

Review

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# Glucose addiction of cholangiocarcinoma: opportunities for therapeutic development

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## Abstract

The association between diabetes mellitus, hyperglycemia, and cholangiocarcinoma (CCA) development and progression has been established. One speculation of the effects of high glucose levels promoting CCA progression is via the feeding of substrate to the aerobic glycolysis or so-called Warburg effects in CCA cells. Several glycolytic enzymes and glucose transporters are upregulated in CCA and further activated by high glucose conditions. However, the increased glucose uptake and the increased aggressive phenotypes of CCA under high glucose conditions might not be solely due to this aberrant energy metabolism. High glucose conditions have been proven to be the activator of the other signaling pathways, as well as the precursors for dysregulated glycosylation of oncoproteins in CCA. The higher requirement of glucose and the abundant glucose availability in diabetic conditions then synergize to promote aggressive CCA phenotypes. Additionally, the glucose avidity could also become the Achilles heel of CCA cells, as they could be sensitive to glucose deprivation. The development of therapeutic agents targeting glucose metabolisms or glucose-activated pathways is promising for CCA treatments. This article reviews and discusses the up-to-date research on how high glucose is involved in CCA progression, both via Warburg effects and other mechanisms.

**Keywords:** Cholangiocarcinoma, diabetes mellitus, glycolysis, hyperglycemia, warburg effect



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## INTRODUCTION

Cholangiocarcinoma (CCA), or malignancy of bile duct epithelia, is one of the emerging cancers whose incidence is increasing worldwide<sup>[1]</sup>. It is the second most prevalent primary liver cancer and accounts for 3% of all gastrointestinal tract cancers<sup>[2]</sup>. CCA can be anatomically classified into intrahepatic and extrahepatic types, in which the latter can be classified into perihilar type when the tumor is located between the second order of the biliary tree to the cystic duct and distal type when the tumor is distal to the cystic duct until the ampulla of Vater. Considering rare malignancy in Western countries, a high incidence rate has been found in East and Southeast Asian countries, up to 85 cases per 100,000 population in Thailand<sup>[1,2]</sup>. Due to late presentation and the aggressive nature of the disease, CCA leads to high mortality each year<sup>[3]</sup>. A lot of efforts have been made to address the biological background of this cancer to get a better understanding, which might lead to the development of effective therapeutic modalities<sup>[4-8]</sup>. One of the approaches that has garnered attention in the research field is metabolic reprogramming<sup>[9-11]</sup>.

Like other cancers, metabolic reprogramming in CCA has been found important for cancer aggression and advancement<sup>[9,11,12]</sup>. CCA cells modify metabolic pathways to benefit their survival and compete with the surrounding normal tissues. The reprogramming of energetic nutrients such as glucose<sup>[12,13]</sup>, amino acids<sup>[14,15]</sup>, and fatty acids<sup>[16,17]</sup> is reported for their involvement with CCA carcinogenesis and progression. In addition, many metabolic factors have been experimentally reported that may be involved in the advancement of CCA and thus lead to poor prognosis of patients. Metabolic disorders, e.g., diabetes mellitus (DM)<sup>[18,19]</sup> and obesity<sup>[20,21]</sup>, have also been studied for their promoting effects on CCA biology. The abnormal metabolic processes in these diseases may favor the metabolism of CCA cells and thus promote cancer progression. Especially in DM, in which patients usually experience chronic hyperglycemia or high blood glucose levels<sup>[22,23]</sup>, which can be utilized in a higher glucose-desired state of aerobic glycolysis or the Warburg effect<sup>[24]</sup>. DM and hyperglycemia are then the emerging factors that might directly play roles in the aggression and progression of CCA.

DM has been studied for decades for its associations with the increased risk of CCA development and also poor survival of CCA patients<sup>[23,25,26]</sup>. It is hypothesized that the effects on CCA risk are probably due to the mitogenic effects of insulin in a state of compensatory hyperinsulinemia<sup>[22,27]</sup>. However, only one study shows an association between using exogenous insulin and the increased risk of extrahepatic CCA to date<sup>[28]</sup>. The effects of other anti-diabetic medications involving increased insulin secretion, such as insulin secretagogue or incretin-based therapy, have not been agreed upon regarding the risk of CCA development<sup>[22,27]</sup>. Thus, the effects of high glucose on CCA progression are suspicious and have been investigated later. CCA cells have been shown to have higher glucose consumption; thus, the glucose transporters (GLUTs) in this cancer are usually upregulated and associated with poor survival of patients<sup>[29-31]</sup>. As mentioned, glucose is a supplier for Warburg effects and, thereby, may straightforwardly increase the glycolysis of CCA cells to meet the high energy requirements. However, glucose has not only been utilized as a nutrient or precursor for glycolysis in CCA cells. High glucose levels have been reported for their regulatory roles in GLUTs<sup>[32]</sup> and glycolytic gene expressions<sup>[18]</sup>. It can also be shunted to the other metabolic pathways, e.g., pentose phosphate pathways (PPP) and hexosamine biosynthetic pathways (HBP), that might also support the aggressive phenotypes of CCA cells<sup>[33-36]</sup>. Moreover, high glucose also activates pro-tumorigenic pathways, resulting in CCA cell aggression<sup>[37-40]</sup>. Therefore, this review aims to update and discuss the increased requirement of glucose in CCA cells for energy metabolisms and other aspects. The opportunity to develop a treatment based on the dysregulation of glucose metabolism is also discussed.

