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REVIEW ARTICLE

PCK1 dysregulation in cancer: Metabolic reprogramming, oncogenic activation, and therapeutic opportunities

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Abstract The last few decades have witnessed an advancement in our understanding of multiple cancer cell pathways related to metabolic reprogramming. One of the most important cancer hallmarks, including aerobic glycolysis (the Warburg effect), the central carbon pathway, and multiple-branch metabolic pathway remodeling, enables tumor growth, progression, and metastasis. Phosphoenolpyruvate carboxykinase 1 (PCK1), a key rate-limiting enzyme in gluconeogenesis, catalyzes the conversion of oxaloacetate to phosphoenolpyruvate. PCK1 expression in gluconeogenic tissues is tightly regulated during fasting. In tumor cells, PCK1 is regulated in a cell-autonomous manner rather than by hormones or nutrients in the extracellular environment. Interestingly, PCK1 has an anti-oncogenic role in gluconeogenic organs (the liver and kidneys), but a tumor-promoting role in cancers arising from non-gluconeogenic organs. Recent studies have revealed that PCK1 has metabolic and non-metabolic roles in multiple signaling networks linking metabolic and oncogenic pathways. Aberrant PCK1 expression results in the activation of oncogenic pathways, accompanied by metabolic reprogramming, to maintain tumorigenesis. In this review, we summarize the mechanisms underlying PCK1 expression and regulation, and clarify the crosstalk between aberrant PCK1 expression, metabolic rewiring, and signaling pathway activation. In addition, we highlight the clinical relevance of PCK1 and its value as a putative cancer therapeutic target.

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Introduction

The global cancer incidence has risen to approximately 19.3 million new cases and nearly 10 million deaths in 2020.[1] During tumorigenesis, metabolic pathways are reprogrammed to promote cancer progression.[2] The Warburg effect, i.e., a preference for aerobic glycolysis and lactate secretion, is a hallmark of malignant persistence in proliferating cancer cells.[3] Multiple reprogrammed metabolic activities, including aerobic glycolysis, glutamine and fat catabolism, redox homeostasis, and macromolecular synthesis, support tumor cell survival and proliferation.[4]

Gluconeogenesis is the generation of glucose from non-carbohydrate substrates.[5] Phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase (FBPase), and glucose-6-phosphatase (G6Pase) catalyze irreversible steps in gluconeogenesis. The first rate-limiting enzyme of gluconeogenesis, PEPCK, has two isoforms: PCK1 (cytosolic, also termed PEPCK-C, PEPCK1) and PCK2 (mitochondrial, also termed PEPCK-M, PEPCK2). Both enzymes catalyze the generation of phosphoenolpyruvate (PEP), CO_2, and GDP from oxaloacetate (OAA) and GTP. The tricarboxylic acid (TCA) cycle, in which acetyl-CoA is oxidized to CO_2 and H_2O, provides energy to the cell. Metabolic intermediates can flow into (anaplerosis) and out of (cataplerosis) the TCA cycle, without oxidative phosphorylation.[6] Cataplerosis is involved in biosynthetic pathways, including gluconeogenesis, fatty acid synthesis, and glyceroneogenesis.[6] As a cataplerotic enzyme, PEPCK has an essential role in the disposal of TCA cycle intermediates. PEPCK is especially important in PEP generation from OAA, which is used for gluconeogenesis.[7] Interestingly, PCK1 has contradictory roles in tumors originating from different tissues or organs. For example, it has an anti-oncogenic role in gluconeogenic organs (the liver and kidneys) but promotes tumors originating from non-gluconeogenic organs. Several metabolic enzymes and metabolites possess non-canonical and moonlighting functions to support tumor formation.[8] Metabolic enzymes aberrantly regulated in cancer govern various cellular activities, including gene expression, DNA repair, apoptosis, cell-cycle progression, and microenvironment remodeling.[9] Emerging evidence demonstrates that PCK1 has metabolic and non-metabolic functions that contribute to tumor initiation and progression.

In this review, we first examine recent findings on PCK1 expression and regulation, and the interplay between PCK1 and metabolic signaling during tumor initiation, progression, and metastasis. We also discuss the non-canonical role of PCK1 in immune function and preclinical research. Understanding these metabolic and non-metabolic functions of PCK1 may broaden our understanding of the crosstalk between metabolic rewiring and signaling pathways, and provide potential therapeutic targets for cancer treatment.

PCK1 structure and function

Functional motifs and inhibitors of PCK1

The human PCK1 gene is located on chromosome 20q13.31 and encodes a 69-kDa cytosolic protein of 622 amino acids.[10] PCK1 possesses putative carboxylate group-binding (aa 87—417), Mn ion-binding (aa 244—311), ribose-binding (aa 287—436), and guanine-binding (aa 298—533) motifs and a 288-aa active site (Fig. 1A). It is a phosphoryl-transfer enzyme that has an unusual nucleotide-binding site for β, γ-methylene GTP binding.[11,12] The GTP nucleotide fits into the binding pocket that comprises guanine- and ribose-binding sites. OAA and PEP bind to carboxylate group-binding sites.[11—13]

3-Mercaptopicolinic acid (3-MPA) is an inhibitor of PCK1[14] that binds PCK1 at two binding sites: one acting in competition with PEP/OAA and one derived from an altered conformation of the ribose/guanine-binding motif that reduces the affinity of PCK1 for GTP.[15,16] The 3-MPA derivative 3-((carboxymethyl)thio)-picolinic acid (CMP) acts as a potentially more selective inhibitor via binding at the OAA/PEP-binding site.[17] Modified 3-alkyl-1,8-dibenzylxanthines inhibit PCK1 activity by GTP-competitive binding.[18,19] PCK1 inhibitors are used to improve glucose homeostasis in anticancer therapy,[20] underscoring the potential importance of this pathway as a novel therapeutic target.

