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Review

Gluconeogenesis in cancer cells – Repurposing of a starvation-induced metabolic pathway?

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ABSTRACT

Cancer cells constantly face a fluctuating nutrient supply and interference with adaptive responses might be an effective therapeutic approach. It has been discovered that in the absence of glucose, cancer cells can synthesize crucial metabolites by expressing phosphoenolpyruvate carboxykinase (PEPCK, PCK1 or PCK2) using abbreviated forms of gluconeogenesis. Gluconeogenesis, which in essence is the reverse pathway of glycolysis, uses lactate or amino acids to feed biosynthetic pathways branching from glycolysis. PCK1 and PCK2 have been shown to be critical for the growth of certain cancers. In contrast, fructose-1,6-bisphosphatase 1 (FBP1), a downstream gluconeogenesis enzyme, inhibits glycolysis and tumor growth, partly by non-enzymatic mechanisms. This review sheds light on the current knowledge of cancer cell gluconeogenesis and its role in metabolic reprogramming, cancer cell plasticity, and tumor growth.

1. Nutrient deprivation in cancer

Metabolic pathways in cancer cells are rewired to support proliferation and tumor growth. Already in the 1920s, Nobel laureate Otto Heinrich Warburg described that tumor slices avidly consumed glucose and produced lactate, even under non-hypoxic conditions (aerobic glycolysis) [1]. The “Warburg effect” is found in many aggressive cancers, but also in normal, highly proliferative cells. As outlined in excellent reviews on the topic [2,3], high rates of glycolysis ensure that glycolytic intermediates are available to be shunted to important biosynthetic pathways including: 1) oxidative and non-oxidative branches of the pentose phosphate pathway (PPP), providing ribose-5-phosphate and NADPH; 2) synthesis of glycerol-3-phosphate for lipid biosynthesis; 3) serine and glycine synthesis; and 4) the hexosamine pathway [2,3]. Still, the tumor's high demand for nutrients is frequently not met by an adequate supply. Although angiogenesis is activated early in cancer growth, the newly formed vascular network is aberrant, with leaky vessels and irregular, fluctuating blood flow (reviewed in [4]). Furthermore, cancers outgrow their supply by continuous proliferation and consumption of nutrients like glucose. At the invasive front of solid tumors, cancer cells co-opt existing microvessels. In this context as well, perfusion is frequently inadequate, since the proliferating tumor cells compress the pre-existing vessels [5]. Local steep gradients for oxygen (O_2), glucose and other nutrients emerge and tumor-cell derived metabolites, like lactate, accumulate [6] (Fig. 1). In fact, a considerable heterogeneity in tumor perfusion and glucose uptake has been shown in rapidly growing tumors, even on a macroscopic level, which greatly impacts tumor cell metabolism [7]. Overall, the microenvironment in solid tumors is considered to be nutrient-poor. Especially glucose deprivation appears to be common, due to its high rate of consumption by

Abbreviations: 1,3-BPG, 1,3-bisphosphoglycerate; 2-PG, 2-phosphoglycerate; 3-PG, 3-phosphoglycerate; α-KG, α-ketoglutarate; AKT, protein kinase B; AMP, adenosine monophosphate; ATP, adenosine triphosphate; Asp, aspartate; cAMP, cyclic adenosine monophosphate; ccRCC, clear cell renal cell carcinoma; Cit, citrate; DHAP, dihydroxyacetone phosphate; EMT, epithelial to mesenchymal transition; ER, endoplasmic reticulum; FADH2, reduced flavin adenine dinucleotide; FBPase, fructose-1,6-bisphosphatase; FBP1, liver FBPase; FBP2, muscle FBPase; FOXO1, forkhead box protein O1; Fru-1,6-P, fructose-1,6-bisphosphate; Fru-6-P, fructose-6-phosphate; G6PC, glucose-6-phosphatase; GA, glutaminase; GAP, glyceraldehyde-3-phosphate; GK, glycerol kinase; Glc-1-P, glucose-1-phosphate; Glc-6-P, glucose-6-phosphate; Gln, glutamine; Glu, glutamate; GLUD, glutamate dehydrogenase; GPL, glycerophospholipid; HCC, hepatocellular carcinoma; HIF, hypoxia-inducible factor; HK, hexokinase; Mal, malate; mTORC, mechanistic target of rapamycin complex; NADH, reduced nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NSCLC, non-small cell lung cancer; OAA, oxaloacetate; O-GlcNAc, O-linked *N*-acetylglucosamine; PC, pyruvate carboxylase; PCK1, cytosolic isoform of PEPCK; PCK2, mitochondrial isoform of PEPCK; PDH, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PEPCK, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; PYK, pyruvate kinase; PPP, pentose phosphate pathway; TCA cycle, tricarboxylic acid cycle

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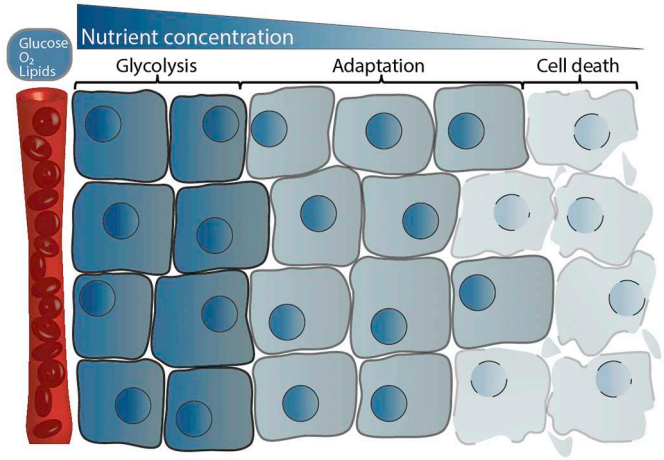

Fig. 1. Effects of nutrient availability on tumor cells.
The concentrations of nutrients like glucose or lipids as well as oxygen (O_2) decline with increasing distance from tumor capillaries. Cancer cells adapt to these conditions, partly by utilizing other nutrients than glucose, *e.g.* lactate. Very low nutrient concentrations and/or oxygen (O_2) deprivation induce cell death.

the neoplastic tumor cells. In numerous studies, glucose concentrations have been found to be significantly lower in human tumors than in corresponding normal tissues [8–14]. The mechanisms underlying cancer cell adaptation to such a fluctuating nutrient supply are under intense investigation, since they might reveal specific vulnerabilities of cancer cells.

2. Metabolic heterogeneity and use of alternative fuels in cancer cells

Cancer cells are increasingly acknowledged to utilize nutrients other than glucose in a context-dependent manner. An unexpected preference for oxidation of exogenous lactate over glucose has been discovered in certain oxidative tumor cells, sparing glucose for hypoxic, glucose-dependent cancer cells [15]. In human head and neck cancers, lactate metabolism was found to be variable, with some tumors showing net lactate release and some net lactate consumption [16]. Recently, a high contribution of circulating lactate and/or glutamine to TCA cycle intermediates has been shown in genetically engineered lung and pancreatic cancer tumors in mice [17]. Intravenous infusion of ^{13}C-labeled glucose in non-small cell lung cancer (NSCLC) patients revealed high intra- and intertumoral variability in glucose metabolism [7]. Tumor areas with high blood perfusion rather resembled normal lung and showed a considerable contribution of non-glucose precursors to different intermediates, while the use of glucose was higher in poorly perfused tumor areas [7]. A conversion of intravenously administered ^{13}C-lactate to TCA cycle intermediates was shown in human NSCLC in a further study by the same group [18]. Besides lactate, glutamine [19,20], acetate [21–23] and fatty acids [24] also emerged as important non-carbohydrate nutrients in different cancers. Their contribution to cancer metabolism has been reviewed extensively elsewhere [3].