## METABOLIC REPROGRAMMING IN CCA

The metabolic reprogramming of cancers has been recognized since Warburg noticed that cancer cells have an increased rate of glycolysis even if they are in an adequate oxygen environment<sup>[41]</sup>. It is primarily hypothesized that this aerobic glycolysis is a result of impaired mitochondria, which compromise the cells for ineffective oxidative phosphorylation (OXPHOS). However, later studies revealed that although the mitochondria of cancer cells are intact, the rates of glycolysis in some specific cancers are still high<sup>[42]</sup>. This led to several “next-generation” studies based on the hypotheses that metabolic alterations in cancer are beneficial and are a choice of cancer cells, not because of the inability to utilize nutrients due to defective mitochondrial machinery<sup>[43]</sup>. This concept also shifts a paradigm of study and expands the discovery of cancer metabolism from glucose in glycolysis to the other metabolic pathways, namely amino acid and fatty acid metabolism.

Metabolic reprogramming has been recognized as one of cancer hallmarks<sup>[44,45]</sup>. In CCA, mutations of genes directly and indirectly involving energy metabolism are reported as driver oncogenes, such as *IDH1*, *IDH2*, *MYC*, and *KRAS*<sup>[6,7,46,47]</sup>. Targeting genes or proteins in the metabolic pathways becomes a promising strategy for cancer treatments<sup>[48]</sup>. Glucose metabolism has been well studied in CCA, similar to other cancers, and it has a high impact on anti-cancer drug development<sup>[24]</sup>. In addition to glucose metabolism, the metabolic pathways involving the catabolism of glutamine and anabolism of lipids also showed their critical roles in CCA progression. As mentioned, the Warburg effect is more likely a choice of cancer metabolism, which benefits cancer cell growth and proliferation. Glycolytic intermediates and their products can serve as precursors for other biomolecule production. For example, pyruvate, a final product of glycolysis, is a precursor for acetyl coenzyme A synthesis, which is a substrate for fatty acid and cholesterol synthesis. Fatty acid and cholesterol are essential molecules for cell proliferation, and thus, the inhibition of the synthetic pathways of both molecules significantly suppresses CCA cell growth<sup>[49-51]</sup>. Moreover, as cancer cells preferably utilize glucose in glycolysis to provide a carbon skeleton for biosynthesis, glutamine is a choice for anaplerosis of the tricarboxylic acid (TCA) cycle to replenish energy production. A higher requirement for glutamine is reported in CCA, together with the upregulation of genes involved in glutaminolysis<sup>[15]</sup>. Inhibition of glutaminolysis is then another promising strategy for CCA treatment, and co-targeting glutaminase and GLUTs showed synergistic effects on CCA inhibition<sup>[14]</sup>. These suggest that glucose metabolism might be the primary pathway that is reprogrammed in cancers, including CCA, and then it is networked with the other metabolic pathways to orchestrate the driving of CCA progression. The following topics in this review are mainly focused on this particular aspect and extend to the recent discovery that reported non-metabolic roles of glucose in CCA, which is another emerging aspect and has the potential for therapeutic development.

## WARBURG EFFECT AND THE PROGRESSION OF CCA

Normal proliferating cells in multicellular organisms display a unique energy metabolism with strict regulation to ensure proper cell functioning and a balance between cell cycle arrest and cell proliferation<sup>[52,53]</sup>. This balance is essential to maintain proper cellular homeostatic responses and requires the process of generating energy in the form of ATP obtained from rather complex molecules. Glucose, a major source of carbohydrates, is a molecule that is primarily utilized for ATP production to meet high metabolic demands. In normal healthy cells, glucose is converted to pyruvate to yield a small amount of ATP via the process of glycolysis. The pyruvate derived from glycolysis is then transported from the cytosol to the mitochondria to be oxidized into acetyl coenzyme A, which is further incorporated into the TCA cycle in the form of citrate and generates abundant ATPs in a flowing mitochondrial OXPHOS.

Cancer cells, including CCA, on the contrary, have the ability to alter this normal metabolic process. This change in cancer cell bioenergetics is also referred to as metabolic reprogramming<sup>[54]</sup>, one of the hallmarks of cancer, which is shown to influence multiple factors to support tumor development and malignant transformation<sup>[44,45]</sup>. Consequently, this aberrant reprogramming facilitates cancer progression, including activation of oncogenes, alteration of receptor-initiated signaling pathways, and deregulation of cellular energetics. Like other cancers, CCA cells require a lot of energy to maintain their continuous growth<sup>[12]</sup>. Some oncogenic mutations lead to metabolic reprogramming of CCA, such as the mutation of *KRAS*. *KRAS* activation can enhance glucose uptake by upregulation of GLUTs and several glycolytic enzymes<sup>[55]</sup>. The overexpression of GLUTs has been reported in *KRAS* mutant CCA cell lines, and the upregulated GLUT1 was associated with progressive carcinogenesis and poor survival of CCA patients<sup>[30,56]</sup>. In addition, an increase in glucose uptake and, thereby, glycolysis, parallel with large amounts of lactate secretion, provides an acidic tumor microenvironment for facilitating CCA invasion, is also observed<sup>[57-59]</sup>. Moreover, Colyn *et al.* also reported that the mutation of *KRAS* can lead to phosphoglycerate dehydrogenase upregulation and, hence, increase the reprogramming of glycolytic metabolites to the serine-glycine synthesis pathway<sup>[60]</sup>. On the other hand, the mutations of tumor suppressor genes, e.g., *TP53*, also play roles in metabolic reprogramming since p53 can act as a transcriptional repressor of key genes in glucose metabolisms such as GLUTs<sup>[61]</sup>.