Gluconeogenic and anaplerotic activities of PCK1

PCK1 plays unique roles in diverse physiological processes. Under low-energy conditions, the binding of phosphate moieties of GTP to the region harboring Mn^{2+}-binding sites triggers the conversion of OAA to PEP, thereby inducing GDP and gluconeogenic activity (Fig. 1B).[11—13] At high glucose levels, hyperacetylation at Lys91 of PCK1 favors anaplerotic activity (PEP → OAA) by improving the kinetic properties for PEP and GDP (Fig. 1C).[21,22]

Non-canonical enzymatic roles of PCK1

Modification of the sulfhydryl group in the Cys288 side chain of PCK1 may cover the surface sites, prevent GTP-binding, and reduce activity.[12] PCK1 is not only capable of transferring a phosphate group from GTP to a metabolite, but it can also use GTP as a phosphate donor to phosphorylate a target protein (Fig. 1D).[23,24] AKT-induced PCK1 phosphorylation at Ser90 inhibits the canonical catalytic function of PCK1 in gluconeogenesis. AKT-phosphorylated PCK1 undergoes a conformational change enhancing its binding to INSIG1/2 and phosphorylates the conjugated protein as a protein kinase.[23] The interaction of PCK1 with lactate dehydrogenase A (LDHA) reduces its stability and activity.[25] Self-acetylation of PCK1 affects enzymatic activity (Fig. 1E).[26] Acetylation of Lys244 in PCK1 blocks manganese coordination with OAA resulting in low enzymatic activity. The binding of acetyl-CoA relies on metal ions to occupy the GTP/GDP-binding site of PCK1.[26] Whether PCK1 functions as an acetyltransferase requires further investigation. Interestingly, PCK1 is enriched in the nuclear fraction of rat liver, without abundant PEP to fulfill glycogenesis,[27] suggesting that PCK1 may have additional unknown functions, such as transcriptional regulation, depending on the nutritional status or environmental stress signals.

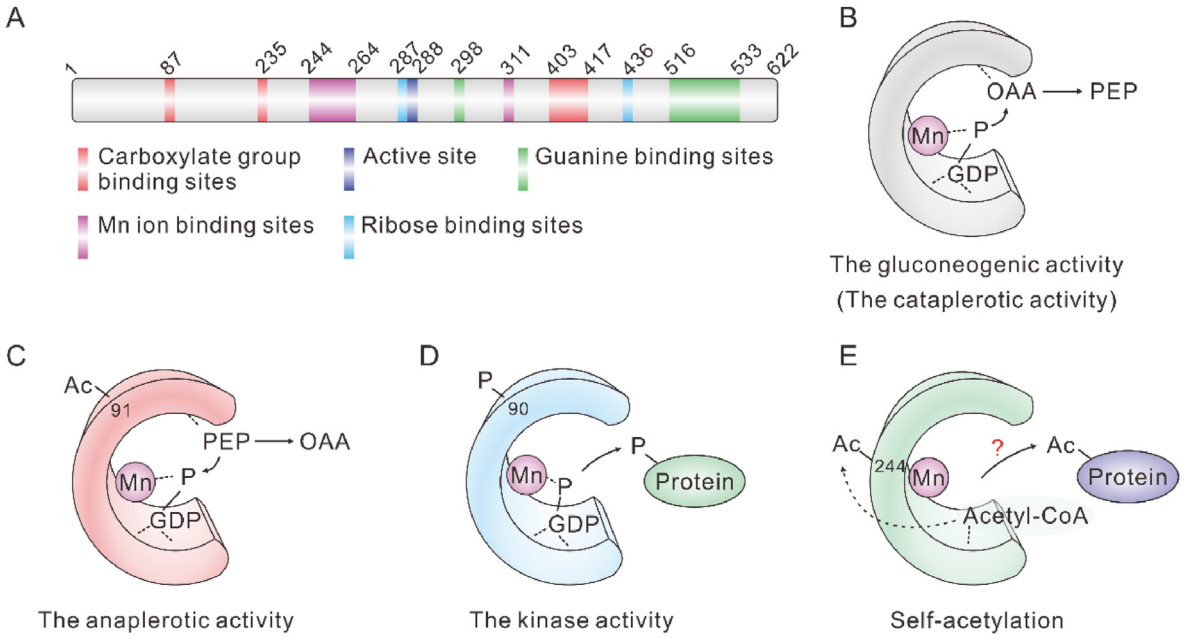

Figure 1 Functional sites and activities of PCK1. **(A)** The 622-aa PCK1 protein has numerous functional sites followed by carboxylate-group binding sites for OAA or PEP, guanine- and ribose-binding sites, which bind GTP nucleotides, Mn^{2+}-binding sites that bind the phosphate moieties of GTP, and an enzymatic active site. **(B–E)** Schematic illustration of enzymatic and non-enzymatic catalysis by PCK1, including gluconeogenic activity, anaplerotic activity, kinase activity, and self-acetylation. Gluconeogenic activity promotes the conversion of OAA to PEP, producing GDP. Anaplerotic activity catalyzes a reversible reaction from PEP to OAA. Kinase activity transfers a phosphate group from GTP to a target protein. Self-acetylation displays low enzymatic activity and may have an acetyltransferase function.

Multilevel PCK1 expression regulation

Hormones and transcriptional regulation

PCK1 expression is regulated at the transcriptional, post-transcriptional, and post-translational levels. Transcriptional regulation is considered the major modality of PCK1 regulation. The *PCK1* promoter harbors numerous regulatory elements (Fig. 2). In normal gluconeogenic tissues, glucagon and glucocorticoids activate *PCK1* transcription, while insulin inhibits PCK1 expression. When glucose falls below or amino acids increase beyond normal levels in the blood, glucagon secreted by the pancreas upregulates *PCK1* transcription in the liver by activating cAMP regulatory element-binding protein (CREB).[28,29] Under stress conditions, such as fasting/starvation, glucocorticoids produced in the adrenal cortex enter hepatocytes, bind to the cytoplasmic glucocorticoid receptor and drive its translocation into the nucleus, and enhance *PCK1* transcription by directly binding to the glucocorticoid-response element.[30–32] Under high-glucose conditions, insulin inhibits Forkhead box protein O1 (FoxO1) and PCK1 expression via the PI3K-PIP3-AKT axis.[33,34] Insulin regulates PCK1 expression by blocking the interaction between CREBP and PGC-1α, decreasing PGC-1α transcriptional activity and enhancing the binding of SREBP to SREBP regulatory element (SRE).[35,36] In addition, it regulates the levels of epigenetic modifications, such as H3K4me3, H3ac, and H4ac, to suppress PCK1 expression.[37,38] The AP-1 superfamily members c-Jun and c-Fos bind to cAMP regulatory element (CRE) to upregulate PCK1 expression and block *PCK1* promoter activity, respectively.[39]

