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Targeting Glycolytic Reprogramming in Cholangiocarcinoma: A Novel Approach for Metabolic Therapy

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Abstract: Cholangiocarcinoma (CCA) is a highly aggressive and poorly prognostic tumor. Due to the lack of early symptoms, diagnosing CCA remains challenging, often occurring at an advanced stage. Therefore, exploring the underlying mechanisms of CCA development and identifying potential biomarkers and therapeutic targets is crucial. Recently, metabolic reprogramming in cancer cells has emerged as a hallmark of the disease. Glycolysis has been identified as a central component of metabolic reprogramming in CCA, with multiple signaling pathways and key enzymes playing significant roles. Additionally, non-coding RNAs (ncRNAs) and post-translational modifications of proteins are also involved in regulating glycolysis in CCA. In this review, we provide a comprehensive summary of the alterations in cancer metabolism and the diverse signaling pathways involved, as they might exert an impact on the development of CCA. Overall, targeting glycolysis holds considerable promise as a crucial strategy for enhancing the therapeutic outcomes of CCA. In addition, we performed a bioinformatic analysis of the relationship between CCA and glycolysis to identify and investigate potential targets. The purpose of this study is to provide a theoretical basis for the development of CCA targets.

Keywords: cholangiocarcinoma, glycolysis, HK2, PKM2, mechanisms

Introduction

Cholangiocarcinoma (CCA) is a malignant tumor that originates from the biliary epithelium.[1] Anatomically, CCA can be classified into two types: intrahepatic (iCCA) and extrahepatic (eCCA), occurring within or outside the liver, respectively. Extrahepatic cholangiocarcinoma can be further divided into perihilar CCA (pCCA) and distal CCA (dCCA).[2] Despite the advancements in surgical techniques and systemic therapies, the prognosis of CCA remains unfavorable due to late diagnosis, frequent recurrence, and resistance to conventional treatments. The complex molecular landscape of CCA, including various genetic and epigenetic alterations, underlies its high metastatic potential and poor response to treatment.

One hallmark of cancer is metabolic reprogramming,[3] and CCA is no exception. Tumor cells often shift their energy production from oxidative phosphorylation to glycolysis, even in the presence of oxygen-A phenomenon known as the "Warburg effect".[4] Glycolysis, the metabolic pathway that converts glucose into pyruvate while generating ATP, plays a critical role in supporting the rapid proliferation of cancer cells. This metabolic switch allows tumor cells to rapidly produce energy and generate the biosynthetic precursors necessary for macromolecule synthesis, facilitating tumor growth and survival under hypoxic conditions.[5] In CCA, the aberrant activation of glycolysis has increasingly been recognized as a driver of tumor progression and therapeutic resistance.[6] Key regulators of glycolysis, such as hexokinase 2 (HK2), and pyruvate kinase M2 (PKM2), are implicated in the metabolic alterations observed in CCA.[7,8] These

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enzymes are often modulated by non-coding RNAs (ncRNAs) and post-translational modifications (PTMs), including phosphorylation, ubiquitination, methylation, succinylation, and lactylation, all of which have a profound impact on protein function and cellular metabolism.[9]

Moreover, the regulation of glycolysis in CCA involves a complex network of oncogenic signaling pathways. These include HIF-1α/PDK1,[10,11] nuclear factor-κB (NF-κB),[12] phosphatidylinositol 3-kinase/protein kinase B/mammalian target of the rapamycin (PI3K/Akt/mTOR),[13] wingless-related integrated site (Wnt),[14] epidermal growth factor receptor (EGFR)/signal transducer and activator of transcription 3 (STAT3),[15] and transforming growth factor-β (TGF-β)/Smad2/3,[16] which collectively drive the metabolic adaptation. These pathways converge to promote a glycolytic phenotype that supports the energy and biosynthetic demands of proliferating CCA cells, further contributing to the aggressive nature of the tumor and poor clinical outcomes.