Although aerobic glycolysis is a less efficient metabolic means of generating ATP compared to OXPHOS in the mitochondria, cancer cells still benefit substantially from high glycolytic rates, enabling them to survive and proliferate accordingly<sup>[43]</sup>. The rationale behind this occurrence is explained by the higher rate of ATP production by glycolysis, which is considered to be much more rapid compared to normal OXPHOS and, thus, can be more efficiently employed to meet the increase in ATP demand, gaining a selective advantage under tumor microenvironment where nutrients have limited availability. Secondly, increased glycolysis results in an increase in the synthesis of glycolytic metabolites that are necessary biosynthetic precursors for many cellular pathways, such as the production of lipids, amino acids, and nucleotides due to the overexpression of pyruvate kinase M2 (PKM2) which causes a reduction in conversion of phosphoenolpyruvate to pyruvate in the final, irreversible step of glycolysis<sup>[62,63]</sup>. The upregulated PKM2 is also evident in CCA, and it is associated with a poor prognosis in CCA patients who have DM<sup>[18]</sup>. High expression levels of PKM2, which causes the retention of glycolytic intermediates, can benefit cancer cells in many ways. For instance, the rise in levels of glucose-6-phosphate (G6P), one of the glycolytic intermediates for shunts in PPP responsible for generating nucleotide synthetic precursors, is indispensable for DNA synthesis and cancer cell progression, while rises in glyceraldehyde-3-phosphate (G3P) account for an increase in triglyceride and serine biosynthesis<sup>[64]</sup>. Hence, this transformed glucose metabolism is highly useful for cancer cells to unlimitedly supply themselves with biosynthetic precursors to satisfy their highly proliferative characteristics. Moreover, subsequent nicotinamide adenine dinucleotide phosphate (NADPH) derived from the PPP provides adequate reduced glutathione for resistance to chemotherapeutic agents, providing additional rationale for how cancer cells may benefit from the Warburg effect.

Apart from altered glucose metabolism, the Warburg effect also influences the expression of signaling molecules and transcriptional regulatory factors, which promotes cancer occurrence. Oncogenic mutations cause upregulation of various cancer-promoting factors such as *HIF1*, *SIRT*s, and *MYC*<sup>[65]</sup>. These genes have been reported in CCA cells exhibiting the Warburg effect, while the downregulation of tumor suppressor genes was also manifested. Reprogramming also affects signal transduction pathways, consequently leading to increased activation of downstream signaling that triggers tumor initiation and progression<sup>[10,12]</sup>. The PI3K/Akt/mTOR regulatory pathway is one of the pathways being explored in CCA cells<sup>[66,67]</sup>. Stimulation of the PI3K/Akt/mTOR pathway indirectly enhances the Warburg effect by activating the transcription factor

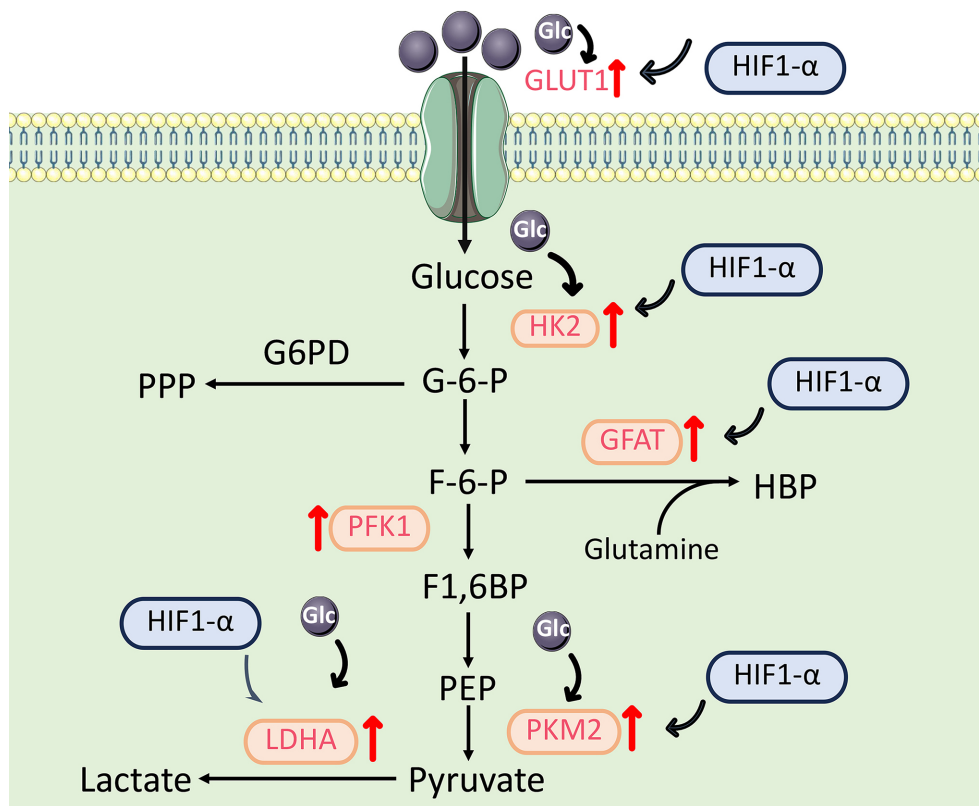
cMyc, HIF-1, and SIRT3<sup>[9]</sup>. Together, this promotes the expression of glycolytic proteins and enzymes such as GLUT1, PKM2, phosphofructokinase 1 (PFK1), hexokinase II (HK2), and lactate dehydrogenase A (LDHA), hence explaining the high glycolytic rates seen in the cancer cells including CCA. Glycolytic inhibitors are therefore considered therapeutic interventions that have an anti-Warburg effect by inhibiting cancer cell growth and progression<sup>[48,68,69]</sup>.