Stress signaling, transcriptional, and post-translational regulation

Stress signals such as nutritional changes, oxidative stress, cytokines, and toxins, regulate PCK1 expression and activity.[22,40–42] Oxidative stress activates p38 MAP kinases and augments the transcriptional activity of activating transcription factor 2 (ATF2) on PCK1 expression.[43] Conversely, ATF3 binds to CAAT/enhancer-binding protein (C/EBP) and represses PCK1 transcription.[44] ATF3 has central functions in the stress signaling network of endoplasmic reticulum stress, cytokines, and toxins.[45] Thus, ATF3 may be a cellular stress sensor. IL1 and TNFα can attenuate *PCK1* transcription by inhibiting glucagon-stimulated signal transduction at the site of cAMP formation.[42] In rat endotoxemia, palmitate increased hepatic *PCK1* mRNA abundance (by ~44%) via the toll-like receptor 4 (TLR4)/NF-KB/CREB pathway.[41] Saturated free fatty acids activate inflammatory cascades, including the TLR4 pathway, which links free fatty acid uptake to altered hepatic gluconeogenesis in type 2 diabetes mellitus.[41] Bile acid, lactate, and pyruvate positively regulate PCK1 expression and hepatic glucose production by binding nuclear bile acid receptor or changing the NAD^+/NADH ratio.[46,47]

Acetylation, ubiquitination, and phosphorylation modulate the biological function of PCK1. In high-glucose

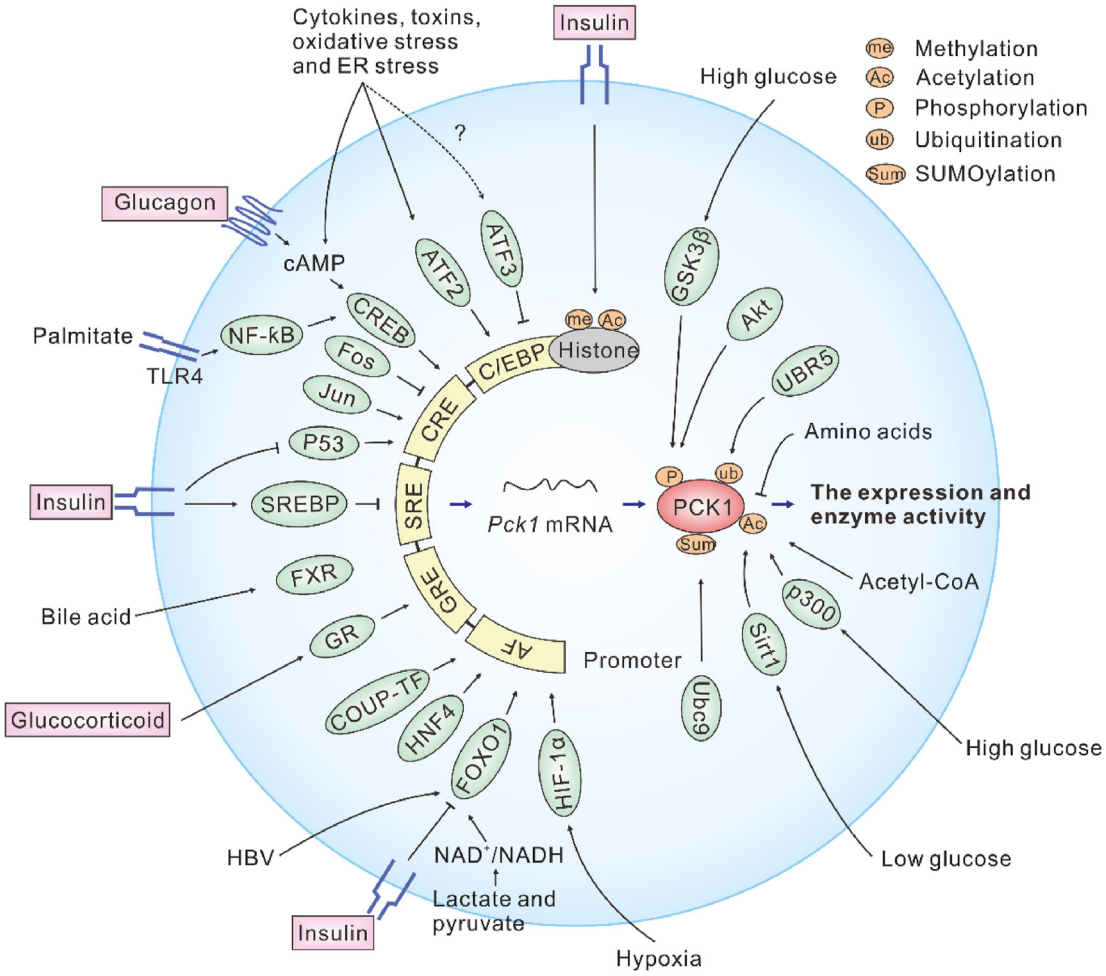

Figure 2 Overview of multilevel PCK1 expression regulation. In normal gluconeogenic tissues, hormones and environmental stress signals modulate PCK1 expression and activity mainly through transcriptional and post-translational regulation. In tumors originating from different organs, cell-autonomous signals may determine the diverse PCK1 expression. Abbreviations of promoters: AF, accessory factor; C/EBP, CAAT/enhancer-binding protein; CRE, cAMP regulatory element; GRE, glucocorticoid regulatory element; SRE, SREBP regulatory element.

environments, PCK1 enhances anaplerotic activity, but lowers gluconeogenic activities.[22] Acetylation of Lys70, Lys71, and Lys594 of PCK1 under high-glucose conditions lowers its stability.[48,49] GSK3β-induced phosphorylation of PCK1 promotes its self-ubiquitination and degradation under high-glucose conditions.[22] Under low-glucose conditions, SIRT1 deacetylates PCK1 and facilitates recovery of its gluconeogenic activity.[22] Acetyl-CoA senses the cell metabolic state through protein acetylation modifications. PCK1 can directly interact with acetyl-CoA and autoacetylate. PCK1 self-acetylation suppresses its gluconeogenic activity,[26] suggesting that the enzymatic activity of PCK1 varies according to the amount of acetyl-CoA, depending on the metabolic status. Thus, PCK1 expression and enzyme activity are regulated by hormone- and stress signaling-mediated transcription and protein modification.