This review endeavors to investigate the role of glycolysis in the progression of cholangiocarcinoma, highlighting the key molecular drivers of this metabolic pathway and discussing potential therapeutic interventions targeting glycolytic enzymes and regulators. Comprehending the interplay between CCA and its metabolic alterations may provide valuable insights into novel treatment strategies for this devastating disease.

Epidemiology and Risk Factors

Epidemiology

CCA represents a rare yet highly aggressive malignancy within the biliary tract. In recent years, its global incidence has been on the rise. This is especially evident in regions where liver fluke infections are endemic, such as Southeast Asia and China.[17] Globally, the annual incidence of CCA varies from 0.3 to 6 cases per 100,000 inhabitants, while the mortality rate ranges from 1 to 6 per 100,000 inhabitants per year. In specific areas like Korea, China, and Thailand, the annual incidence rates surpass 6 cases per 100,000 inhabitants.[18] The geographical distribution of this disease shows marked variation, with Southeast Asia-especially northeastern Thailand-exhibiting significantly higher incidence rates due to infections with Opisthorchis viverrini.[19] In contrast, Western countries have lower incidence rates, though they are also gradually increasing, presumably due to changes in metabolic diseases and lifestyle factors.[20]

Risk Factors

With regard to risk factors, chronic biliary diseases such as primary sclerosing cholangitis are considered the most common causes of CCA in Western countries.[17] In Southeast Asia, parasitic infections, particularly with Opisthorchis viverrini and other liver flukes, represent the primary etiological factors.[21] Other associated conditions include intrahepatic bile duct stones, liver cirrhosis, chronic hepatitis B and C infections, and congenital biliary abnormalities such as Caroli disease.[22–24] Metabolic conditions, including metabolic syndrome, obesity, and diabetes, as well as long-term exposure to industrial chemicals like dichloroethane and vinyl chloride, have also been implicated as potential risk factors.[25] Like other tumors, CCA strongly depends on glucose metabolism. Fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) is the main diagnostic tool for staging and prognosis of CCA.[26] The glycolytic pathway plays a central role in CCA metabolism, as indicated by the deregulation of several enzymes. HK2 is upregulated in CCA tissue specimens, and its inhibition significantly reduces the invasiveness of CCA cells.[27] High levels of PKM2 are detected in tumor tissues of CCA patients, which is associated with a poor clinical prognosis. Moreover, PKM2 promotes the proliferation, migration and angiogenesis of CCA cells.[28] The highly invasive nature of CCA, coupled with its frequent late-stage diagnosis, results in poor prognosis, with a 5-year survival rate of typically less than 10%. Consequently, comprehending the epidemiological features and risk factors is of crucial importance for both disease prevention and for directing early screening and treatment approaches.

Metabolic abnormalities are closely related to the development of cholangiocarcinoma. Among them, diabetes, obesity, and NAFLD have interrelated risk factors. The pathogenic mechanisms are as follows: Excessive fat leads to excessive excretion of leptin, which promotes the growth of cholangiocarcinoma cells.[29] Excessive adipose tissue releases inflammatory cytokines, causing chronic liver inflammation, etc.,[30] leading to insulin resistance and type 2 diabetes.[23] Insulin resistance leads to hyperinsulinemia and increased production of insulin-like growth factor-1 (IGF-1)

in the liver.[31,32] The binding of IGF-1 to its receptor upregulates related genes.[33,34] The association between type 1 diabetes and cholangiocarcinoma may be related to the high prevalence of NAFLD in patients with type 1 diabetes.[35]

Altered Glucose Metabolism

In normal cells, glucose metabolism predominantly adheres to the aerobic respiration pathway (Figure 1). Glucose undergoes glycolysis to produce pyruvate, generating a small amount of ATP in the cytoplasm.[36] Subsequently, pyruvate enters the mitochondria, where it is converted to acetyl-CoA by the pyruvate dehydrogenase complex (PDH), entering the tricarboxylic acid (TCA) cycle and driving oxidative phosphorylation (OXPHOS) to efficiently produce a large amount of ATP. This process is reliant on oxygen, and the complete oxidation of one glucose molecule can yield up to 38 ATP molecules.[36] However, in cancer cells, a significant alteration occurs in glucose metabolism. Even in the presence of oxygen, cancer cells depend on glycolysis for energy production.[37]