Hypoxia-inducible factor 1 (HIF-1) is another master regulatory protein for glycolysis by upregulating genes of GLUTs and glycolytic enzymes<sup>[70,71]</sup>. Under physiological oxygen level, HIF-1 is modified by prolyl hydroxylation by the enzyme prolyl hydroxylase (PHD) and interacts with tumor-suppressor protein von Hippel-Lindau (vHL) to be targeted for proteasomal degradation<sup>[72]</sup>. However, PHD is inhibited during hypoxic conditions, and thus, HIF-1 is stabilized. As seen in cancer cells, mutations in oncogenes and tumor suppressor genes such as vHL ultimately lead to the stabilization of HIF-1, suppressing mitochondrial activity. HIF-1 not only promotes cancer progression and malignant transformation by potentiating transcriptions of GLUT1, HK2, PKM2, PFK1, and LDHA, but also increases the expression of pyruvate dehydrogenase kinase (PDK), which inactivates the pyruvate dehydrogenase complex. Additionally, HIF-1 upregulates vascular endothelial growth factor (VEGF), thereby resulting in increased angiogenesis. The upregulation of HIF-1 $\alpha$ , a subunit of HIF-1, in a normoxic and hypoxic condition of CCA cells was also reported<sup>[63-76]</sup>. The current findings of these effects in CCA are summarized in [Figure 1](#).

Emphasizing the Warburg effect, studies suggest that mitochondrial dysfunction is essential in promoting tumor progression<sup>[77]</sup>, as several underlying mechanisms regarding deregulated cellular energetics are associated with this phenomenon. Oncogenes such as *HIF1* and tumor suppressor gene *TP53* impose a direct link toward mitochondrial activity. Thus, impairments of these genes due to metabolic reprogramming consequently alter normal cellular metabolism<sup>[78]</sup>. Firstly, increased expression of pyruvate dehydrogenase kinase 1 (PDHK1) inhibits the conversion of pyruvate to acetyl coenzyme A by phosphorylating the pyruvate dehydrogenase, resulting in less substrate entering the TCA cycle and thus repressing mitochondrial oxidative mechanism. Secondly, HK2, a prevalent isoform with the most enzymatic activity that catalyzes the conversion of glucose to G6P, can interact with voltage-dependent anion channels (VDACs) on the outer membrane of the mitochondria; it is this VDAC-HK2 interaction that gives cancer cells an anti-apoptotic property by blocking cytochrome c release into the cytoplasm, leading to no caspase activation. Thirdly, the upregulation of GLUTs, especially GLUT1 in CCA cells, which divert its metabolic flux from OXPHOS to glycolysis as a response to hypoxia and inhibition of mitochondrial respiration, may be explained by the increased activation of AMP-activated protein kinase (AMPK) upon decreased ATP production as a subsequent result of mitochondrial dysfunction. Moreover, the supplementation of glycolysis in CCA was also supported by the uptake of fructose, as reported in the study that showed the upregulation of GLUT5<sup>[79]</sup>.

In addition, SIRT3, a nuclear-encoded mitochondrial protein deacetylase, is shown to regulate several mitochondrial protein activities, including OXPHOS, and is thought to play an antitumor role. Loss of SIRT3 function results in mitochondrial dysfunction, increased intracellular reactive oxygen species (ROS) levels, and tumorigenesis. Interestingly, one study shows that decreased expression of SIRT3 corresponds to an increase in the glycolytic rate in CCA, highlighting an inverse correlation with HIF-1, therefore suggesting SIRT3 as a possible molecule exhibiting an anti-Warburg effect<sup>[80]</sup>. Another study also showed that high glucose levels can increase the levels of ROS, which promotes the aggressiveness of CCA<sup>[81]</sup>. Taken together, Warburg effects might be a linkage among these pro-tumorigenic pathways that enhance the aggressiveness of CCA cells and promote the disease progression.





**Figure 1.** Regulatory roles of HIF1 in the expression of genes associated with glycolysis in CCA. HIF1, especially a subunit HIF1- $\alpha$ , is a master regulatory protein that upregulates many genes encoding the enzymes and transporters that play roles in glucose metabolism, such as GLUT1, HK2, PFK1, PKM2, and GFAT. Glc: Glucose; G-6-P: glucose-6-phosphate; F-6-P: fructose-6-phosphate; F1,6BP: fructose-1,6-bisphosphate; PEP: phosphoenolpyruvate; G6PD: glucose-6-phosphate dehydrogenase; PPP: pentose phosphate pathway; HBP: hexosamine biosynthetic pathway; HIF1: hypoxia-inducible factor 1; CCA: cholangiocarcinoma; GLUT1: glucose transporter 1; HK2: hexokinase II; PFK1: phosphofructokinase 1; PKM2: pyruvate kinase isoform M2; LDHA: lactate dehydrogenase A; GFAT: glutamine fructose-6-phosphate amidotransferase.