The TCA cycle functions as a central metabolic hub, linking glycolysis, respiration and anabolic biosynthetic pathways (Fig. 2). TCA cycle intermediates are diverted into multiple pathways *e.g.* nucleic acid, amino acid or fatty acid synthesis (Fig. 2, blue boxes). If glucose is missing, other nutrients entering the TCA cycle can feed into these biosynthetic pathways. However, *de novo* synthesis of several important cellular building blocks in proliferating cells, including nucleic acids and (glycero-) lipids, requires glycolytic intermediates [25]. Non-carbohydrate precursors can be shuttled *via* the TCA cycle to glycolytic intermediates, but this pathway requires the action of the gluconeogenesis enzymes phosphoenolpyruvate carboxykinase (PEPCK) and to some extent fructose-1,6-bisphosphatase (FBPase) [25] (Fig. 2, red boxes). Until recently, it was unclear whether glycolytic intermediates are generated in cancer cells *via* the PEPCK pathway. The emerging role of gluconeogenesis in adaptation of cancer cells to nutrient deprivation on the one hand, and the regulatory properties of gluconeogenesis enzymes on cancer cell metabolism, on the other hand, are discussed here. Highlighting the differential roles of gluconeogenesis enzymes in cancer cells under nutrient-poor as opposed to nutrient-rich conditions and on the metabolic pathways fed by (abbreviated) gluconeogenesis, this review emphasizes other aspects than a recent review on the topic by Wang et al. [26].

3. Physiological functions of gluconeogenesis enzymes

Gluconeogenesis is the synthesis of glucose from non-carbohydrate precursors. It primarily occurs in the liver, kidney, intestine and skeletal muscle [25,27]. Though many steps in gluconeogenesis are glycolytic reactions run in the reverse direction, three reactions are catalyzed exclusively by gluconeogenesis enzymes. The initial enzyme of gluconeogenesis, PEPCK, forms a bottle neck in cell metabolism linking the TCA cycle and glycolysis/gluconeogenesis (Fig. 2). Two isoforms of PEPCK exist, a cytosolic isoform, PCK1 (also known as PEPCK-C, PEPCK1), and a mitochondrial isoform, PCK2 (PEPCK-M, PEPCK2). PCK1 and PCK2 both catalyze the conversion of oxaloacetate (OAA) and GTP to phosphoenolpyruvate (PEP), CO_2 and GDP [25,27]. OAA is generated either in the TCA cycle or by pyruvate carboxylase (PC), which mediates the conversion of pyruvate to OAA. OAA is exported from the mitochondria to the cytosol in the form of malate or aspartate [25,28] (Fig. 2). This is a pre-requisite for further conversion *via* PCK1, but not *via* PCK2. Further downstream, gluconeogenesis requires FBPase, which hydrolyzes fructose-1,6-bisphosphate into fructose-6-phosphate and P_i. The two isoforms of FBPase are known, liver FBPase (FBP1) and muscle FBPase (FBP2). The final step of gluconeogenesis is mediated by glucose-6-phosphatase (encoded by *G6PC*), which hydrolyzes glucose-6-phosphate to glucose and P_i [25] (Fig. 2). Although the role of PCK1 in gluconeogenesis is well known, it has only recently been demonstrated that PCK2 also contributes to gluconeogenesis. Adenoviral overexpression of PCK2 in liver-specific PCK1 knockout mice showed that PCK2 supports hepatic gluconeogenesis, but is less efficient than PCK1 [28]. Silencing of PCK2 with antisense oligonucleotides in rats reduced blood glucose levels, particularly in the fed state, and diminished gluconeogenesis from lactate or amino acids in isolated hepatocytes [29]. PCK1 and PCK2 mediate not only gluconeogenesis but also glyceroneogenesis, an abbreviated metabolic pathway for the generation of glycerol-3-phosphate from non-carbohydrate precursors. Glyceroneogenesis plays an important role in re-esterification of fatty acids in adipose tissue and in the liver [30].

Functionally linked to their downstream anabolic pathways, PCK1 and PCK2 also control the removal of TCA cycle anions (cataplerosis). If anaplerosis (entry of anions into the TCA cycle) is increased *e.g.* after conversion of glutamine to glutamate and finally to α-ketoglutarate (α-KG), the influx must be balanced by cataplerosis. Accordingly, PCK1 was shown to promote TCA cycle flux in the liver [31,32] and in the small intestine [33]. PCK2 was found to be involved in regulating TCA cycle flux in insulin-producing β-cells [34]. Immunohistochemical analysis of different human tissues suggests that PCK1 and FBP1 are quite broadly expressed [35], but the function of PCK1/2 or FBP1 in non-gluconeogenic or non-glyceroneogenic tissues remains still elusive. Interestingly, PCK1 overexpression in *Caenorhabditis elegans* extends lifespan [36], however the mechanism is not entirely clear.

It is well known that under glucose starvation, many cells can generate energy (ATP) from alternative fuels in the presence of functioning mitochondria, *e.g.* from ketone bodies or fatty acids. However, glycolytic intermediates are needed in highly proliferative cells that require ribose-5-phosphate to synthesize nucleotides or glycerol-3-phosphate to synthesize membrane glycerophospholipids [2,3]. Given