Cell-autonomous regulation of PCK1 in tumor cells

In tumor cells, due to environmental stress and genetic alterations, PCK1 is regulated in a cell-autonomous manner, relying less on hormonal feedback regulation.[50,51] PCK1 is downregulated in tumors originating from gluconeogenic organs, such as hepatocellular carcinoma (HCC) and renal cell carcinoma (RCC).[25,52,53] Aberrant transcriptional and post-translational regulation are the main reasons for PCK1 dysregulation.[54] Aberrantly hyperactive mTOR2 eliminates PCK1 expression by triggering the nuclear export of FoxO1.[55] p53 enhances PCK1 expression.[56] Mutant p53 is involved in HCC carcinogenesis,[57] and may be an important regulator of PCK1 expression. Hepatitis B X-interacting protein suppresses PCK1 by targeting FoxO1 mRNA and FoxO1 nuclear export.[52] In HCC cells, PCK1 SUMOylation is increased, enhancing PCK1 ubiquitination and degradation.[53] Post-translational modifications, such as acetylation, phosphorylation, SUMOylation, and ubiquitination, may play critical roles in regulating PCK1 expression in tumors.

In cancers originating from non-gluconeogenic organs, such as colon, lungs, breast, and skin cancers, PCK1 expression is upregulated.[36] Oncogenic *KRAS* mutations are found in 30—50% of colorectal cancers and increase PCK1

expression.[58] SIRT2 is markedly increased in gastric cancer and deacetylates and stabilizes PCK1.[59] In breast cancer, hypoxia-inducible factor 1α and FoxO1 induce epigenetic reprogramming to upregulate PCK1.[60] In tumors originating from different organs, the interplay between environmental stress signals and genetic alterations may determine the diverse PCK1 expression. In various cancer types, PCK1 acts as a specific metabolic modulator of biomolecule synthesis under nutrient-limited conditions. Metabolic reprogramming in cancer cells regulates PCK1 expression and activity likely in a cell-autonomous fashion.

PCK1, gluconeogenesis, and cancer cachexia

Gluconeogenesis, which mainly occurs in the liver and kidney cortex, maintains the blood glucose level during fasting/starvation.[61] Cancer cells exhibit a high rate of anaerobic glycolysis, thereby consuming glucose to produce lactate.[61,62] The excess lactate is absorbed by the liver and used to produce glucose through gluconeogenesis, which is then returned to the circulation and reused for glycolysis, a metabolic pathway known as the Cori cycle. The unique feature of this cycle is that glycolysis and gluconeogenesis do not occur in the same tissues.[63,64] Thus, the liver and kidney cortex exhibit a high gluconeogenesis rate and low glycolysis rate, whereas the malignant sites generally show high glycolysis and low gluconeogenesis rates.[61,65] High ATP-consuming Cori cycle activity is a common cause of cancer cachexia.[66–68] Accordingly, Cori cycle inhibitors have been used to treat cancer cachexia.[69] If tumors gain their energy from anaerobic glycolysis, the inhibition of gluconeogenesis would be an effective therapeutic strategy.[62]

The conversion of OAA to PEP by PCK1 is the first rate-limiting, and likely the most important step in gluconeogenesis. Tumor growth inhibition by PCK1 suppression has been demonstrated in patients with non-gluconeogenic organ tumors and cachexia.[20] The PCK1 inhibitors hydrazine sulfate, tryptophan, and pyridine-2,3-dihydrazide significantly inhibit the growth of Walker carcinosarcoma and leukemia, lymphosarcoma, and melanoma.[70] Patients with HCC or RCC present with high-grade hypoglycemia due to low PCK1 expression and activity.[7,25,71–74] Gluconeogenesis induced by glucocorticoids can enhance glucose synthesis and blood glucose levels in patients.[75] Depending on the tumor type, proper modulation of PCK1 activity and gluconeogenesis may improve the quality of life of cancer patients.

PCK1 directly mediates signaling pathways

PCK1 as a protein kinase

PCK1 expression and activity affect the biological processes of tumor cells (Fig. 3). Metabolism feeds the regulation of signaling pathways via metabolic enzymes and metabolites. PCK1 and its metabolites may directly affect cancer signaling pathways.[76] A moonlighting function of PCK1 has been recently described in HCC. Insulin-like growth factor 1-induced Akt activates the protein kinase activity of PCK1 by phosphorylating Ser90.[23] Phosphorylated PCK1 translocates to the endoplasmic reticulum and phosphorylates INSIG1/2, thereby contributing to the movement of the SCAP/SREBP complex to the Golgi apparatus. SREBP activation promotes the expression of lipogenesis-related genes and tumorigenesis.[23]

PEP as a metabolic inhibitor

PCK1 converts OAA and GTP into PEP, GDP, and CO_2. Due to PCK1 dysregulation in tumor cells, the concentrations of PCK1 substrates and products increase or decrease.[7,77] PEP can be transported into the intracellular space via the erythrocyte membrane,[78] suggesting the existence of a PEP transporter. PEP can markedly attenuate the decrease in cell viability.[79] In melanoma, PEP directly binds and represses sarco/ER Ca^{2+}-ATPase (SERCA) activity to mediate Ca^{2+}-NFAT signaling while sustaining T-cell functions, thereby exerting anti-tumor effects.[80] O-GlcNAcylation is a nutrient-sensitive posttranslational modification that regulates protein function and subcellular localization.[81] We found that PEP treatment suppressed O-GlcNAcylation in hepatoma cells.[82] As O-linked N-acetylglucosaminyltransferase (OGT) is the only enzyme known to transfer N-GlcNAc into target proteins,[83] we speculated that PEP may act as a metabolic inhibitor by binding OGT, suppressing its activity; however, further studies are required to validate this hypothesis.