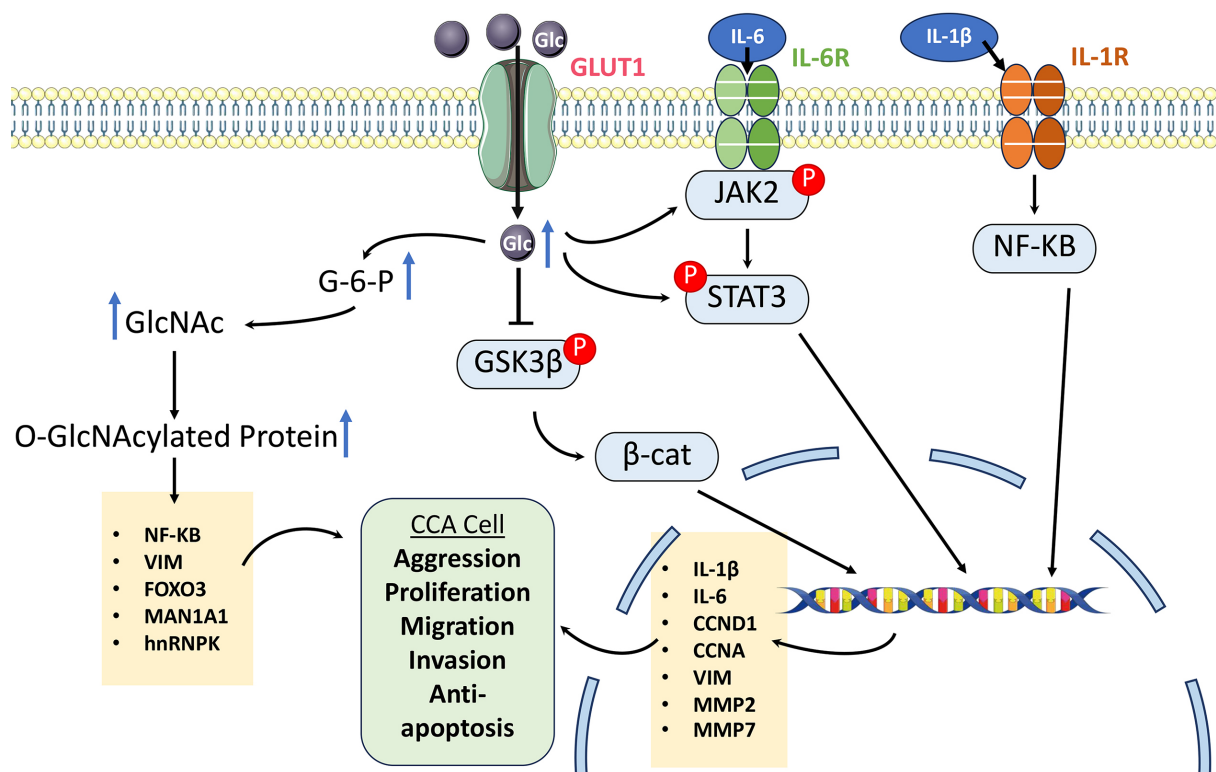
Another pathway that is demonstrated to inhibit apoptosis in CCA, thereby promoting cancer aggressiveness, is the SIRT2/cMYC pathway<sup>[82]</sup>. SIRT2 is classified as a class III histone deacetylase (HDAC), responsible for post-translational modification, an important step in controlling a variety of metabolic regulatory processes. There has been growing evidence that SIRT2 can promote malignant tumor transformation by upregulating its downstream target transcription factor-cMYC. Pyruvate is a known inhibitor of HDAC3, such as SIRT2. So, with a low sustained level of pyruvate due to overexpression of lactate dehydrogenase (LDH) and PKM2, SIRT2 is stabilized. This, in turn, allows SIRT2 to deacetylate cMYC, thereby inhibiting its ubiquitin degradation. Conversely, the mechanisms by which the SIRT2/cMYC pathway has been shown to contribute to metabolic reprogramming in CCA have been explored. A study using CCA cell lines revealed that these molecules, in coordination, target PDHA1 activity in response to site-specific phosphorylation, thus inhibiting the TCA and OXPHOS. The increased lactate secretion levels in the CCA cell line with overexpressed SIRT2 further reinforce the effect of this pathway on the Warburg effect. Alternatively, in a similar study, the SIRT2/cMYC pathway is also revealed to promote serine anabolic metabolism by increasing the conversion of glucose to serine via activation of the serine synthesis pathway (SSP). As aforementioned, the transformed glucose oxidative mechanism led to an increase in serine biosynthesis<sup>[82,83]</sup>. However, the specific role of SSP and its interplay with cancer has not been largely explored. Nevertheless, a recent study indicates that downstream activation of SSP plays a

crucial role in antioxidant production (T-GSH/GSSG/GSH) and anti-apoptotic effect in CCA cell lines. Serine is a non-essential amino acid, and its function in cancer progression has been assessed. It is shown that serine can act as the ROS scavenger, contributing to redox homeostasis. Consequently, this provides CCA with an anti-oxidative tumor microenvironment enhanced for growth and proliferation and protects CCA cells from oxidative stress-induced apoptosis. The inhibition of SSP is then a promising strategy to inhibit CCA cell growth, particularly by inhibiting a gain of benefit from metabolic reprogramming<sup>[84]</sup>. Collectively from these findings, the metabolic reprogramming functions of the SIRT2/cMYC pathway may attract potential attention for further anti-Warburg therapies.