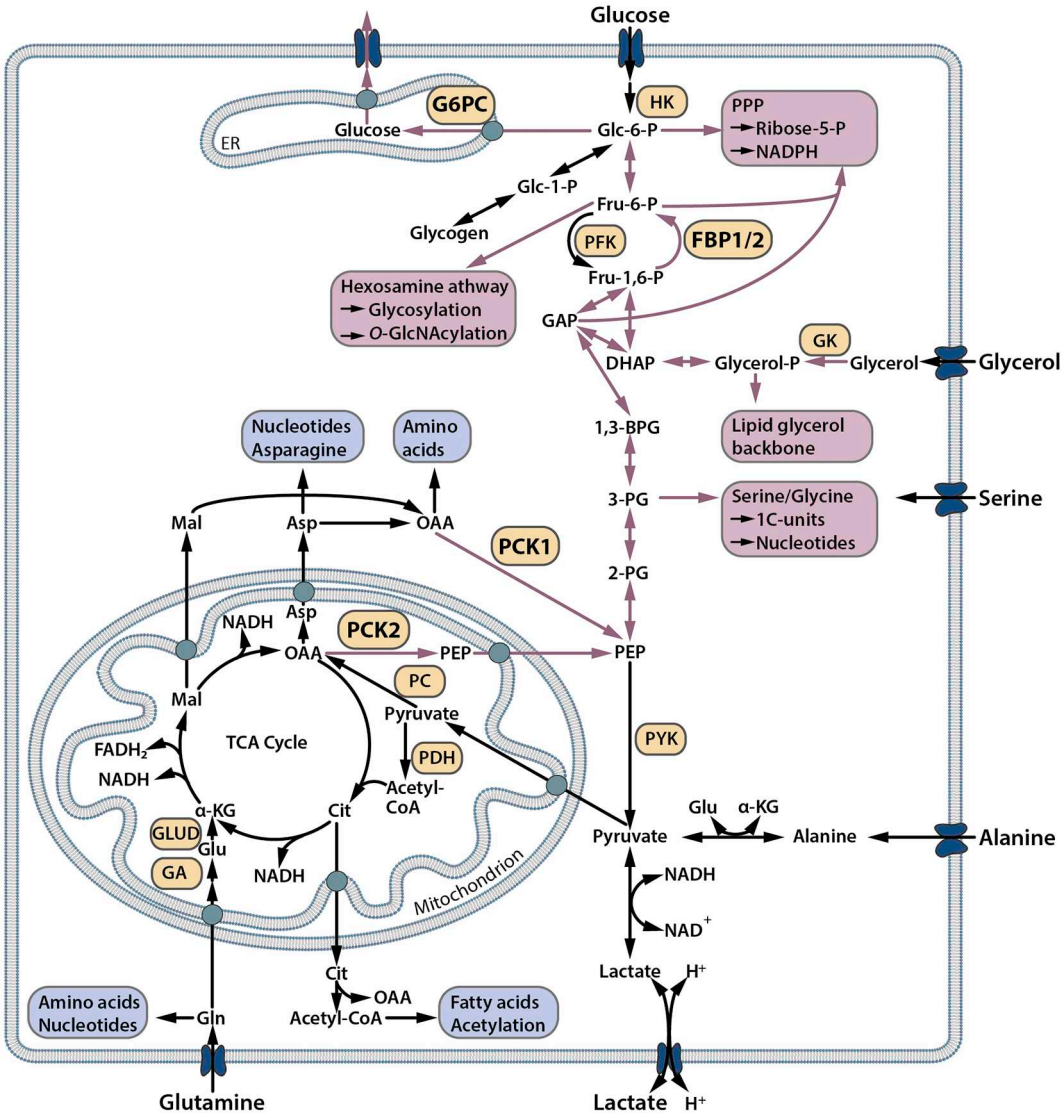

Fig. 2. Scheme of glycolysis, gluconeogenesis and branching biosynthetic pathways.
Gluconeogenesis, the formation of glucose from non-carbohydrate precursors such as amino acids or lactate, is in part the reversal of glycolysis. Phosphoenolpyruvate carboxykinase, which exists as a mitochondrial (PCK2) and a cytosolic isoform (PCK1), mediates the initial step of gluconeogenesis and links the TCA cycle (citric acid cycle, Krebs cycle) and glycolysis/gluconeogenesis. Further steps in gluconeogenesis (red arrows) are mediated by bi-directional glycolytic/gluconeogenesis enzymes and the downstream enzymes fructose-1,6-bisphosphatase (FBPase) and glucose-6-phosphatase (G6PC). Abbreviated gluconeogenesis allows the synthesis of cellular building blocks from non-carbohydrate precursors (red boxes) and requires the action of PCK1/2 and to some extent also FBP1/2. Biosynthetic pathways independent of gluconeogenesis are depicted in blue.

the fact that gluconeogenic and glycolytic pathways share common intermediates, gluconeogenesis might potentially be an alternative source of biosynthetic precursors under glucose deprivation. It has long been recognized that gluconeogenesis is indispensable for growth of bacteria or yeast on non-fermentable (hexose sugar free) carbon sources [37,38]. Recently, our group and others have identified such an adaptive mechanism in certain tumor cells.

4. Role of PCK1/2 in cancer

Although gluconeogenesis enzymes were previously assumed to be absent from cancers not arising in gluconeogenic organs, several studies have demonstrated their functional expression in diverse cancers as mediators of abbreviated forms of gluconeogenesis. As will be outlined in detail, gluconeogenesis enzymes allow the synthesis of crucial intermediates and biomass in cancer cells under glucose deprivation, while they also regulate glycolysis and the TCA cycle.

4.1. PCK1 and PCK2 enhance metabolic flexibility in cancer cells

Elevated expression of the upstream gluconeogenesis enzyme PCK2 has been noted in the context of mutant *KRAS* in colon cancer cells and PCK2 was upregulated in colon carcinoma samples compared to normal colon tissue [39]. In a proteomics analysis PCK2 has been found to be highly elevated in brain metastatic cells derived from breast cancer compared to the parental breast cancer cells or bone metastatic cells [40]. The functional significance of PCK2 has not been analyzed in these studies. In 2014 our group reported that the gluconeogenic pathway is active in cancer cells not arising from a gluconeogenic organ [41]. We found that PCK2 mRNA expression and activity were increased in human lung cancer (NSCLC) samples compared to normal lung tissue, although PCK2 was also detectable in bronchial epithelial cells in normal lung, but not in alveolar cells [41]. Low glucose conditions led to upregulation of PCK2 expression and activity in lung cancer cell lines [41]. PCK1 was expressed only at very low levels.

While a net production of lactate under high glucose medium was found in different NSCLC cell lines, there was a net consumption of lactate under low glucose conditions [41]. In fact, stable isotopic labeling showed that lactate was converted to PEP in glucose-starved cancer cells, confirming PCK2 activity in the direction of gluconeogenesis [41]. Silencing of PCK2 significantly compromised cancer cell survival under glucose deprivation in two of the three NSCLC cell lines. PCK1/2 inhibition further enhanced apoptosis in NSCLC cells growing as 3-dimensional spheroids, which are known to exhibit gradients for glucose and O_2 [41].

Mendez-Lucas et al. [42] reported functional PCK2 expression in cancer cells and its regulation by stress pathways shortly thereafter. PCK2 was abundant in different cancer cell lines, while PCK1 expression was low [42]. *KRAS* transformed NIH-3T3 fibroblasts showed enhanced PCK2 mRNA compared to the non-tumorigenic parental cell line. In breast cancer cells, silencing of PCK2 slightly reduced glucose consumption, lactate production and proliferation under normal conditions. Apoptosis induction by glutamine deprivation or by endoplasmic reticulum (ER) stress was significantly increased [42]. Thus, PCK2 was identified as a component of the amino acid response and unfolded protein response in cancer cells.

In an unbiased metabolomics and gene expression analysis of lung cancer cells, PCK2-mediated gluconeogenesis and serine synthesis were found to be upregulated in glucose-free medium [43]. PCK2 silencing reduced proliferation of different NSCLC cell lines under these conditions [43]. Importantly, in two different NSCLC cell lines, PCK2 silencing clearly reduced growth of subcutaneous xenografts in mice [43]. PCK2 silencing prevented lung cancer xenografts from growing beyond microscopic size *in vivo,* as recently published [44]. *In vitro* analyses showed reduced colony forming ability of PCK2 silenced lung cancer cells under glucose- and serum starvation [44].

PCK1 was found to be expressed in the majority of colon cancers and moderate to high PCK1 immunohistochemistry scores were more frequent in colon carcinoma than in normal colon mucosa [45]. Silencing of PCK1 decreased proliferation and clonogenic growth of colon cancer cells in glucose-containing medium and clearly reduced colon cancer xenograft growth [45]. PCK1 was found to operate in both, the gluconeogenic or the reverse, anaplerotic direction, depending on the glucose concentration in the medium [45]. These properties of PCK1 may further enhance the metabolic flexibility of cancer cells. Full conversion of gluconeogenic precursors to glucose was not observed [45], which is not surprising since the production and release of glucose would be of no apparent benefit for the cancer cells. Interestingly, PCK1 silencing has been shown to reduce cancer growth in a drosophila brain tumor model that was rescued by increasing NAD^+ or oxidizing cytosolic NADH [46].

4.2. Metabolic downstream pathways of PCK1 and PCK2 in cancer cells

Under glucose starvation, cancer cells appear to redistribute glycolytic/gluconeogenic intermediates to downstream pathways that are crucial for survival and/or proliferation. As expected, only a minor proportion of PEP generated by PCK2 was converted (back) to pyruvate and to the TCA cycle in glucose-deprived lung cancer cells [43]. Instead, serine and glycine were synthesized *de novo* [43]. Abbreviated gluconeogenesis *via* PCK2 generated 40% of cellular serine under glucose depletion in lung cancer cells [43]. Thus, a large proportion of serine may derive from gluconeogenesis in cancer cells. Amplification of the serine synthesis enzyme phosphoglycerate dehydrogenase and a dependency of cancer cells on *de novo* serine synthesis have been described in different cancers [47,48]. Serine is a precursor in numerous biosynthetic pathways, including the synthesis of glycine, cysteine and sphingosine [47–49]. Together with its downstream product glycine, serine fuels one-carbon metabolism, which is important for nucleotide synthesis and restoration of the $NADPH/NADP^+$ ratio [49]. In fact, glutamine-derived carbons were detected in the purine nucleotide ATP in cells grown under glucose-free conditions, suggesting that PCK2 contributes to nucleotide synthesis *via* serine *de novo* formation and one-carbon metabolism [43].