Non-covalent interactions with OAA and target proteins

In neuronal cells, the addition of OAA enhances glycolysis and oxidative phosphorylation via increasing the levels of cytosolic malate dehydrogenase 1 and total and phosphorylated SIRT1.[84] In addition, OAA ameliorates chemical liver injury via increasing ATP level and inhibiting MAPK pathways.[85] *In vitro* and *in vivo* studies have shown that the hepatoprotective effect of OAA is related to enhanced liver bioenergetics.[86] OAA binds to succinate dehydrogenase to inhibit its enzymatic activity.[87,88] As a competitive inhibitor of human LDHA, OAA inhibits its activity and the Warburg effect in cancer cells.[89] OAA may have more unknown functions in protein binding and biology.

Intracellular GTP/GDP levels

Ectopic PCK1 expression significantly increased AMPK phosphorylation under low-glucose conditions.[7] We have found that, due to GTP consumption, PCK1 overexpression enhances AMPK phosphorylation by reducing the intracellular ATP/ADP ratio, thereby decreasing Rb phosphorylation, which contributes to cell-cycle arrest at G1.[90] Intracellular GTP/GDP levels regulated by PCK1 may mediate signaling pathways in cancers. This is corroborated by mitochondrial GTP levels regulating signals involved in mitochondrial maintenance, insulin secretion, nutrient sensing, and cell health.[91] Moreover, we have found that PCK1 deficiency is associated with the development of hepatic steatosis and fibrosis through elevated GTP levels facilitating RhoA/AKT signaling and activating hepatic

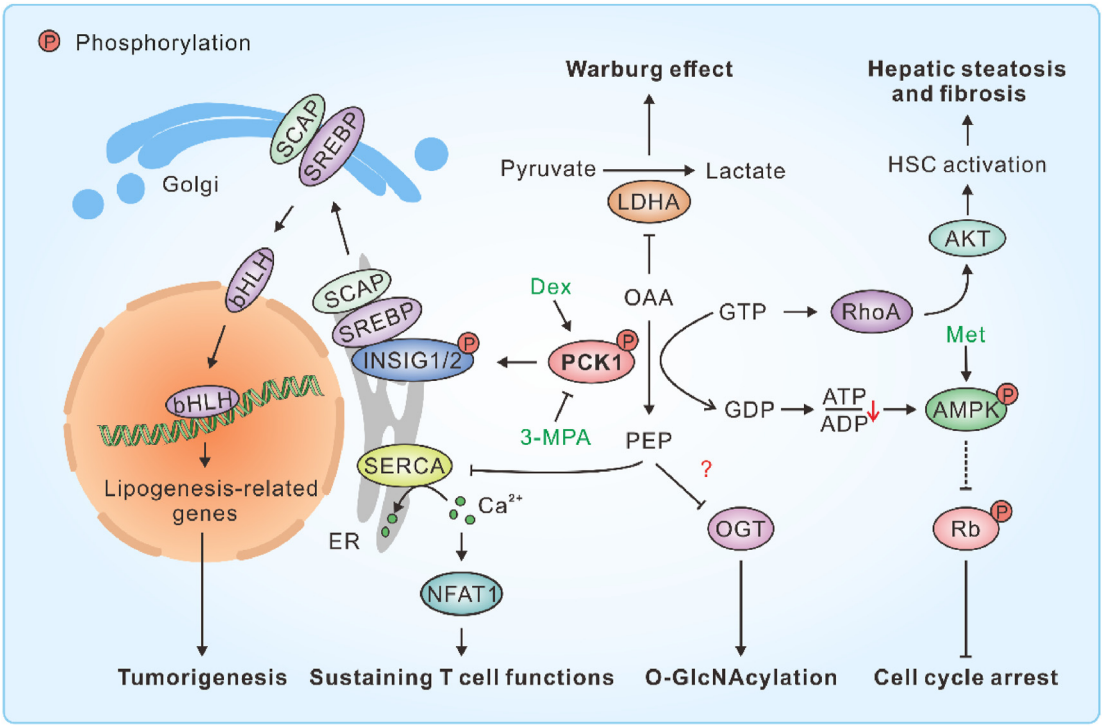

Figure 3 PCK1 and its metabolites directly mediate signaling pathways. Schematic overview of the signaling networks directly regulated by PCK1 and its metabolites, including OAA, PEP, GTP/GDP, and ATP/ADP. Abbreviations: AKT, RAC-alpha serine/threonine-protein kinase; AMPK, AMP-activated protein kinase; bHLH, basic helix-loop-helix protein; Dex, dexamethasone; INSIG1/2, insulin-induced gene 1 protein; LDHA, lactate dehydrogenase A; Met, metformin; 3-MPA, 3-mercaptopicolinic acid; NFAT1, nuclear factor of activated T cells 1; OGT, O-linked N-acetylglucosaminyltransferase; Rb, retinoblastoma-associated protein; RhoA, small GTP-binding protein; SCAP, sterol regulatory element-binding protein cleavage-activating protein; SERCA, sarco/ER Ca^{2+}-ATPase; SREBP, sterol regulatory element-binding protein.

stellate cells.[92] These experimental data indicate that PCK1 itself and its metabolites may mediate signaling networks, providing non-canonical functions to support malignant transformation.

PCK1 induces metabolic reprogramming

Cancer metabolic reprogramming involves complex biochemical reactions that generate macromolecules, energy, redox equivalents, and activating signals.[2] The physiological significance of PCK1 lies in initiating gluconeogenesis under low-sugar conditions to produce glucose in the liver and kidneys. *Pck1*-knockout mice displayed severe hypoglycemia by day two after birth and then died.[93] Even glucose supplementation failed to improve mouse survival,[36,93] indicating that PCK1 has a critical role besides glucose generation.