## DIABETOGENIC GLUCOSE AND ITS NON-WARBURG'S ROLES IN CCA

In addition to being an energy source, glucose at a supraphysiological level may have other functions rather than a simple nutrient<sup>[37]</sup>. Some straightforward reasons are that glucose can be shunted into collateral pathways such as the PPP and the HBP, which are important for other biomolecule anabolisms<sup>[85]</sup>. The HBP is responsible for synthesizing an amino sugar called *N*-acetylglucosamine (GlcNAc), a precursor of an *O*-link GlcNAc glycosylation or *O*-GlcNAcylation<sup>[86]</sup>. The glycosylations of GlcNAc to proteins have several crucial biological functions, such as activation of the proteins, increasing protein stability, or even activating the *O*-GlcNAcylated proteins for cellular degradations. The changes in protein properties become significant for CCA development and progression when dysregulated *O*-GlcNAcylation occurs at tumor suppressors or oncoproteins<sup>[33,34,87]</sup>. High glucose level was shown to promote global *O*-GlcNAcylation in CCA cells by upregulating the expression of glutamine fructose-6-phosphate amidotransferase (GFAT)<sup>[33]</sup>, the rate-limiting enzyme in HBP, thus resulting in an increase in glucose flux for GlcNAc production in CCA cells. The increased GlcNAcylation of oncoproteins, namely nuclear factor- $\kappa$ B (NF- $\kappa$ B)<sup>[34]</sup>, vimentin<sup>[33]</sup>, (Forkhead Box O3) FOXO3<sup>[88]</sup>, mannosidase alpha class 1A member 1<sup>[88]</sup>, and heterogeneous nuclear ribonucleoprotein-K (hnRNP-K)<sup>[89]</sup>, have been reported for their involvement with aggressive phenotypes of CCA, e.g., proliferation, migration, and invasion [Figure 2]. Thus, glycosylations are another underlying mechanism of high glucose promoting the progression of CCA, which is non-negligible.

With unclear mechanisms, high glucose also affects the intracellular signaling pathways of CCA cells. High glucose levels activate the signal transducer and activator of transcription 3 (STAT3) pathways by increasing STAT3 phosphorylation and promoting nuclear translocation in CCA cell lines cultured in high glucose and tumor tissues from patients with CCA who had DM<sup>[37,38]</sup>. The activation of STAT3 in high glucose was also found to crosstalk with NF- $\kappa$ B by the upregulation of communicating cytokines, interleukin-6 (IL-6) and interleukin-1 $\beta$  (IL-1 $\beta$ ), which reciprocally activate STAT3 and NF- $\kappa$ B pathways<sup>[39,90]</sup>. The activation of STAT3 has been shown to have several aggressive consequences in CCA cells, such as increased proliferation and metastatic potential. Moreover, upregulated IL-1 $\beta$  in CCA cells under hyperglycemia was shown as one of the factors that promote CCA growth *in vitro* and *in vivo*<sup>[90]</sup>. High glucose also regulates other transcription factors, such as  $\beta$ -catenin<sup>[40]</sup>. By controlling several transcription factors, high glucose can upregulate many proteins that enhance CCA's aggressive phenotypes, i.e., cell cycle regulatory proteins<sup>[19]</sup>. Altogether, glucose in the hyperglycemic ranges is one factor that promotes CCA progression. Rather than being utilized by glycolysis, multiple pathways respond to the increased glucose levels, which leads to the higher aggression of CCA. Concerning these points, CCA patients with DM who have chronic hyperglycemia might then be prone to have a poorer prognosis than those with euglycemia<sup>[18,19]</sup>, as evidenced by the association between high-glucose-induced molecules and poorer survival of CCA patients<sup>[18,19]</sup>. The development of novel therapeutic agents should thus take the underlying DM and hyperglycemia into consideration as factors that might compromise the effectiveness of treatment. In addition, the development of any therapeutic agents that target high-glucose-induced molecules would help reverse the aggressive phenotypes of CCA cells in hyperglycemic conditions as reported by using static