Cancer cells display abnormally elevated rates of glucose uptake and glycolysis. Instead of pyruvate entering the mitochondria, the majority of it is converted to lactate in the cytoplasm by lactate dehydrogenase (LDH), and the lactate is then promptly expelled from the cell.[38] This metabolic reprogramming not only facilitates the rapid generation of ATP to sustain the high proliferation rate of cancer cells but also leads to the accumulation of glycolytic intermediates. These intermediates provide crucial building blocks for the synthesis of nucleotides, lipids, and amino acids.[39] Furthermore, the accumulation of lactate causes acidification of the tumor microenvironment, promoting cancer cell invasion and immune evasion. In CCA and various other cancers, LDH-particularly LDHA is significantly upregulated, further enhancing

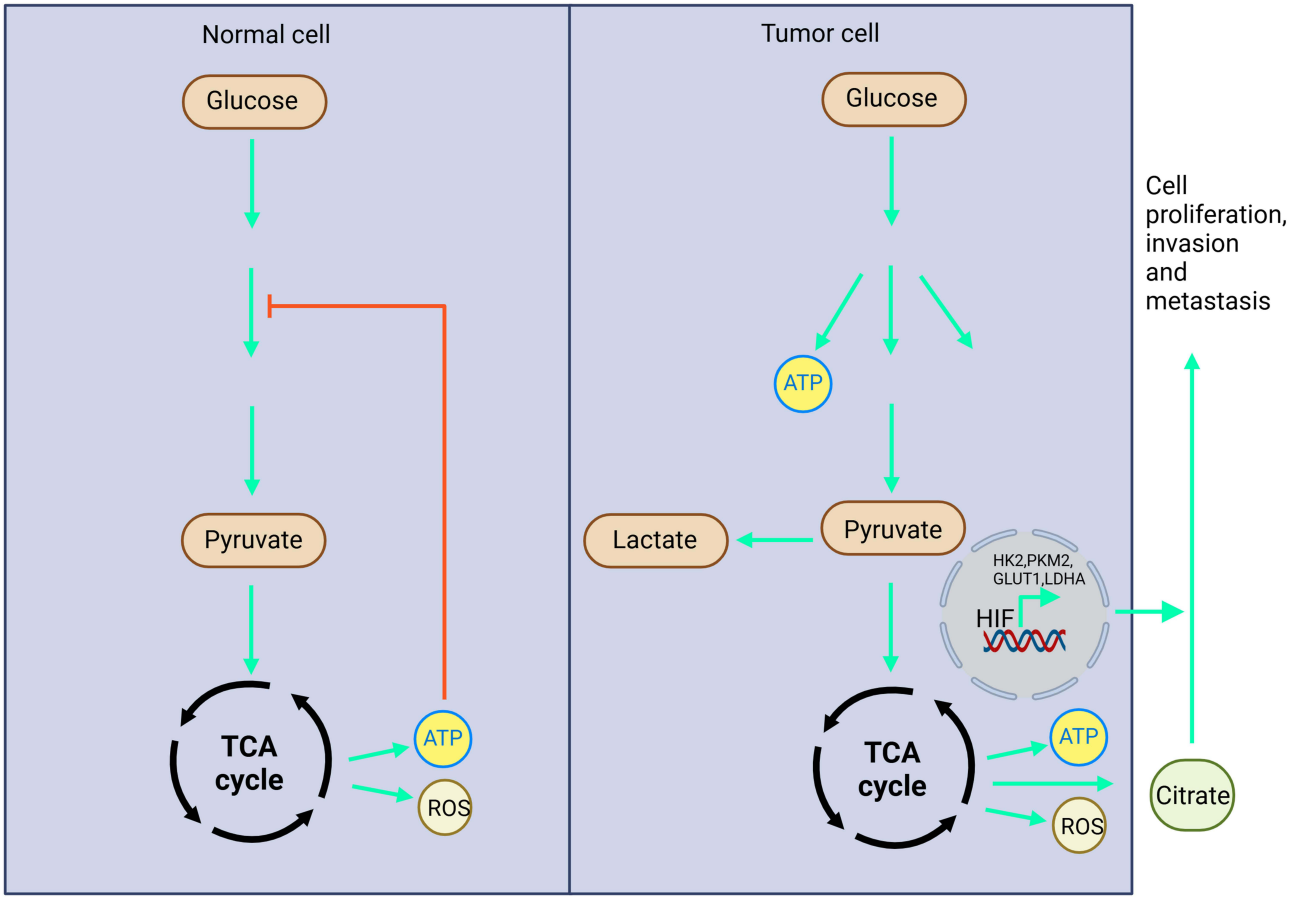

Figure 1 Glucose metabolism alterations in normal and tumor cells. In normal cells, glucose undergoes glycolysis and is thereby converted into pyruvate, which subsequently enters the tricarboxylic acid (TCA) cycle for the production of adenosine triphosphate (ATP) and reactive oxygen species (ROS). Conversely, in tumor cells, even in the presence of oxygen, glucose is predominantly converted into lactate, with a diminished involvement in the TCA cycle. Citrate is shuttled out from the TCA cycle to support anabolic processes that foster cell proliferation, invasion, and metastasis. This metabolic shift augments the production of ATP, thereby facilitating the survival and growth of cancer cells. Created in BioRender. Hu, X. (2024) https://BioRender.com/y99v474.

glycolytic activity.[40] This metabolic adaptation enables cancer cells to grow rapidly in diverse oxygen conditions and presents a promising target for metabolic-based cancer therapies. For example, research has found that key glycolytic enzymes such as LDHA, HK2 and PKM2 are upregulated in CCA cells, promoting glucose metabolism and lactate production.[40–42] Furthermore, inhibiting the expression of these key enzymes can effectively reduce the proliferation and migration of CCA cells, suggesting that targeting the glycolysis pathway may offer a new therapeutic strategy for CCA.

Glycolysis Reprogramming in Cholangiocarcinoma