Recently, our group has shown that PCK2 mediates glyceroneogenesis and thereby contributes to biomass synthesis in cancer cells under glucose deprivation [44]. Carbons derived from glutamine and lactate were transferred onto the glycerol backbone of glycerophospholipids (GPL) *via* PCK2 under low but not under high glucose conditions [44]. Importantly, PCK2 silencing led to a 30–50% reduction of absolute levels of the GPL phosphatidylethanolamine under glucose- and serum deprivation [44]. Colony formation was inhibited by different constructs of PCK2 shRNA under glucose starvation, and the effect was partly reversed by exogenous phosphatidylethanolamine [44]. These results show that glyceroneogenesis *via* PCK2 is an important pathway necessary for *de novo* GPL synthesis and GPL homeostasis in glucose-deprived cancer cells.

The PPP generates ribose-5-phosphate for nucleotide synthesis and/or NADPH, required for cellular antioxidant functions. A series of stable isotopic tracer experiments demonstrated that in PCK1 positive colon cancer cells, there is carbon flow from glutamine *via* the TCA cycle to (RNA) ribose, which was enhanced by low glucose [45]. Similarly, ribose-5-phosphate generation from ^{13}C-glutamine was found in glucose-deprived metastatic breast cancer cells, mediated by FBP2 [50]. The hexosamine pathway, which branches from fructose-6-phosphate, generates *N*-acetylglucosamine (GlcNAc), an intermediate required for a posttranslational modification (O-GlcNAcylation) as well as protein glycosylation [51]. O-GlcNAcylation, which is increased in cancer cells, has been linked to nutrient sensing and is required for signaling and transcription [51]. Whether gluconeogenesis contributes to maintaining the hexosamine pathway in cancer cells remains unknown. Together these studies show that metabolic flexibility of cancer cells is highly increased by PCK1/2 and suggest that PCK1 or PCK2 are required in different cancers to maintain the levels of precursors for biomass production under glucose deprivation (Fig. 3).

4.3. Role of PCK1 and PCK2 beyond anabolism

Increased expression of PCK2 in neuroendocrine tumors of the pancreas compared to non-neoplastic islet cells and a proliferation promoting effect of PCK2 were found in pancreatic neuroendocrine cancer cells associated with increased expression of tRNA processing genes [52]. In prostate cancer, a higher expression level of PCK2 has been reported in metastases compared to primary tumors or normal

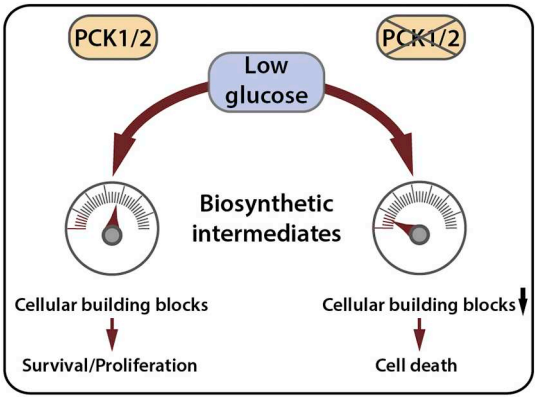

Fig. 3. PCK1/2 confer metabolic plasticity under glucose starvation.
PCK1/2 allow numerous biosynthetic pathways (the generation of serine/glycine, glycerol-3-phosphate or ribose-5-phosphate) to be fed from alternative nutrients under glucose starvation. If PCK1 or PCK2 are absent or silenced, gluconeogenesis is inhibited, resulting in a lack of cellular building blocks and decreased cell survival or proliferation, as has been shown in several studies on non-hepatic cancer cells.

prostate [53]. High PCK2 expression in prostate cancer tissue was associated with worse overall survival [53]. In this study prostate cancer tumor initiating cells (TIC) showed greatly elevated PCK2 expression compared to their non-stem cell like counterparts. PCK2 silencing reduced the proportion of TIC, diminished their sphere-forming ability and inhibited growth of prostate cancer nodules *in vivo* [53]. Glycolysis was decreased by PCK2 silencing, suggesting a role of PCK2 in promoting the glycolytic switch [53]. Interestingly, PCK2 silencing slightly enhanced survival of TIC in medium without glucose [53].

Similarly, an upregulation of PCK1 has been observed in tumor-repopulating (stem-cell like) cells from murine hepatocarcinoma (liver cancer), melanoma and lymphoma [54]. PCK1 expression promoted glycolysis and glucose consumption in melanoma tumor-repopulating cells [54]. Silencing of PCK1 in these cells reduced the capacity to form colonies in 3D fibrin gels *in vitro*, slowed nodule growth in the skin and impaired metastasis formation in the lungs [54]. However, a subsequent study by the same research group showed that PCK2 was rather reduced in melanoma tumor-repopulating cells in contrast to PCK1 [55]. Overexpression of PCK2 decreased glucose uptake, reduced the levels of fumarate, citrate and lipids in high glucose medium and reduced tumorigenesis *in vivo* and colony formation *in vitro* [55]. Thus, in some cancer cells PCK1/2 might play a role in regulating TCA cycle flux, both, under glucose starvation or normal glucose conditions.

4.4. Crosstalk of PCK1/2 with signaling and epigenetics

Metabolites are continuously sensed in cells and their abundance can greatly impact signaling and cell fate. Mechanistic target of rapamycin complex 1 (mTORC1) is one of the most important nutrient-regulated signaling nodes, which activates protein synthesis and cell growth if amino acids and glucose are available [56]. On the other hand, adenosine monophosphate-activated protein kinase (AMPK) is activated under low energy stress by an increase in AMP (adenosine monophosphate) and downregulates energy consuming processes, while activating autophagy [56]. Both, PCK1 and PCK2, have been shown to modulate cell signaling, which was at least partially attributable to alterations in metabolite levels. PCK1 overexpression in colon cancer cells led to an enhancement, while PCK1 silencing inhibited glucose uptake and lactate production [45]. The effect was due to enhanced mTORC1 activation by PCK1 following increased glutamine uptake [45]. In tumor-repopulating melanoma cells, overexpression of PCK2 decreased levels of fumarate under nutrient-replete conditions [55]. As a consequence, the stability of hypoxia-inducible factor 1α (HIF-1α) under hypoxia was decreased, since fumarate regulates prolyl hydroxylase activity [55]. Additionally, the reduction of fumarate by PCK2 overexpression altered DNA methylation due to modified activity of Ten-eleven translocation (TET) enzymes [55]. In gastric cancer cells, PCK1 enhanced extracellular signal-regulated kinase 1/2 activation and expression of matrix metalloproteinase 9 [57]. This effect was associated with an increased uptake of glucose and glutamine and with increased invasion and migration in normal glucose containing medium [57]. PCK2 has been found to have an interesting role in regulating protein acetylation in prostate TIC [53]. PCK2 silencing elevated acetyl-CoA levels due to enhanced production from citrate *via* ATP citrate lyase (ACLY), increasing histone and total protein lysine acetylation [53]. Importantly, reducing protein acetylation by inhibiting ACLY reversed the reduction in TIC numbers by PCK2 silencing, showing that PCK2 elevates TIC numbers in prostate cancer by suppressing protein acetylation [53].

4.5. PCK1/2 downregulation in liver and kidney cancer

In contrast to the predominant tumor-promoting role of PCK2 or PCK1 observed in non-hepatic cancers, PCK1 was downregulated in hepatocellular carcinoma (HCC) (Table 1). PCK1 overexpression induced apoptosis or inhibited proliferation and suppressed growth of HCC *in vivo* in different studies (Table 1). In one study, the ability to survive under low glucose conditions was decreased under forced PCK1 and partially under PCK2 overexpression, due to a considerable decrease in ATP levels, while effects under high glucose were quite variable [68]. These results are contradictory to the data obtained in different non-hepatic cancer cells and might be related to the reported pivotal role of PCK1 in regulating TCA cycle flux in the liver [31,32]. In clear cell renal cell carcinoma (ccRCC), the most common type of kidney cancer, PCK1 was decreased in several studies (Table 1). Thus, downregulation of gluconeogenesis enzymes appears to be advantageous for cancer development in gluconeogenic tissues and constitutes part of the metabolic rewiring necessary to support growth and proliferation; in contrast, abbreviated gluconeogenesis facilitates tumor cell proliferation and survival in cancers originating in non-gluconeogenic tissues.

