significant role in drug resistance (Table 4).

Reducing the expression of PGK1 will be a new strategy to overcome drug resistance in the future. For example, PGK1 is upregulated in radioresistant astrocytomas [83] and cisplatin-resistant ovarian cancer [84]. Moreover, inhibition of PGK1 increases the sensitivity of gastric adenocarcinoma cells to chemotherapeutic

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**Table 4.** The essential role of PGK1 in drug and radiation resistance

Cancer types	Aberrant expression	Drugs or radiation	Underlying pathways	References
Leukemia	↑	1,4-benzoquinone	PGK1 can protect K562 cells from 1,4-benzoquinone-induced toxicity by scavenging ROS and inhibiting ROS-stimulated apoptosis.	[41]
Astrocytoma	↑	Radiation	Not mentioned.	[83]
Ovarian cancer	↑	Cisplatin	Not mentioned.	[84]
Gastric cancer	↑	5-fluorouracil and mitomycin-C	Not mentioned.	[85]
Breast cancer	↑	Paclitaxel	Sustained chemotherapy drugs treatment leads to tumor starvation and intratumoral hypoxia, inducing the expression of HIF-1 $\alpha$ and its downstream target gene PGK1.	[86]
Osteogenic sarcoma	↑	Paclitaxel, vincristine, adriamycin and mitoxantrone	PGK1 induces a multidrug resistant phenotype through an MDR-1 independent mechanism.	[88]
Gallbladder carcinoma	↑	Doxorubicin	PGK1 induces chemoresistance of cancer cells by upregulating autophagy.	[13]
Endometrial cancer	↑	Cisplatin	PGK1 facilitates cisplatin chemoresistance by triggering HSP90/ERK pathway mediated DNA repair and methylation.	[92]
-	↑	H <sub>2</sub> O <sub>2</sub>	PGK1 can protect RAW 264.7 cells from H <sub>2</sub> O <sub>2</sub> -induced cell death by enhancing the chaperone activity of HSP90.	[94]
-	↑	Menadione	PGK1 knockdown induces higher susceptibility to menadione-induced cell death by producing a mass of ROS.	[6]

†: PGK1 expression is upregulated in drug or radiation resistant cells/PGK1 promotes the drug or radiation resistance of cells. -: The study is not carried out in cancer cells.

drugs 5-fluorouracil and mitomycin-C [85]. Although the exact molecular mechanisms for PGK1-involved drug resistance are not clearly clarified in the above research.

In breast cancer patients who undergo paclitaxel chemotherapy, patients with high expression of PGK1 have a shorter overall survival than those with low level of PGK1 [86]. It indicates that high PGK1 expression may be the key reason responsible for poor prognosis of breast cancer patients treated with paclitaxel. A hypothesis about the mechanism of PGK1 promoting drug resistance is proposed in this research. The sustained chemotherapy drug treatment leads to tumor starvation and intratumoral hypoxia, supporting the selection of resistant cell clones adapted to the deficiency of oxygen and nutrients. Therefore, response to hypoxia, PGK1 and related target gene of HIF-1 $\alpha$  are initiated to increase glycolysis in drug-resistant cells (**Figure 3A**).

The human multidrug resistance 1 (MDR1) gene encodes the plasma membrane P-glycoprotein that pumps lipophilic anticancer drugs out of the cancer cell and lead to drug resistance [87]. Overexpression of PGK1 induces a multidrug resistance phenotype in human osteogenic sarcoma cells, but the expression level of MDR1 has no change with the overexpression of PGK1 [88]. This suggests the generation of drug resistance phenotype induced by PGK1 is independent on MDR1 (**Figure 3B**).