Macromolecule synthesis

In cancer cells, abnormal PCK1 may indirectly fuel macromolecule biosynthesis to support cancer cell proliferation and metastasis under nutrient-limited conditions (Fig. 4). Upregulated PCK1 in melanoma cells did not mediate gluconeogenesis, but promoted side-branches of glucose metabolism, such as serine biosynthesis.[94] Serine plays a key role in one-carbon metabolism, which supports nucleotide synthesis for cell proliferation and S-adenosylmethionine synthesis for protein methylation.[95] In colorectal cancer, elevated PCK1 expression does not contribute to the conversion of intermediates into glucose.[77] Furthermore, PCK1 promotes anaplerosis of glutamine into the TCA cycle, and mTORC1 activation.[77] In PCK1-overexpressing colorectal cancer cells, citrate fluxes into palmitate and myristate for lipogenesis.[77] Additionally, PCK1 enhances the PPP pathway, which produces ribose-5-phosphate for nucleotide synthesis and NADPH for biosynthetic pathways.[77] PCK1 plays an important role in glyceroneogenesis, which converts PEP to glyceraldehyde-3-phosphate, the precursor of triglyceride, in adipose tissue and the liver.[96] In tumors with high PCK1 expression, side-branch metabolism along the direction of gluconeogenesis promotes the synthesis of required macromolecules, while in tumors with low PCK1 expression, metabolic pathways of the opposite direction promote the synthesis of other macromolecules. For example, in HCC, PCK1 deficiency increases the pool of OAA and aspartate (critical for *de novo* nucleotide synthesis) for orotate synthesis under low-glucose conditions.[82] Overexpression of PCK1 causes cataplerosis and severely reduces TCA intermediates,[7] which can participate in transamination. Alanine transaminase catalyzes the amination of glutamate and pyruvate to produce α-KG and alanine. Aspartate transaminase catalyzes the conversion of glutamate and OAA to α-KG and aspartate.[97] Thus, metabolic reprogramming through PCK1 deficiency may influence the levels of

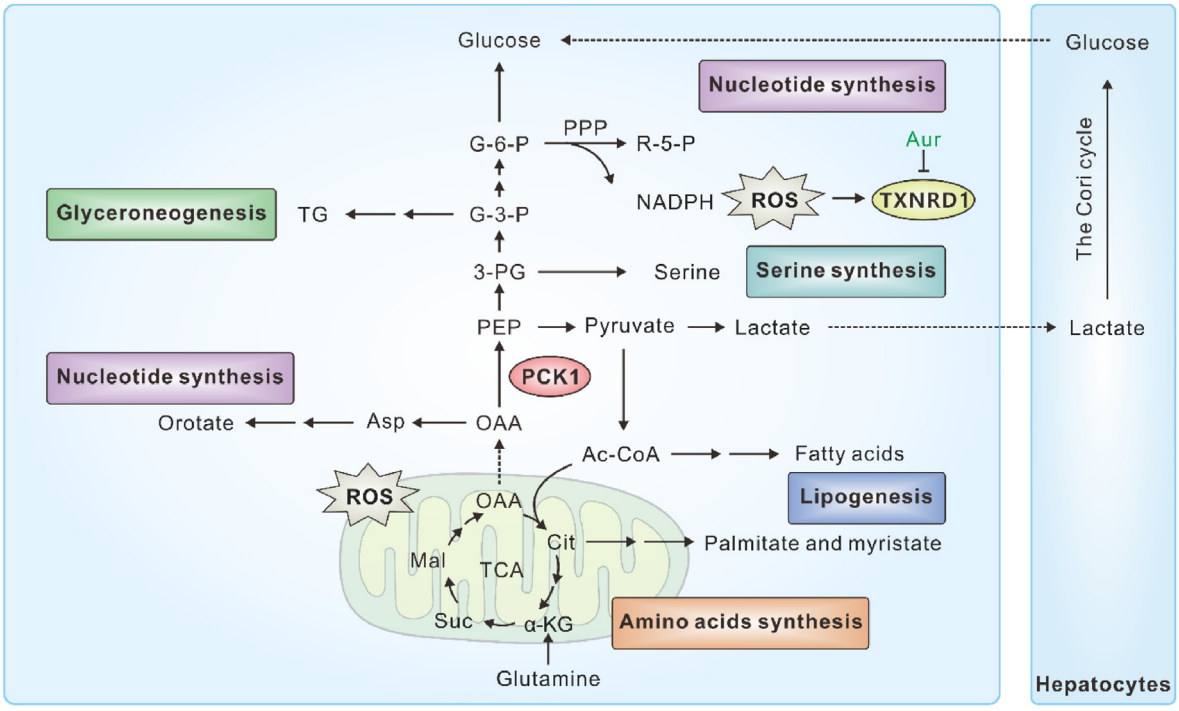

Figure 4 PCK1-induced metabolic reprogramming fuels macromolecule synthesis and energetics, and maintains redox homeostasis. Schematic representation of metabolic pathways regulated by PCK1. Abbreviations: Ac-CoA, acetyl-coenzyme A; Asp, aspartate; Aur, Auranofin; Cit, citrate; G-6-P, glucose-6-phosphate; G-3-P, glyceraldehyde-3-phosphate; α-KG, α-ketoglutarate; Mal, malate; OAA, oxaloacetate; PCK1, phosphoenolpyruvate carboxykinase 1; PEP, phosphoenolpyruvate; 3-PG, 3-phosphoglycerate; R-5-P, ribose-5-phosphate; Suc, succinate; TG, triglyceride; TXNRD1, thioredoxin reductase 1.

amino acids required for polypeptide synthesis.[93] Acetyl-CoA is a central metabolic intermediate linked with fatty acid synthesis.[98] Acetyl-CoA levels were higher in liver tumors of *Pck1*-knockout mice than in those of wild-type mice,[82] suggesting that *PCK1* deletion may enhance lipogenesis via acetyl-CoA accumulation.[99] Thus, PCK1-induced metabolic reprogramming may meet the requirements for specific nutrients of different tumors under nutrient-limited conditions.

Energy generation

Tumor cells have an enhanced energy demand to meet rapid cell proliferation. Glucose is a primary energy source, which is converted to ATP via multiple steps, including aerobic and anaerobic respiration. However, glucose synthesis is an energy-consuming process that utilizes at least 6 ATPs via gluconeogenesis.[62] In HCC cells, PCK1 overexpression increases glucose generation and restricts the production of lactate, pyruvate, malate, and citrate,[100] reducing viability, causing apoptosis, and restraining migration.[100] PCK1-overexpressing hepatoma cells have dramatically reduced cellular ATP levels, causing an energy crisis due to TCA cataplerosis and GTP consumption, and triggering cell death.[7,90] In melanoma cells, elevated PCK1 expression increases lactate production and secretion.[94] In tumors originating from non-gluconeogenic organs, lactate is released into the blood, absorbed by the liver and kidneys, and converted into glucose to provide energy for the tumor via the Cori cycle.[62] PCK1 overexpression in tumors derived from gluconeogenic organs may strengthen gluconeogenesis and dampen glycolysis, restraining tumor growth.[101] Conversely, tumors originating from non-gluconeogenic organs can reuse the lactate induced by PCK1 through the Cori cycle to produce glucose.