Alterations in Key Glycolytic Enzymes

The activity of key enzymes in the glycolytic pathway plays a crucial role in metabolic reprogramming of tumors and is closely associated with the initiation and progression of various malignancies. HKs are the first rate-limiting enzymes in glycolysis, catalyzing the initial step of glucose utilization.[43] Among them, HK2 is predominantly expressed in insulin-sensitive tissues such as the heart, skeletal muscles, and adipose tissues. Moreover, HK2 is upregulated in many tumor types exhibiting enhanced aerobic glycolysis.[44] In iCCA, HK2 promotes tumor migration and invasion by enhancing cancer stem cell-like properties and resistance to anoikis.[8] Additionally, the dual inhibition of fibroblast growth factor receptor (FGFR) and vascular endothelial growth factor receptor (VEGFR) has been demonstrated to synergistically suppress HK2-dependent lymphangiogenesis and immune evasion in iCCA.[45]

Pyruvate kinase (PK), which serves as the final rate-limiting enzyme in glycolysis, catalyzes the conversion of phosphoenolpyruvate to pyruvate while simultaneously generating ATP.[46] There are four isoforms of PK: L, R, M1, and M2.[47] PKM2 is the isoform that is highly expressed in cancers. It regulates aerobic glycolysis by reprogramming the metabolic pathways of cancer cells, thereby conferring a metabolic advantage for tumor growth.[47] In iCCA, the high expression of PKM2 promotes cell proliferation, migration, and invasion, leading to poor prognosis.[28] Targeting PKM2 has been shown to enhance the sensitivity of iCCA cells to gemcitabine by inhibiting the β-catenin signaling pathway.[48] Additionally, in iCCA patients with diabetes, the expression of PKM2 is even more pronounced, and this upregulation is associated with reduced overall survival and disease-free survival rates.[49] Both hypoxia-inducible factor-1 α (HIF-1α) and PKM2 are significantly upregulated in human CCA QBC939 cells, playing critical roles in elevating glycolytic activity in CCA.[50] Leptin stimulates epithelial-mesenchymal transition (EMT) and pro-angiogenic capabilities of CCA cells through the miR-122/PKM2 axis. Therefore, increasing miR-122 expression and inhibiting PKM2 may serve as potential therapeutic approaches for cholangiocarcinoma in the future.[51]