Moreover, accumulating evidences suggest autophagy is also contributed to the resistance of cancer cells to anticancer drugs [89]. PGK1 has been demonstrated to play an essential role in modulating autophagy initiation [10-13]. Therefore, PGK1 seems to induce chemoresistance of cancer cells by upregulating autophagy (**Figure 3C**). For example, the latest study shows that knockdown of PGK1 can inhibit the autophagic flux of doxorubicin (Dox)-resistant GBC cancer cells at initial stage, which enhances the sensitivity of Dox-resistant GBC cancer cells to Dox *in vitro* and *in vivo* [13].

Tumor cells exposed to chemotherapy agents often improve global DNA hypermethylation, and this drug-induced DNA hypermethylation can cause drug resistance by inactivating the genes whose products are needed for chemotherapy agents to kill cancer cells [90]. Mo-

reover, DNA hypermethylation can be induced by overexpression of DNA methyltransferase (DNMTs), mainly DNMT1, DNMT3A and DNMT3B [91]. Recent studies have found that PGK1 promotes the expression of DNA repair-associated proteins (FOSL1, c-JUN, and POLD1) and DNA methylation-related enzymes (DNMT1, DNMT3A and DNMT3B) through the HSP90/ERK pathway. And this eventually enhances the chemoresistance of endometrial cancer cells to cisplatin [92, 93] (**Figure 3D**). Another research also indicates that PGK1 can activate HSP90 to enhance stress tolerance [94].

Moreover, drug-induced apoptosis is linked with the enhanced levels of ROS, and a prolonged drug treatment reduces ROS to make cells resistant to drugs [97]. PGK1 knockdown cells show higher susceptibility to ROS-induced cell death [6], and PGK1 upregulation protects K562 cells from 1,4-benzoquinone-induced toxicity by inhibiting ROS-stimulated apoptosis [41]. These results indicate that PGK1 has ability to scavenge ROS for drug resistance (**Figure 3E**).

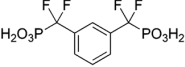
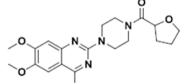
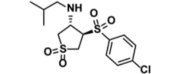
### Potential inhibitors of PGK1

Considering close associations of PGK1 with carcinogenesis and drug resistance, the development of PGK1 inhibitors is of great value for anticancer therapy. Due to lack of promising lead compounds, the research on small molecule inhibitors of PGK1 is comparatively weak by now [5]. We summarize several potential PGK1 inhibitors and compare their advantages and disadvantages in **Table 5**.

Current strategies to inhibit PGK1 in cancer are often through an indirect approach. The enzymatic activity of PGK1 is inhibited by modulating PTMs of PGK1. For example, K323 acetylation of PGK1 is thought to promote the enzymatic activity of PGK1, so the use of acetyltransferase inhibitors or sirtuin activators can repress PGK1 function effectively [5]. Similarly, KAT9 activators or HDAC3 inhibitors can increase the level of PGK1 K220 acetylation, which inhibits PGK1 enzyme activity by blocking its binding with substrate ADP [6]. Moreover, several antioxidants, including  $\alpha$ -tocopherol, butylated hydroxytoluene and Trolox, can inhibit the increase of PGK1 protein expression induced by hydrogen peroxide [37]. However, the specificity of these indirect approaches is

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**Table 5.** Potential inhibitors for PGK1