Redox homeostasis maintenance

The intracellular redox state, which provides cells with an optimal capacity to counteract the highly oxidizing environment, is vital for maintaining physiological homeostasis in cells. In hepatoma cells, PCK1 markedly reduces TCA intermediates, contributes to energy crisis and reactive oxygen species (ROS) production, and increases cell death.[7] Overexpression of PCK1 can enhance NADPH synthesis to suppress ROS production, nuclear translocation of Nrf2, and hepatoma cell growth.[102] Supplementation with α-ketoglutarate (α-KG) effectively rescued PCK1-caused energy crisis and oxidative stress in HCC cells, indicating that PCK1 may disrupt the redox balance in HCC.[7] However, in breast and colorectal cancers, PCK1 confers cells with a powerful capacity to maintain redox balance. Hypoxic breast cancer cells trigger self-growth through PCK1-induced glycogen metabolic reprogramming, leading to NADPH production and the maintenance of a moderate ROS level.[60] In colorectal cancer, PCK1 enhances NADPH production for antioxidant activities via the PPP pathway to promote tumor growth.[77] The function of PCK1 in redox regulation in different tumors likely depends on the origin of the tumor and its redox status.

Crosstalk with signaling pathways

Emerging evidence indicates a complex interaction between metabolic reprogramming and signaling pathway activation in cancer (Fig. 5). Metabolic reprogramming can trigger tumor progression by generating oncometabolites to hijack growth-promoting signaling cascades through gene expression regulation. Post-translational modification is a critical step in signal transduction in which chemical groups, such as acetyl, phosphate, or glycosyl groups, derived mainly from cellular metabolites, are added on donor proteins.[103] It mediates pathological processes, cancer metastasis, and chemotherapy resistance,[104] and involves various proteins, including transcription factors, enzymes, oncoproteins, and tumor suppressors, which may be effective therapeutic targets or clinical biomarkers.[104]

Metabolic rewiring in PCK1-deleted hepatoma cells induces OAA accumulation and AMPK-GFAT1 axis inactivation, and increases *de novo* UTP synthesis, leading to uridine diphosphate-N-acetylglucosamine biosynthesis and elevated O-GlcNAcylation.[82] Enhanced O-GlcNAcylation activates oncogenic signaling, facilitates tumor cell growth and metastasis, and may be a cancer hallmark.[105] Our previous studies indicated that PCK1 deficiency promotes O-GlcNAcylation of CHK2 and KAT5 in HCC cells.[82,106] O-GlcNAcylation of CHK2 contributes to its stability and dimer formation, which enhances Rb phosphorylation and HCC growth.[82] PCK1 depletion-mediated KAT5 O-GlcNAcylation contributes to the acetylation of histone H4 and c-Myc, promoting the expression of TWIST1, MMP9, and MMP14, and epithelial—mesenchymal transition in HCC.[106]

PCK1 aggravates serine and S-adenosylmethionine (SAM) synthesis for protein methylation to inhibit HCC growth and metastasis (unpublished data). Additionally, PCK1 overexpression markedly reduces cellular ATP/ADP levels in HCC cells.[90] Protein phosphorylation is catalyzed by protein kinases that transfer a phosphate group from ATP to the donor protein.[107,108] PCK1 may be related to protein phosphorylation. Moreover, PCK1 utilizes GTP to phosphorylate INSIG1/2 protein,[23] suggesting that GTP may be an important substrate for PCK1-mediated phosphorylation to regulate cancer progression. PCK1 deficiency markedly increases TCA intermediates and reduces glycolysis intermediates, such as succinate, Ac-CoA, and lactate,[7,82,100] which serve as substrates for succinylation, acetylation, and lactylation. Therefore, PCK1 may affect post-translational modification by influencing intracellular metabolites to regulate signaling pathways.

PCK1 and immunoregulation

Immune cells are associated with host resistance to infection and tumors. Activated immune cells undergo metabolic reprogramming, affecting their contribution to immune