5. Role of FBP1/2 and G6PC in cancer

The expression of the distal gluconeogenesis enzymes FBP1, FBP2 and G6PC is highly variable in tumors. FBP1 or FBP2 are frequently downregulated compared to normal tissue; however, in some contexts FBPase activity is protumorigenic. Here we summarize the present body of literature on the function of FBP1, FBP2 and G6PC in cancer, focusing on the distinction between enzymatic and non-enzymatic effects under different nutritional conditions.

5.1. FBP2 is required for glucose-independent growth in metastatic breast cancer cells

FBP2, the muscle isoform, was shown to be upregulated in human breast cancer brain metastases compared to paired samples of primary breast cancer [50]. It was enhanced in brain metastatic cancer cells, in contrast to the parental breast cancer cells and mediated *de novo* ribose-5-phosphate and purine synthesis from ^{13}C-labeled glutamine under glucose limitation [50]. FBP2 silencing greatly compromised viability and proliferation of the metastatic cancer cells under glucose deprivation and delayed the growth of orthotopically implanted brain metastases in mice [50].

5.2. Tumor-suppressive function of FBP1

In contrast, numerous studies suggest a tumor-suppressive role of FBP1, and partly also FBP2 (summarized in Table 2). FBP1 expression was shown to be downregulated in different cancers and low FBP1 expression was frequently associated with poor overall survival (Table 2). FBP1 overexpression suppressed proliferation in HCC cells and other tumor types and significantly reduced tumor growth *in vivo* in numerous studies (Table 2). When the role of FBP1 was studied *in vitro* under nutrient-replete conditions, it was observed to inhibit glycolysis in different cancer cells, including breast cancer [71,72], pancreatic cancer [73,74], lung cancer [75,76] and HCC cells [77–79]. The reduction of proliferation by FBP1 appears to be related to the decrease in glucose uptake. Likewise, overexpression of FBP1 in ccRCC diminished proliferation and migration and led to a reduction of glycolysis [69]. Importantly, the metabolic and proliferation-promoting effects were partially independent of its enzymatic activity but mediated by the interaction of FBP1 with HIFs [69] (Fig. 4). A non-enzymatic function of FBP1 regulating extracellular signal-regulated kinase (ERK) signaling has been shown to be responsible for the suppression of growth of pancreatic cancer xenografts *in vivo* [74]. Besides inhibiting proliferation, FBP1 was shown to affect epithelial to mesenchymal transition (EMT), an important step in tumor metastasis. Restoration of FBP1 expression suppressed the EMT phenotype, migration and tumor growth in Snail overexpressing HCC cancer cells [80] and reduced EMT in gastric cancer cells [81]. Only limited knowledge exists on the role of FBP2 in cancer. In contrast to the tumor promoting role of FBP2 in

Table 1
Tumor promoting and tumor suppressor activity of PCK1/2.

	Alteration in tumor tissue	Biological effect
I. Tumors originating in non-gluconeogenic tissues		
PCK1	*Primary tumors* Increased in colon carcinoma [45] and gastric cancer [57] *Metastases/tumor initiating cells* Increased in tumor-repopulating cells from liver cancer, melanoma and lymphoma [54] Increased in liver metastases from human pancreatic cancer [58]	*In vitro* Silencing inhibits proliferation of colon carcinoma cells [45] and reduces invasion in gastric cancer cell lines [57] in normal glucose Silencing decreases colony formation of murine melanomas [54] *In vivo* Silencing inhibits growth of colon carcinoma xenografts [45] and murine melanomas [54] Silencing inhibits tumor growth in a drosophila model [46]
PCK2	*Primary tumors* Increased in colon carcinoma [39], lung cancer (NSCLC) [41], and neuroendocrine tumors of the pancreas [52] High expression associated with worse overall survival in prostate cancer patients [53] Increased in the course of human gastric cancer development [59] *Metastases/tumor initiating cells* Increased in metastatic prostate cancer compared to primary cancer or normal prostate [53] Increased in brain metastatic cells derived from breast cancer compared to the parental breast cancer cells [40] Increased in liver metastases from human pancreatic cancer [58] Downregulated in melanoma tumor-repopulating cells [55]	*In vitro* Silencing inhibits survival, colony formation [41,44] and proliferation [43] of lung cancer cells under low glucose Silencing inhibits survival of breast cancer cells under low glutamine [42] and sphere formation of prostate cancer cells in normal glucose [53] Silencing reduces proliferation of pancreatic neuroendocrine cancer cells [52] Silencing inhibits proliferation of colon carcinoma cells and increases resistance towards 5-fluorouracil/radiation [60] Silencing enhances colony formation of murine melanoma cells [55] *In vivo* Silencing inhibits tumor formation of lung cancer cells [43,44] and prostate cancer cells [53] Silencing enhances growth of murine melanomas [55]
II. Liver and kidney cancer		
PCK1	Downregulated in HCC [61–68] and renal cell carcinoma [63,69] Low expression associated with worse survival in HCC patients [65,66,68]	*In vitro* Overexpression exerts variable effect in HCC cells in high glucose and reduces survival in low glucose [68] Overexpression induces apoptosis and suppresses migration of HCC cells in normal glucose [67] Silencing inhibits clonogenic growth in hepatoma cells in normal medium [45] *In vivo* Overexpression inhibits *in vivo* growth of HCC [62,65,66,68]
PCK2	Downregulated in HCC [63,68,70] and renal cell carcinoma [63], low expression associated with worse overall survival in HCC patients [68]	Overexpression reduces survival of HCC cells in low glucose [68]

Tumor promoting and tumor suppressor activities are highlighted in green and red, respectively.

metastatic breast cancer [50], FBP2 inhibited glycolysis and growth of gastric cancer cells [82].

Together, these studies suggest that FBP1 and possibly also FBP2 act to inhibit glycolysis and may play a tumor-suppressive role in tumor cells, both *via* enzymatic and non-enzymatic mechanisms (Fig. 3). This is in contrast to the finding that FBP2 may be required to confer metabolic flexibility under glucose deprivation. Interestingly, FBP1 expression inhibited tumor growth also in cancer types that displayed growth enhancement by PCK2, *e.g.* in breast and lung cancer (Table 2). In the non-oxidative PPP, *de novo* synthesis of ribose-5-phosphate starts from both, fructose-6-phosphate and glyceraldehyde-3-phosphate (GAP) (Fig. 2). Thus, even in the absence of FBPase, abbreviated gluconeogenesis can contribute carbons to ribose-5-phosphate. Alternatively, nucleotide degradation *via* salvage pathways might ensure sufficient ribose-5-phosphate for biosynthetic purposes.

5.3. G6PC activity is linked to glycogen turnover in cancer cells

G6PC hydrolyzes glucose-6-phosphate and thus mediates the final step in gluconeogenesis but also in glycogen degradation *e.g.* in the liver. In HCC, G6PC expression [61–63] and activity [61] were reported to be downregulated compared with adjacent tumor-free tissues, and similar results were obtained in kidney cancer [63,69]. G6PC overexpression reduced HCC growth in mice *in vivo* [62]. These results are

Table 2
Tumor promoting and tumor suppressor activity of FBP1/2.