Inhibitors	Chemical structure	Inhibitory mechanism	Advantages	Disadvantages	References
Acetyltransferase inhibitors or sirtuin activators	-	Inhibit PGK1 K323 acetylation to inactivate PGK1	Inhibit tumor growth effectively <i>in vivo</i>	Poor specificity	[5]
KAT9 activators or HDAC3 inhibitors	-	Increase the level of PGK1 K220 acetylation, which inhibits PGK1 enzyme activity by blocking its binding with substrate ADP	Inhibit PGK1 activity significantly <i>in vitro</i>	Not reported in cancer research; Poor specificity	[6]
Antioxidants	-	Inhibit the H <sub>2</sub> O <sub>2</sub> -induced PGK1 overexpression	Inhibit PGK1 level in cancer cells effectively <i>in vitro</i>	Lack of <i>in vivo</i> experiments; Poor specificity	[37]
Bisphosphonate analogues		Function as analogues of 1,3-BPG and reduce the enzymatic activity of PGK1 by weakening the binding ability of PGK1 to 1,3-BPG	One of the earliest synthesized PGK1 competitive inhibitors; Relatively high specificity	Need to be further modified and optimized; Not reported in cancer research	[96, 97]
Terazosin		Terazosin occupies the ADP/ATP binding site of the PGK1	Relatively high specificity	Only inhibit PGK1 at high dosages; Need to be further modified and optimized; Not reported in cancer research	[94]
CBR-470-1		Reduce the stability of PGK1	The latest identified PGK1 inhibitor	Uncertain inhibitory mechanism; Not reported in cancer research	[98]

Note: '-' means there are no detailed information.

controversial, mainly because PGK1 is not the only target of these inhibitors.

PGK1 catalyzes the conversion of 1,3-BPG and ADP into 3-PG and ATP, and this important catalytic reaction in glycolysis requires the binding of PGK1 to substrates (1,3-BPG and ADP). Therefore, the design of inhibitors for PGK1 has generally focused on analogues of the natural substrate 1,3-BPG. Bisphosphonate analogues of 1,3-BPG are one of the earliest synthesized PGK1 competitive inhibitors [96, 97]. These bisphosphates can competitively bind to PGK1, which weakens the binding ability of PGK1 to 1,3-BPG, thus greatly reducing the enzymatic activity of PGK1. Terazosin is also considered as a potential inhibitor of PGK1. Terazosin can significantly inhibit PGK1 at high dosages (2.5-25  $\mu$ M) [97]. Co-crystal structure data from PGK1 with terazosin suggests that the terazosin-binding site overlaps with the ADP/ATP binding site of the PGK1. Therefore, terazosin is supposed to be a competitive inhibitor of PGK1. Recently, a small-molecule compound, CBR-470-1, has been identified as a new novel inhibitor of PGK1 [98]. However, the specific mechanism of CBR-470-1 inhibiting PGK1 is unclear. So far, whether CBR-470-1, bisphosphonates, terazosin or their derivatives, the effects of these PGK1 inhibitors on cancer cells have not been reported. That is to say, whether they can inhibit the growth of tumors by inhibiting PGK1 needs to be further evaluated by experiments.

In brief, there is still a long way to develop novel PGK1 inhibitors for clinical cancer treatment. Future strategies for developing PGK1 inhibitors may be through virtual screening (i.e., similarity search, pharmacophore model, shape-based model and molecular docking) based on the X-ray structure of PGK1/terazosin complex [99]. Meanwhile, designing strong inhibitors for PGK1 and then seeking to modify and optimize them is also a trend in the future.

### Future perspective

Cancer is considered as a metabolic disease. Therefore, it is important to understand metabolic related genes in tumors. Even under oxygen-sufficient conditions, cancer cells prefer glycolysis than mitochondrial oxidative phosphorylation for energy acquisition during proliferation and metastasis. As a key enzyme in