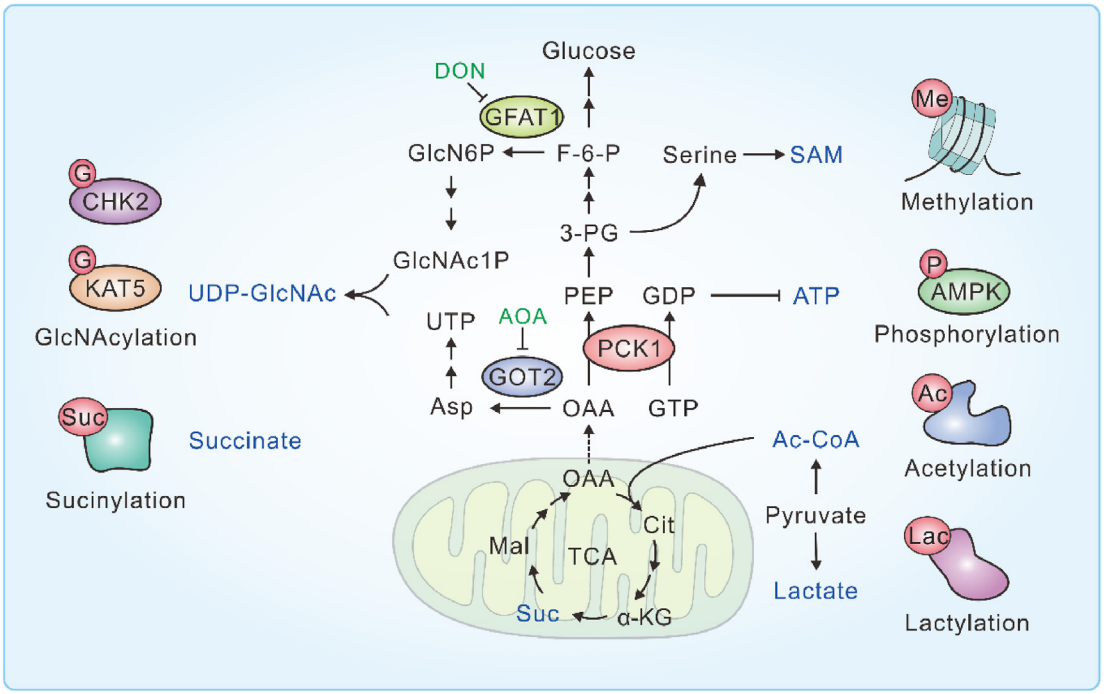

Figure 5 Post-translational modification links oncogenic signaling pathways and PCK1-induced metabolic remodeling. Aberrant PCK1 can induce the accumulation of several metabolites, including UDP-GlcNAc, SAM, ATP, GTP, Ac-CoA, succinate, and lactate, which can function as substrates to modify chromatin conformation and protein functions. Abbreviations: Ac-CoA, acetyl-coenzyme A; AOA, aminooxyacetic acid hemihydrochloride; Asp, aspartate; Cit, citrate; DON, 6-diazo-5-oxo-L-norleucine; F-6-P, fructose-6-phosphate; GFAT1, glutamine-fructose-6-phosphate aminotransferase 1; GlcNAc1P, N-acetylglucosamine-1-phosphate; GlcN6P, glucosamine-6-phosphate; GOT2, the mitochondrial glutamate-oxaloacetate transaminase; α-KG, α-ketoglutarate; Mal, malate; OAA, oxaloacetate; PEP, phosphoenolpyruvate; 3-PG, 3-phosphoglycerate; SAM, S-adenosylmethionine; Suc, succinate; UDP-GlcNAc, UDP-N-acetylglucosamine.

functions in cancer progression.[109] Regulation of PCK1 expression induces the upregulation of peroxisome proliferation-activated receptor alpha, which may provide hepatocellular protection through anti-inflammatory pathway activation.[110,111] $CD8^+$ memory T (Tm) cells play a fundamental role in antitumor immunity.[112] $CD8^+$ Tm cells increase PCK1 expression to enhance glycogen production for generating G-6-P and NADPH, and recovering the high levels of reduced glutathione for survival. In melanoma, PCK1 deletion increases ROS levels, contributing to the impairment of $CD8^+$ Tm formation and its maintenance.[113] PCK1 expression is upregulated in $CD8^+$ Tm cells as more Ac-CoA is diverted to ketogenesis, which causes ketogenesis-derived β-hydroxybutyrylation of Lys9 histone H3 of FoxO1 and PGC-1α, activating PCK1 expression.[114] Additionally, PEP sustains T-cell receptor-regulated Ca^{2+}-NFAT signaling and effector functions by inhibiting SERCA activity. Thus, PCK1-overexpressing T cells limit melanoma growth.[80]

PCK1-deleted macrophages show M1 polarization with a more proinflammatory phenotype and increased expression of the cytokines IL-6, IL-1β, and TNFα, resulting in an antitumor effect.[115] In PCK1-deleted myeloid cells, altered macrophage metabolism reduced citrate and malate levels and increased lactate and ROS levels.[115] There may exist heterogeneity in the tumor microenvironment because of the different immune cell functions and multiple levels of cytokines regulated by PCK1. Whether PCK1 regulates broader and essential immunomodulatory functions is being investigated.

PCK1 and preclinical research

In HCC and RCC cells, PCK1 overexpression markedly inhibits proliferation and migration, whereas PCK1 deficiency enhances tumor progression.[7,25,82,100] Dexamethasone can restore gluconeogenesis and induce PCK1 expression, and has therapeutic efficacy against hepatocarcinoma in mice.[101] It may improve hypoglycemia in patients with adverse symptoms. HCC growth could be suppressed with inhibitors of GOT2 (aminooxyacetic acid hemihydrochloride, AOA) or GFAT1 (6-diazo-5-oxo-L-norleucine, DON) to reduce *PCK1* knockout-induced O-GlcNAcylation *in vivo*.[82] Auranofin, a TXNRD1 inhibitor, enhances the sensitivity of PCK1-deleted hepatoma cells to sorafenib-induced apoptosis.[102] Metformin (an AMPK activator) markedly inhibited the growth of HCC with PCK1 deficiency-induced AMPK inactivation.[90] These inhibitors may be useful for cancer treatment through inhibiting PCK1 deficiency-induced oncogenic pathways.

In non-gluconeogenic organ tumors, such as colorectal cancer and melanoma, inhibiting gluconeogenesis and PCK1 expression is a potentially effective therapeutic strategy.[77,94,116] The PCK1 inhibitor 3-MPA strongly inhibited tumor growth, reduced tumor metastasis, and prolonged the long-term survival of mice with colorectal or breast cancer.[60,116] Further, 3-MPA inhibits gluconeogenesis in the liver and kidneys to reduce the amount of glycolytic energy available in cancer cells via the Cori cycle. Thus, PCK1 inhibitors or activators may improve adverse symptoms in patients and become a potential cancer therapeutic strategy.

Conclusions

PCK1 is downregulated and has antitumorigenic effects in cancers originating from gluconeogenic organs (HCC and RCC), whereas it has promoting effects in tumors originating from non-gluconeogenic organs. The regulation of PCK1 expression and activity establishes an interplay between metabolism and oncogenic pathways in tumors. PCK1 and its metabolites (PEP, OAA, and GTP/GDP) can directly regulate signaling pathways and indirectly induce metabolic reprogramming to generate macromolecules and energy, maintain redox homeostasis, and activate signaling. Possibly, tumors have autonomous requirements for proliferation and metastasis, which contribute to the divergent roles of PCK1 in tumors arising from different organs. Considering the differential PCK1 expression and activity in various tumor types, individualized therapies should be administered to patients as precision medicine. Future research should continue to explore the unacknowledged functions of PCK1 and its metabolites to provide fundamental insights and novel therapeutic strategies for human malignancies.

Author contributions

NT and KW provided direction and guidance throughout the preparation of this manuscript. JX, KW, and NT conducted the literature review, drafted, and revised the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors declare no competing interests.

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