	Alteration in tumor tissue	Biological effect
I. Tumors originating in non-gluconeogenic tissues		
FBP1	*Primary tumors* Enhanced in different histological breast cancer subtypes [72] Reduced in cholangiocarcinoma [83], gastric cancer [84], pancreatic cancer [73], NSCLC [75] and cervical carcinoma [85] Reduced in basal-like breast cancer but not luminal type breast cancer [71] Low expression associated with poor survival in breast cancer [72], gastric cancer [81], pancreatic cancer [73], NSCLC [75], and cervical carcinoma patients [85] Inversely correlated with tumor grade in pancreatic cancer tissue [74] *Metastases/tumor initiating cells* Reduced in lung cancer stem cells compared to non-stem cell cancer cells [76]	*In vitro* Overexpression reduces proliferation and invasion in NSCLC cells, particularly under hypoxia [75] Overexpression reduces tumorsphere formation and suppresses proliferation of breast cancer cells in severe hypoxia [71] Overexpression reduces/silencing enhances proliferation in breast cancer [72], cholangiocarcinoma [83], cervical carcinoma [85], pancreatic cancer [73,74] and colon carcinoma cells [86] Overexpression reduces/silencing enhances migration in breast cancer cells [72,87] Overexpression enhances/silencing reduces apoptosis in breast cancer [88] and cholangiocarcinoma cells [83] Restoration of FBP1 in gastric cancer cell lines inhibits proliferation and EMT [81] *In vivo* Overexpression reduces cholangiocarcinoma [83], NSCLC [75] and breast cancer xenograft growth [71] Overexpression reduces tumor growth from lung cancer stem cells [76]
FBP2	*Primary tumors* Reduced in gastric cancer [82] Low expression associated with poor survival in gastric cancer patients [82] *Metastases/tumor initiating cells* Increased in human breast cancer brain metastases compared to paired samples of primary breast cancer [50] Increased in brain metastatic cancer cells compared to parental breast cancer cells [50]	*In vitro* FBP1/2 silencing reduces survival and proliferation of metastatic breast cancer cells under glucose deprivation [50] Overexpression reduces proliferation of gastric cancer cells [82] *In vivo* FBP1/2 silencing delays the growth of breast cancer metastases in the brain [50] Overexpression reduces gastric cancer xenograft growth [82]
II. Liver and kidney cancer		
FBP1	Reduced in HCC [61,63,69,77–80], and renal cell carcinoma [63,69,89] Low expression associated with poor survival in HCC [77–80] and renal cell carcinoma patients [69]	*In vitro* Overexpression reduces spheroid formation [77], proliferation [78,79,86,90,91], colony formation [78] and EMT [80] in HCC cells Overexpression reduces proliferation and migration in renal cell carcinoma cells [69] *In vivo* Overexpression reduces tumor growth of HCC [77–79,90,91] and renal cell carcinoma [69]

Tumor promoting and tumor suppressor activities are highlighted in green and red, respectively.

reminiscent of the findings on PCK1 in tumors arising in gluconeogenic tissues. In contrast, G6PC expression was enhanced in glioblastoma, a highly malignant brain cancer, compared to noncancerous human cortex [92]. TIC isolated from glioblastoma showed an upregulation of G6PC by the glycolysis inhibitor 2-desoxyglucose (2DG) [92]. G6PC silencing reduced proliferation and migration in glioblastoma cells and invasion *in vivo*, which was especially pronounced after 2DG treatment and recovery. Mechanistically, G6PC silencing led to glycogen accumulation and a reduced activation of AKT (protein kinase B) [92]. High G6PC expression was found to be associated with poor overall and disease-free survival in ovarian cancer [93]. Silencing or inhibition of G6PC in ovarian cancer cells led to reduced proliferation and invasion and an accumulation of glycogen *in vitro* [93]. In both studies a tumor-promoting role of G6PC has been observed in the presence of abundant glucose, suggesting a role of G6PC in glucose cycling (glycogen degradation) rather than in gluconeogenesis.

6. Regulation of gluconeogenesis enzyme expression in normal and cancer cells

The regulation of PCK1 expression in gluconeogenic tissues upon fasting, which has been studied for decades, has been reviewed in detail elsewhere [94,95]. In summary, glucagon and glucocorticoids are the best characterized activators of PCK1 transcription in the liver, while insulin inhibits PCK1 expression. Glucagon activates cAMP regulatory element-binding protein (CREB), which acts in concert with multiple

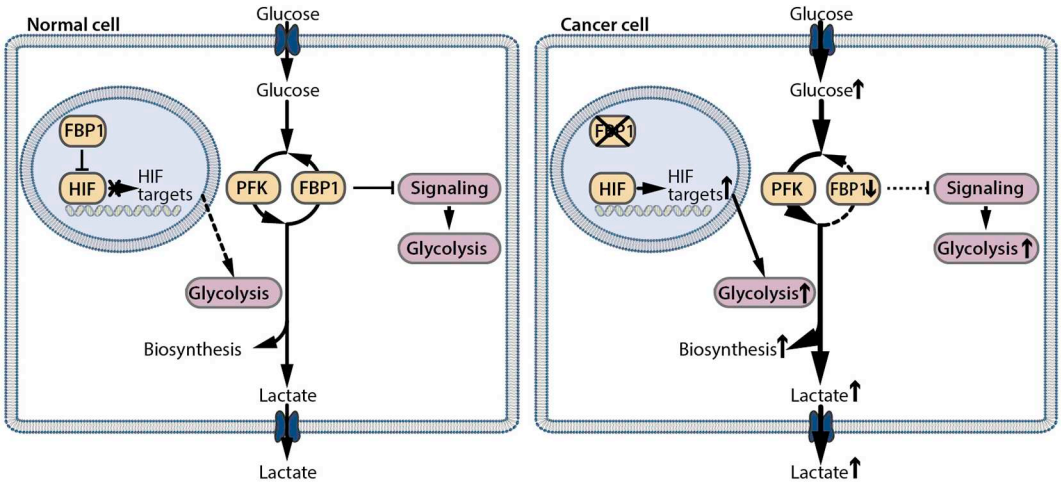

Fig. 4. Effects of FBP1 on glycolysis by canonical and non-canonical activities.
Besides its gluconeogenic function that opposes glycolysis (canonical function), FBP1 inhibits glycolytic gene expression by a direct action on different signaling pathways (non-canonical function). In kidney cancer cells FBP1 binds to HIF-1α or HIF-2α in the nucleus and reduces HIF-induced expression of glycolytic genes. In cancer cells, FBP1 expression is frequently decreased to promote glycolysis and the Warburg effect.

co-activators to induce PCK1 [94,95]. Acting antagonistically, insulin leads to the recruitment of phosphoinositide 3-kinase (PI3K) to the plasma membrane upon binding to its receptor, which generates phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 in turn inhibits different transcription factors *via* AKT, including forkhead box protein O1 (FoxO1) [94,95]. In tumor cells, PCK1 and PCK2 are rather regulated in a cell-autonomous manner than by insulin or glucagon. In HepG2 liver cancer cells, insulin had no effect on PCK1 expression [96]. Accordingly, levels of PCK1 or G6PC were unaffected by fasting in liver cancer tissue in mice, although an increase by exogenous glucocorticoids was observed, both *in vivo* and *in vitro* [62]. PCK1 levels and activity are also regulated by post-translational modification. The turnover of PCK1 protein has been found to be controlled by acetylation [97]. High glucose levels destabilize PCK1 by stimulating its acetylation which promotes its ubiquitinylation and subsequent degradation, while deacetylation *e.g.* by sirtuin 2 stabilizes PCK1 [97]. The different signaling pathways regulating expression of gluconeogenesis enzymes in cancer cells have been reviewed elsewhere in detail [26] and are briefly summarized here.

The tumor suppressor p53 activated the expression of PCK2 and G6PC in HepG2 liver cancer cells [98]. Contrary to these results, ectopic expression of p53 efficiently downregulated the expression of PCK1 and G6PC, by sirtuin 6-mediated nuclear exclusion of transcription factor FoxO1, in different cancer cell lines, and in mouse liver [99]. The effect of p53 on gluconeogenesis gene expression thus appears to be context-dependent. A posttranslational mechanism of PCK1 regulation has been described in HCC cells involving increased degradation *via* sumoylation [65]. PCK1 sumoylation was enhanced in HCC cells due to repressed Nur77 [65]. Notably, PCK1 and G6PC were downregulated in liver and kidney cancer cells, but not in a panel of tumor cell lines from other tissues, by mTORC2, one of the complex forming members of mTOR [63]. Silencing of the mitotic kinase polo-like kinase 1 (PLK1) dramatically reduced levels of PCK1 mRNA and protein in melanoma cells, while FBP1 expression was increased, suggesting that the two enzymes are not regulated in the same direction in certain cancer cell types [100].