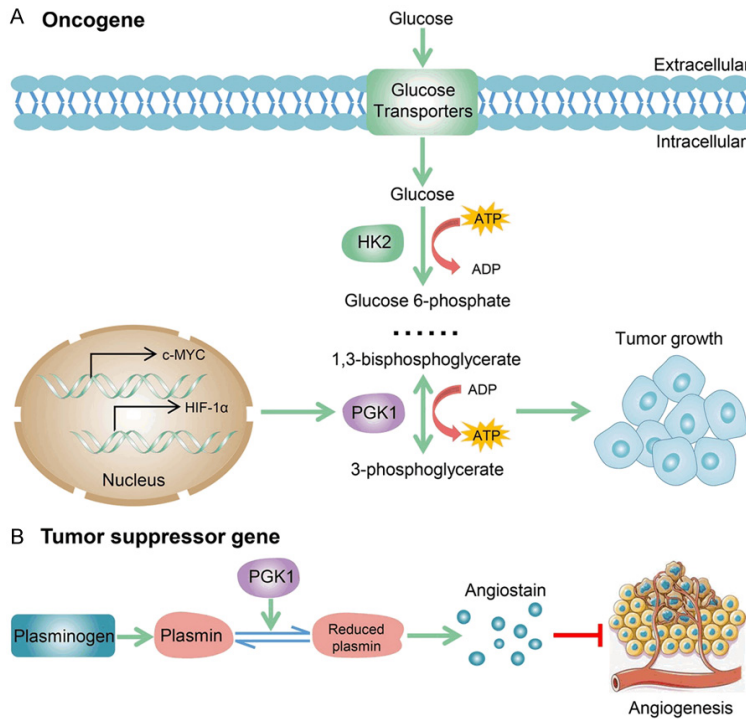
glycolysis, PGK1 catalyzes the reversible conversion of 1,3-BPG to 3-PG, generating one molecule of ATP to meet the energy requirements for rapid growth of cancer cells. PGK1 and PKM2 are the only two enzymes that generate ATP during aerobic glycolysis in cancer cells. However, the enzyme activity of PKM2 in cancer cells is very low [100]. Low activity PKM2 can increase the accumulation of upstream glycolysis metabolites, which can be converted into nucleic acids, amino acids and lipids through corresponding pathways for biosynthesis. Therefore, it can be inferred that PGK1 controls a considerable part of ATP synthesis in cancer cells and plays an important role in promoting tumor growth.

However, under hypoxic conditions, PGK1 production by tumor cells is enhanced, but its secretion is inhibited [20]. In addition, patients with stronger PGK1 expression in the ITF of OSCC have a higher risk of local relapse and worse survival, but the low expression of PGK1 in saliva samples from OSCC patients is associated with lymph node metastasis and advanced clinical stage [76]. These contradictions may imply that the functions of intracellular and extracellular PGK1 are different. Extracellular PGK1 can serve as a disulfide reductase, which can generate angiostatin by promoting the reduction of disulfide bonds in plasmin. Regulation of glucose metabolism (**Figure 4A**) and angiogenesis (**Figure 4B**) are the two main mechanisms of PGK1-mediated tumor growth and migration. High levels of PGK1 are essential for tumor growth but inhibit angiogenesis when released extracellularly. The functional role and regulatory mechanism of PGK1 in cancer progression are still not particularly clear. How to make a balance and utilize the important role of PGK1 in glycolysis and angiogenesis remains to be further studied for targeted cancer therapy.

PGK1 secretion into cell extracellular space provides a convenient way of liquid biopsy. The abnormal level of PGK1 is detectable conveniently not only in tumor tissues, but also in peripheral blood and saliva of patients. Therefore, as a promising diagnostic and prognostic biomarker, PGK1 has a good application prospect in liquid biopsy. Combined detection of PGK1 and other tumor markers can significantly improve the sensitivity and specificity of



## PGK1-mediated cancer progression and drug resistance



**Figure 4.** Molecular mechanisms of PGK1-mediated glucose metabolism and angiogenesis. A. PGK1 has an essential role in metabolic reprogramming induced by c-MYC and HIF-1 $\alpha$ , leading to an enhanced Warburg effect and tumorigenesis. B. Extracellular PGK1 serves as a disulfide reductase, which can promote the reduction of disulfide bonds in plasmin to generate angiostatin for angiogenesis inhibition.

diagnosis. For example, the high serum level of PGK1 is closely related with HCC early relapse and worse prognosis, and the serum PGK1 level is complementary to monitor AFP for improving the sensitivity and specificity of predicting HCC recurrence [70].