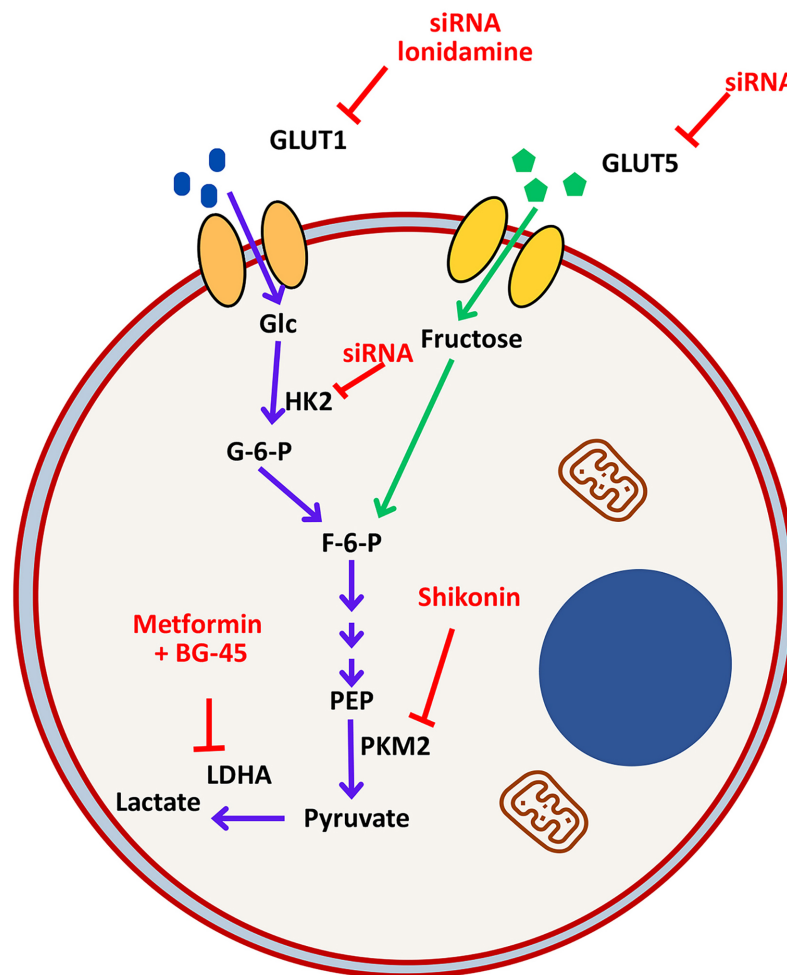
**Figure 2.** Non-energetic roles of glucose in the progression of CCA. Apart from being metabolized in glycolysis to yield ATP, glucose can be shunted to the hexosamine biosynthetic pathway to be a precursor for GlcNAc synthesis. GlcNAc is utilized in an O-GlcNAcylation, which alters the stability of several oncoproteins and hence promotes the aggressive phenotypes of CCA cells. On the other hand, glucose can activate several intracellular signaling pathways such as GSK3β/β-Cat, JAK2/STAT3, and NF-κB. Moreover, high glucose also upregulates IL-1β and IL-6, which are the upstream activators of those signaling pathways. Altogether, these mechanisms explain in part how diabetogenic glucose promotes CCA progression. However, the in-depth molecular mechanisms remain elucidated. *VIM*: Vimentin; *FOXO3*: Forkhead Box O3; *MAN1A1*: Mannosidase Alpha Class 1A Member 1; *hnRNP*K: Heterogeneous Nuclear Ribonucleoprotein K; *CCND1*: cyclin D1; *CCNA*: cyclin A; *MMP2*: matrix metalloproteinase-2; *MMP7*: matrix metalloproteinase-7; CCA: cholangiocarcinoma; GlcNAc: *N*-acetylglucosamine; O-GlcNAcylation: O-link GlcNAc glycosylation; GSK3β/β-Cat: glycogen synthase kinase-3β/β-catenin; JAK2/STAT3: Janus kinase 2/signal transducer and activator of transcription 3; NF-κB: nuclear factor-κB; IL-1β: interleukin-1β; IL-6: interleukin-6.

(STAT3 inhibitor)<sup>[37]</sup>, dehydroxymethylepoxyquinomicin (NF-κB inhibitor)<sup>[39]</sup>, and anakinra (IL-1 receptor antagonist), to inhibit CCA cell proliferation under diabetic condition in the *in vitro* and *in vivo* model<sup>[90]</sup>.

## OPPORTUNITIES FOR THERAPEUTIC DEVELOPMENT TARGETING GLUCOSE METABOLISM IN CCA

The current treatments of CCA in clinical practice are facing several limitations. The only highly effective and curative treatment for CCA is radical surgical resection. However, this curative treatment can be expected only when the tumor is localized, which is limited to the very early stage of CCA. Standard chemotherapeutic drugs can prolong the survival of patients in a few months, but most patients experience undesired adverse effects<sup>[2]</sup>. The approved targeted therapies against fibroblast growth factor receptor 2 (FGFR2) are effective only in patients with FGFR2 mutation, which is found to be approximately 15% worldwide and less than 1% in liver fluke-associated CCA<sup>[91]</sup>. Therefore, the research and development of therapeutic agents for CCA remains needed, and targeting metabolic reprogramming is one of the hopes to improve the therapeutic outcomes of the patients.





**Figure 3.** Potential CCA therapeutic targets based on glucose metabolism. Upregulation of genes involving glycolysis in CCA provides opportunities to develop targeted therapies. Previous reports showed that inhibiting the expression of GLUT1, GLUT5, and HK2 by siRNA, and LDHA by metformin combined with BG-45, significantly reduced CCA cell proliferation and induced cell apoptosis. Additionally, inhibition of the activities of GLUT1, HK2, and PKM2 by their inhibitor also retarded CCA cell growth. Glc: Glucose; G-6-P: glucose-6-phosphate; F-6-P: fructose-6-phosphate; PEP: phosphoenolpyruvate; CCA: cholangiocarcinoma; GLUT1: glucose transporters 1; GLUT5: glucose transporter 5; HK2: hexokinase II; LDHA: lactate dehydrogenase A; PKM2: pyruvate kinase isoform M2.

High glucose uptakes in CCA cells are correlated with the high expression of glucose transporter families such as GLUT1<sup>[30,31]</sup>, GLUT2<sup>[31]</sup>, and GLUT5<sup>[79]</sup>, which provide opportunities to develop therapeutic agents targeting these molecules. The gradual upregulation of GLUT1 was demonstrated along with cholangiocarcinogenesis in the liver-fluke-associated CCA in a hamster model<sup>[30]</sup>. High expressions of GLUT1 in tumor tissues from patients with CCA were also associated with shorter survival of patients<sup>[29,30]</sup>, and thus, silencing GLUT1 in CCA cell lines showed a significantly decreased CCA cell proliferation<sup>[30]</sup>. Apart from GLUT1, which is mainly responsible for glucose uptake, immunohistochemistry staining of CCA tissue microarrays and the study of CCA cell lines discovered significantly higher SLC2A5 (GLUT5) expression in CCA than in normal biliary epithelial cells<sup>[79]</sup>. Upon knocking down the expression of GLUT5 using siRNA, suppression of CCA cell proliferation was observed in a time-dependent manner, along with the inhibition of tumor migration and invasion. Since GLUT5 has a higher affinity to fructose than the other sugars, these results suggested the dependency on the fructose of CCA cells. Fructose is another monosaccharide that replenishes glycolysis by being metabolized to glycolytic intermediates. Targeting these GLUTs and glycolysis might be promising for developing CCA treatment.