In contrast to the large body of literature on the regulation of PCK1 expression, especially in liver cells and adipocytes, little is known about the regulation of PCK2. PCK2 was upregulated under low glucose conditions in different cancer cell lines [41–44]. Moreover, ER stress and glutamine deprivation increased PCK2 expression [42]. The upregulation of PCK2 by HIFs in lung cancer cells [43] points to a crosstalk of hypoxia-induced pathways and gluconeogenesis in cancer cells. More research is warranted to characterize the factors regulating the expression of PCK1 or PCK2 in cancer cells *in vivo*.

In an interesting study using tumor tissue derived from rapid autopsies, PCK1 and PC were found to be among the most highly upregulated genes in liver metastases from human pancreatic cancer compared to a standard reference tissue [58]. In contrast, PCK1 or PC mRNA were not elevated in primary pancreatic cancer [58]. PCK2 was also upregulated in liver metastases, but downregulated in skeletal muscle metastases [58]. These site-specific alterations of gluconeogenic gene expression in metastases, that have also been observed in breast cancer metastases [40,50] and prostate cancer metastases [53] (Tables 1 and 2), are of major interest. These results suggest that the specific environment at the metastatic site might contribute to the upregulation of gluconeogenic genes.

Physiologically, FBPase activity is tightly regulated by metabolic intermediates. FBP1 and FBP2 are inactivated by fructose-2,6-bisphosphate and AMP [101]. In different cancers, including gastric cancer [84], HCC [77,86], colon carcinoma [86], NSCLC [102] and breast cancer [103] hypermethylation of the *FBP1* promoter decreased expression of FBP1. Similar findings have been obtained for *FBP2* promoter hypermethylation in breast cancer [103]. In ccRCC [104] and HCC [77] there is also gene copy number loss. NSCLC cell lines showed reduced FBP1 expression, which was reversed by silencing of Zinc finger *E*-box-binding homeobox 1 (ZEB1) [75]. FBP1 protein may also be post-translationally regulated in cancer cells by ubiquitination and proteasomal degradation [90]. Interestingly, FBP1 was significantly downregulated following *HRAS*-induced transformation in NIH3T3 mouse fibroblasts [84]. The regulation of FBPase activity in the direction of gluconeogenesis and the modulation of FBP1 or FBP2 expression by the glucose availability in cancer cells is still unclear and should be addressed in future studies.

7. Present concepts

The studies discussed here show that gluconeogenesis enzymes exert context-dependent and highly important functions in cancer cells (Fig. 5). When glucose is abundant, most cancer cells are glycolytic and the suppression of FBP1 mediated inhibition of glycolysis is an advantage (Fig. 5A). Under these conditions PCK1/2 may play a role by regulating the flux through the TCA cycle in certain cancers (Fig. 5A). This cataplerotic function of PCK1/2 may be either tumor-promoting or tumor-suppressive, depending on the cancer type and context.

In contrast, under low glucose conditions, PCK1 and PCK2 confer

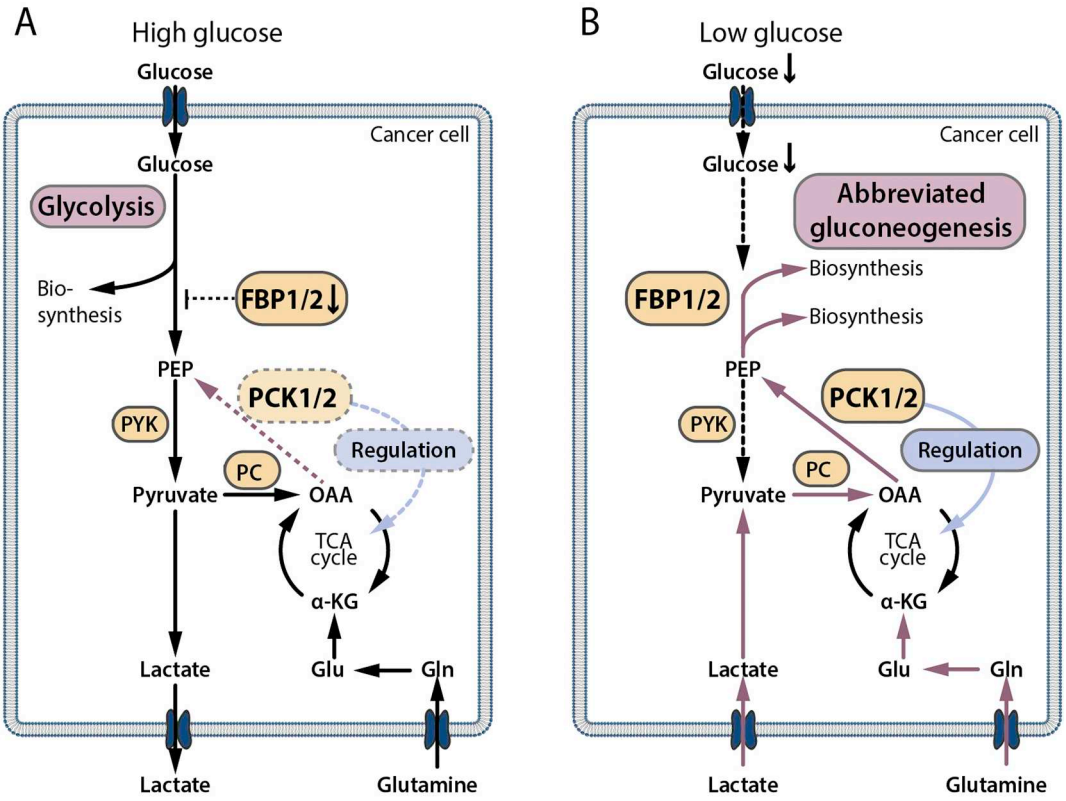

Fig. 5. Present concepts of gluconeogenesis in cancer cells under different microenvironmental conditions.
(A) Under high glucose supply, most cancer cells perform glycolysis for biosynthesis, lactate is released. In many cancers, this aerobic glycolysis is facilitated by a downregulation of FBP1, since FBP1 inhibits glycolysis and glycolytic gene expression *via* enzymatic and non-enzymatic mechanisms. In some tumors, PCK1 or PCK2 act under high or moderate glucose concentrations to regulate TCA cycle flux. (B) Under glucose deprivation, many cancer cells exhibit abbreviated forms of gluconeogenesis *via* PCK1 or PCK2 and to some extent FBPase, which allows feeding of biosynthetic pathways from lactate or glutamine, if glucose is missing. This promotes cancer cell survival in the hostile, glucose-deprived microenvironment of solid cancers. Additionally, PCK1/2 have a regulatory function on the TCA cycle flux. PCK1/2 and/or FBP1/2 expression occurs in a context-dependent manner and is highly heterogenous among different cancers.

metabolic flexibility in different cancer types by allowing the synthesis of ribose-5-phosphate, glycerol-3-phosphate and serine and hence nucleotides and lipids from non-carbohydrate precursors (Fig. 5B). The preference for PCK1 or PCK2 in glucose-deprived cancer cells appears to be largely determined by the tissue of origin. An upregulation of PCK1/2 or FBP2 was found in metastases (Tables 1 and 2), which might be related to the nutrient-poor metabolic microenvironment in progressed cancers or to altered nutrient availability at the metastatic site. However, in some tumors, PCK1 or PCK2 are absent or expressed at very low levels. Alternative pathways or nutrient scavenging strategies might be activated in these cells, as will be discussed below. The metabolic pathways mediated by gluconeogenesis enzymes in cancer cells are reminiscent of the physiological abbreviated gluconeogenesis in adipocytes and liver cells (glyceroneogenesis). In cancer cells, however, abbreviated gluconeogenesis is not hormonally regulated but activated in a cell-autonomous manner, especially under low glucose conditions. In rapidly growing cancers like lung cancer, expression of PCK1 or PCK2 might be important for cancer growth, because of the high anabolic demand and frequently insufficient supply. Many different gluconeogenic precursors, like lactate, glutamine, and several other amino acids can serve as precursors for (abbreviated) gluconeogenesis in glucose-starved cancer cells *in vivo*, which leads to enormous versatility of PCK1/2 mediated anabolic pathways.