PGK1 is considered to be a drug resistance-related protein in cancer cells. High expression of PGK1 protein may promote drug resistance through complicate molecular mechanisms. Therefore, predicting sensitivity to chemotherapeutic drugs by detecting the expression of PGK1 before commencing chemotherapy will be beneficial to those patients who are unlikely to respond to drugs, and will protect patients from unnecessary drug toxicity. In addition, decreasing the expression of PGK1 will be a new strategy to overcome drug resistance in the future. The combination of PGK1 inhibitors and chemotherapeutic drugs may be effective in reversing drug resistance and improving therapeutic effect.

Unfortunately, due to the lack of promising lead compounds, the research on small molecule

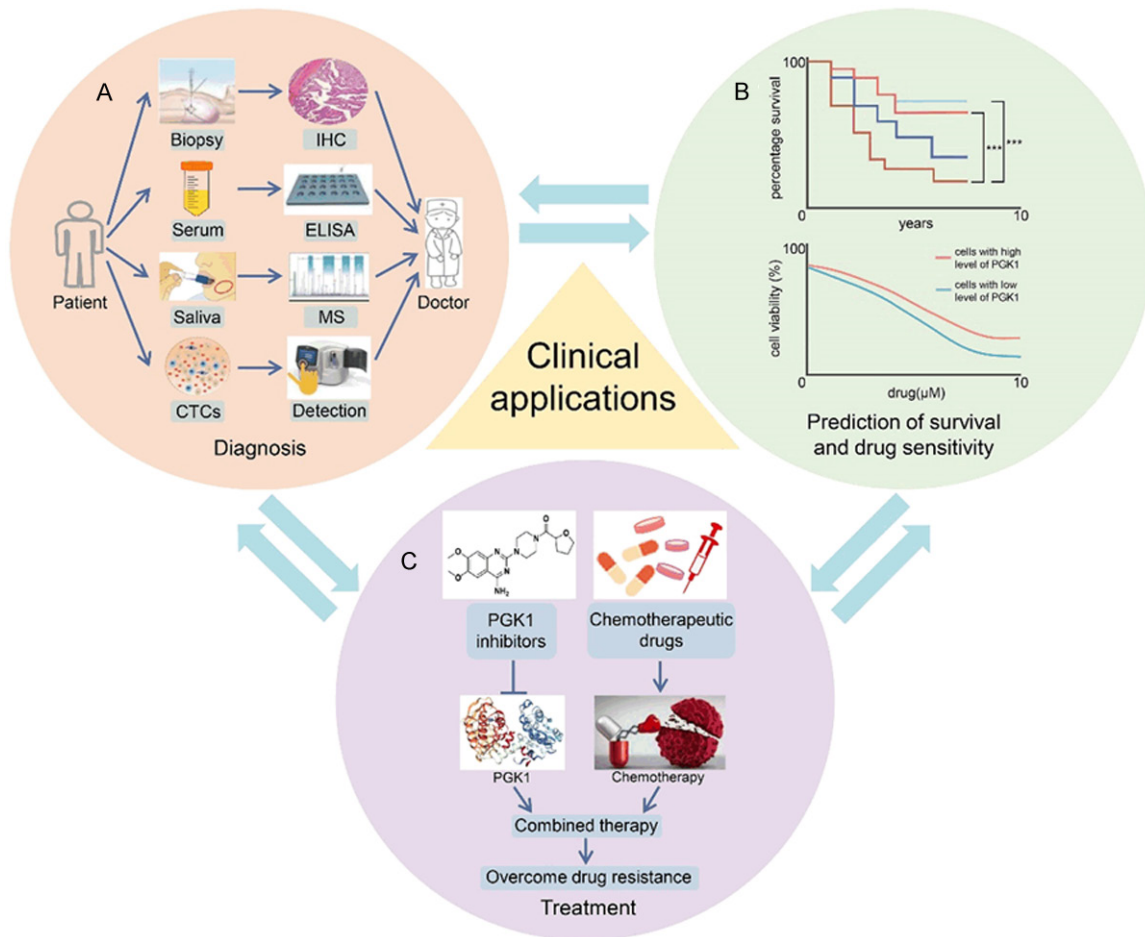
inhibitors of PGK1 is relatively weak. The small molecule inhibitors of PGK1 have some shortcomings, such as poor activity, low druggability and bad specificity, which bring great challenges to drug discovery and development. At present, PGK1-specific inhibitor is still not available [5]. Some antioxidants, acetyltransferase inhibitors and sirtuin activators have been shown to significantly reduce the enzyme activity of PGK1 to inhibit tumor growth [5, 37]. However, the specificity of these inhibitors is controversial, mainly because PGK1 is not the only target of them. On the other hand, some competitive inhibitors of PGK1, such as bisphosphonates, terazosin and their derivatives can specifically inhibit PGK1 *in vitro*. Despite this, the effects of these PGK1 competitive inhibitors on cancer cells have not been reported. In the future, a large number of *in vivo* experiments are needed