HK2, the first rate-limiting step of glucose metabolism, is another potential target for drug development. Overexpression of HK2 in CCA tumor tissues was significantly associated with poor overall survival<sup>[68]</sup>. Using siRNA to silence the expression of HK2 in CCA cell lines resulted in a significant reduction in clonogenicity along with their migration capacity. The requirement of the Warburg effect in CCA was shown in CCA cells with high expression of PKM2, the isoform of pyruvate kinase that promotes glycolysis and the retention of glycolytic intermediate<sup>[18,69]</sup>. PKM2 depletion sensitized CCA cells, HCC9810 and RBE, to gemcitabine, a chemotherapeutic drug<sup>[92-94]</sup>. Using PKM2 inhibitors also exhibits significant antitumor effects on CCA cells<sup>[69]</sup>. On the contrary, another study by Tang *et al.* also shows that metformin can inhibit the Warburg effect in CCA cells<sup>[95]</sup>. Still, it exerts a minimal impact on inducing CCA cells to apoptosis. However, metformin, in combination with BG-45, a HDAC 3 inhibitor, significantly increased CCA cell apoptosis and decreased the expression of LDHA along with metabolite fluctuations. Some potential molecular targets that have been reported in CCA are summarized in [Figure 3](#).

Although promising results were obtained in the preclinical study, clinical trials of the agents targeting GLUT, glycolytic enzymes, and the associated molecules remained limited in CCA. This might result from a lack of a definite tumor-specificity of these enzymes, and then those agents may affect the normal, highly proliferative tissues<sup>[96]</sup>. A clinical trial phase I using metformin for *IDH1/2* mutation CCA also showed unfavorable results<sup>[97,98]</sup>. Similarly, clinical trials of GLUT1 and HK2 inhibitors in other cancers also showed unsatisfactory outcomes, and some were terminated<sup>[96]</sup>. A combination of devimistat, pyruvate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase inhibitors, with gemcitabine/cisplatin for CCA treatment, did not meet a satisfactory overall response rate<sup>[99,100]</sup>. Comparisons of the advantages and limitations of currently available treatment strategies and the proposed metabolic targeted therapy from the aforementioned part could be summarized in [Table 1](#). The limitation of less specific targeting by synthetic compounds may be overcome by using other strategies, such as the siRNA that could be designed to suppress the expression of specific or preferable isoforms of genes involving metabolic reprogramming, together with the development of effective delivery systems. To date, targeting glucose metabolism remains a significant challenge for developing cancer treatment, including CCA, which needs further investigations for a better outcome with minimal adverse effects. Some clinical trials are underway investigating targeting metabolisms in CCA, such as the inhibitors of IDH1 and IDH2<sup>[101]</sup>, as well as sphingolipid metabolism<sup>[102]</sup>.

## CONCLUSION

Like other cancers, metabolic reprogramming also occurs and benefits CCA's development and progression. High glucose consumption of CCA cells, favored with high blood glucose conditions in patients with DM, is part of the driving force for Warburg effects. On the other hand, high glucose also promotes CCA progression via a collateral metabolic pathway such as PPP and HBP. Moreover, high glucose can activate several pro-tumorigenic intracellular signaling pathways, resulting in aggressive phenotypes of CCA. The attempt to target molecules involving Warburg effects is promising in preclinical experiments. However, the success rate of clinical studies remains limited. Further development of therapeutic agents targeting the Warburg effects and related molecules remains a challenge that may require more efforts to overcome this metabolic dysregulation in CCA.

**Table 1. Comparisons of advantages and limitations among currently available treatments and proposed targeted metabolic treatment**

Therapeutic methods	Advantages	Limitations
Surgical removal of tumors	- Curative in the early, non-invasive stage	- Not applicable for patients with metastatic disease - Require a skillful hepato-biliary specialist for a radical curative resection
Chemotherapy	- Can be used for metastatic cancer - Widely available at an affordable cost	- Less specific to cancer cells, usually causes adverse effects for highly proliferative tissues - Prolong a few months survival time
FGFR targeted therapy	- Effective and highly specific targeting at FGFR fusion or rearrangements	- Only a small proportion (approximately 15%) of CCA patients possess this mutation - Fewer than 1% of liver-fluke-associated CCA cases possess this mutation
Targeted metabolic treatment	- Potential to develop tumor-specific isoforms - Theoretically useful for metastatic cancer - Developing small RNA targeting at the transcription level of metabolic genes could be a benefit	- Absolutely specific isoforms for tumors are rare; most metabolic genes expressed in cancer are expressed in highly proliferative tissues - Have not been extensively studied at clinical levels

FGFR: Fibroblast growth factor receptor; CCA: cholangiocarcinoma.

## DECLARATIONS

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All authors contributed to editorial changes in the manuscript.

All authors read and approved the final manuscript.

All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

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Not applicable.

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### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

**Consent for publication**

Not applicable.

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