As already outlined in the review by Wang et al. [26], contradictory findings on PCK1/2 have been reported in cancers originating from gluconeogenic organs as opposed to non-gluconeogenic organs. Mostly anti-tumorigenic effects of PCK1 or PCK2 have been described in gluconeogenic organs, liver and kidney, while in cancers arising from non-gluconeogenic organs mostly tumor promoting effects have been found (Table 1). These controversial results may be related to the fact that the physiological function of PCK1 and PCK2 as gluconeogenesis enzymes is lost during carcinogenesis. HCC tissue and kidney cancer tissue do not contribute to maintaining blood glucose levels. Lower PEPCK activities have been measured in the context of abbreviated gluconeogenesis in tumor cells, than in starved mouse liver [41]. This indicates that physiological gluconeogenesis requires higher gluconeogenesis enzyme activities than abbreviated gluconeogenesis in tumor cells. Kidney cancers, especially ccRCC, are typically well perfused tumors due to constitutive HIF stabilization and angiogenesis [105]. Therefore, differences in tumor perfusion and the metabolic tumor microenvironment might also play a role. Moreover, metabolic requirements for growth might differ across different cancer types. For example, a downregulation of the serine synthesis pathway enzymes and an upregulation of pyruvate dehydrogenase, the enzyme diverting pyruvate away from the glycolytic pathway to form acetyl-CoA, have been noted in HCC [70], which is in contrast to other tumor types.

8. Open questions and future challenges

Inhibition of gluconeogenesis enzymes could potentially be exploited to prevent tumor cell adaptation to the nutrient-starved microenvironment. This model is supported by the observed decline in biosynthetic intermediates as well as end products, such as specific classes of phospholipids [44], in glucose-deprived cancer cells. The efficacy of gluconeogenesis inhibition, however, may likely depend on the extent of nutrient deprivation in the specific tumor and on the availability of non-carbohydrate precursors (lactate, amino acids). The existing literature suggests that a possible effect of gluconeogenesis

enzyme inhibition might be context-related and highly dependent on the target. Especially the canonical and non-canonical functions of FBP1 as inhibitor of glycolysis and cell signaling must be considered. Conversely, any treatment options aiming at FBP1 re-expression in FBP1-low cancer might promote cancer cell metabolic plasticity and a potential growth advantage under starvation.

Robust anticancer effects upon silencing of PCK1 or PCK2 have been observed in different *in vivo* models of primary or metastatic non-hepatic cancers (Table 1), however data on the effect of inducible silencing or inhibition of these enzymes in already established tumors are missing. Moreover, the contribution of PCK1 or PCK2 to biosynthetic activities in cancer cells in their 3D context and tumor microenvironment is still poorly understood. In a study on glucose metabolism in patient-derived lung cancer xenografts, there was a considerable scrambling of the tracer (the appearance of intermediates with only partial labeling) at the level of the glycolytic intermediate fructose-6-phosphate [106]. This was suggested by the authors to implicate active gluconeogenesis in the tumor tissue [106]. However, the authors pointed out that the PPP could contribute to the ^{13}C scrambling in sugar phosphates [106]. Lactate has been found to contribute to TCA cycle intermediates in different cancers [7,17,18]; however, a possible further conversion along the gluconeogenesis pathway is still elusive. When NSCLC patients or tumor-bearing mice were infused with ^{13}C-lactate, ^{13}C-lactate was in fact taken up by the tumors and converted to TCA cycle metabolites, but only a minor pool of 3-phosphoglycerate (3-PG) or PEP was labeled, likely from systemic gluconeogenesis [18]. According to the authors, the 3-PG and PEP label might have derived from labeled plasma glucose due to systemic gluconeogenesis [18]. While this study does not support a major contribution of gluconeogenesis from lactate in NSCLC, the utilization of glutamine, as suggested by several *in vitro* studies, has not been addressed. Future studies using different stable isotope labeled gluconeogenic precursors are warranted.

Alternative pathways feeding the pools of glycolytic/gluconeogenic intermediates besides gluconeogenesis might potentially play a role in glucose-deprived cancer cells. Fructose and galactose are less abundant in blood than glucose, however both may eventually enter glycolysis. Still, the availability of fructose may be high in some settings. A high fructose diet has recently been shown to enhance intestinal tumor growth in mice [107]. Fructose enhanced glucose uptake by the tumor cells, but it was also used as a fuel. When tumors where incubated in ^{13}C-fructose in the absence of glucose *ex vivo*, a considerable conversion of fructose to glycolytic intermediates was found [107]. Recently, a novel metabolic pathway has been described in cancer cells, that leads to the generation of glycolytic intermediates from thymidine catabolism, similar to a known degradation pathway in prokaryotes [108]. These pathways may act in addition to gluconeogenesis to enhance metabolic plasticity in glucose deprived cancer cells. Nutrient scavenging *via* macropinocytoysis followed by digestion of the scavenged macromolecules is an important, oncogene-activated mechanism [109,110]. Many different components of the extracellular space can be scavenged, including albumin, as well as necrotic cell debris [111]. Macropinocytosis has been shown to enhance proliferation and survival of cancer cells under starvation and to be important for cancer growth *in vivo* [109,110]. It is unclear today, whether PCK1/2 activation and macropinocytosis represent different strategies of cancer cell adaptation to starvation or whether they act in concert. A recent study suggests that macropinocytosis-derived amino acids are precursors for gluconeogenesis in cancer cells [112].

Microvascular density [113] and high fluorodeoxyglucose positron emission tomography (FDG-PET) signals [114,115] have been shown to be correlated with a poor prognosis in different cancers. Although these findings do not support nutrient starvation as major driver of cancer progression, still the local or temporal adaptation to nutrient-poor conditions might be a prerequisite for metabolically highly active tumors to thrive. Abbreviated gluconeogenesis may be a survival promoting metabolic pathway in a subset of cancer cells that continue anabolic activity despite poor perfusion. Interestingly, stem-cell like tumor subpopulations and metastases showed high PCK1/2 expression across different tumor entities and gluconeogenesis promoted metastasis and colony forming capacities. To clarify, which intrinsic (cell-autonomous) or extrinsic (microenvironment-driven) signals regulate gluconeogenesis in different cancer types is of major interest and should be addressed in future studies. Moreover, it is unclear, how the gluconeogenic phenotype is linked to the glycolytic switch, and which intratumoral subpopulations, *e.g.* invasive *versus* proliferative cells preferentially utilize this pathway.

In the hostile tumor microenvironment, tumor cells and tumor-infiltrating immune cells compete for nutrients [116] and anti-tumor effector functions might potentially be influenced by the activities of gluconeogenesis enzymes in the immune cells as well. Little is known about the abundance and function of the different gluconeogenesis enzymes in tumor-infiltrating immune cells. Overexpression of FBP1 in natural killer cells inhibited their tumoricidal activity [117]. In contrast, abrogation of PCK1 dependent PPP decreased levels of glutathione, increased levels of reactive oxygen species and depleted $CD8^+$ memory T-cells [118]. Lactate released from colon carcinoma cells induced PCK1 in monocytes [119]. It will be important to determine the role of gluconeogenesis enzymes not only in the tumor cells, but also in the immune cells.

In summary, a growing body of literature on gluconeogenesis in cancer shows that tumor cells exploit or regulate gluconeogenesis enzymes that also have distinct functions in normal cells, allowing growth and survival in their specific metabolic microenvironment. Gluconeogenesis enzymes, which mediate important anabolic biosynthetic pathways and regulate multiple cellular functions, thus might represent interesting therapeutic targets for cancer therapy.

Declarations of interest

None.

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