to study whether they have inhibitory effects on tumors and whether they have toxic and side effects on the body. At the same time, proper levels of PGK1 expression are essential for normal cell function, and PGK1 deficiency has been associated with multiple diseases [15-18]. It can be inferred that PGK1 inhibitors have some toxic and side effects on normal cells. Therefore, it is urgent to construct a targeted drug delivery system to allow PGK1 inhibitors targeting cancer cells.

Moreover, several somatic variants of PGK1 have been identified in cancer cells, but more detailed experiments are needed to explore the function of mutated PGK1 in cancer cells. Meanwhile, multiple PTMs of PGK1 are found to be able to regulate tumor metabolism and growth. The crosstalk between various PTMs of PGK1 in the development of cancer deserves a further study. In addition, how to design drugs according to the functions of PGK1 PTMs is also a difficult problem.

Overall, PGK1 has great value in the diagnosis, treatment and prognosis of various types of

## PGK1-mediated cancer progression and drug resistance



**Figure 5.** Potential clinical value of PGK1 for diagnosis, treatment and prognosis of cancers. A. Monitoring PGK1 in clinical samples such as tissue, serum, saliva and circulating tumor cells (CTCs) is helpful for doctors to diagnose and treat patients better. B. PGK1 level is related to patient survival and drug sensitivity. C. Combined therapy with PGK1 inhibitors and chemotherapeutic drugs in the treatment of cancer significantly overcome drug resistance.

cancer (Figure 5). It is of great significance to develop therapeutic drugs targeting PGK1 in the future.

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### Disclosure of conflict of interest

None.

### Abbreviations

PGK, phosphoglycerate kinase; PKM2, pyruvate kinase M2; 1,3-BPG, 1,3-bisphosphoglycer-

ate; 3-PG, 3-phosphoglycerate; PTM, post-translational modification; HPA, human protein atlas database; PCa, prostate cancer; lncRNA, long noncoding RNA; HCC, hepatocellular carcinoma; NBD, nucleotide-binding domain; CD, catalytic domain; uPAR, urokinase-type plasminogen activator receptor; GBC, gallbladder carcinoma; HIF-1 $\alpha$ , hypoxia inducible factor 1 $\alpha$ ; EMT, epithelial-mesenchymal transition; GBM, glioblastoma; ROS, reactive oxygen species; ccRCC, clear cell renal cell carcinoma; CXCR4, C-X-C chemokine receptor 4; CXCL12, C-X-C chemokine ligand 12; CTCs, circulating tumor cells; NSCLC, non-small cell lung cancer; AFP, alpha-fetoprotein; OSCC, oral squamous cell carcinoma; ITF, invasive tumor front; TOM, translocase of the outer mitochondrial membrane; MDR1, multidrug resistance 1; Dox, doxorubicin; DNMTs, DNA methyltransferase.

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