LDHA is a key enzyme in the glycolytic pathway, responsible for converting pyruvate to lactate while oxidizing NADH.[52] LDHA is overexpressed in various types of tumors, including cervical cancer,[53] breast cancer,[54] and pancreatic cancer[55], and its upregulation is closely associated with the initiation and progression of iCCA. Studies have demonstrated that LDHA overexpression correlates with decreased survival in iCCA patients.[40] Knockdown of LDHA significantly inhibits proliferation, migration, and tumor growth in iCCA cell lines.[56] Furthermore, downregulation of LDHA expression has been shown to suppress the proliferation of CCA Hucct1 cells and enhance apoptosis.[57]

The glucose transporter isoform 1 (GLUT1) gene encodes a key rate-limiting factor responsible for transporting glucose into cancer cells.[58] Furthermore, the high expression of GLUT1 is significantly associated with non-papillary tumors, larger tumor size, and shorter overall survival.[59] KRAS mutations are associated with GLUT1 expression and volumetric parameters of 18F-FDG-PET in iCCA tumors. KRAS mutations affect the prognosis of iCCA patients undergoing surgical resection and are linked to tumor glucose uptake.[26]

Aldolase A (ALDOA) is a critical enzyme in the glycolytic pathway, catalyzing the conversion of fructose-1,6-bisphosphate to glyceraldehyde 3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP).[60] During the progression of ICC, ALDOA mainly exerts a regulatory role in tumor progression relying on its enzymatic activity. High expression of ALDOA in iCCA is closely associated with tumor malignancy and poor prognosis. Knocking down its expression inhibits iCCA cell proliferation and migration, while high expression promotes them. Studies show ALDOA regulates iCCA cell biology and metabolism via its enzyme activity. Blocking this activity can be a strategy to inhibit iCCA as it promotes iCCA proliferation and migration through enhanced glycolysis.[61,62] Reducing ALDOA expression

or inhibiting its enzymatic activity can significantly suppress tumor progression. Accelerated glycolysis is one of the biochemical characteristics of cancer cells.

The pyruvate dehydrogenase complex (PDC) irreversibly decarboxylates pyruvate into acetyl-CoA through decarboxylation, thereby connecting glycolysis to the tricarboxylic acid (TCA) cycle. The phosphorylation of PDC, which results in the inhibition of its activity, has been witnessed in several types of cancers. In patients with iCCA, elevated serum levels of pyruvate dehydrogenase kinase 3 (PDK3) are significantly associated with shorter survival durations.[63] Studies have shown that three glycolysis/gluconeogenesis-related enzymes, triosephosphate isomerase 1 (TPI1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and phosphoglycerate kinase 1 (PGK1), are significantly downregulated in the large-duct type (LD-type) of iCCA. The knockdown of these proteins mediated by small interfering RNA (siRNA) led to a significant increase in the proliferation of two cholangiocarcinoma cell lines. This suggests that the effective downregulation of glycolysis and gluconeogenesis might represent a novel mechanism underlying the development of LD-type iCCA.[64] Glycolysis and gluconeogenesis-related genes (G6PC, TPI1, ALDH1B1, PGAM1, etc.) are significantly correlated with the maximum standard uptake value (SUVmax). A high SUVmax value is usually associated with the high metabolic activity of tumors. The expression changes of genes related to glycolysis and gluconeogenesis may affect the energy metabolism process of cells. The significant correlation between these genes and SUVmax probably implies that they play a crucial role in regulating the uptake and utilization of glucose by cells. For example, if the expression of these genes is upregulated, it may lead to an increased uptake of glucose by cells, thereby presenting a higher SUVmax value in PET scans. This may provide important clues for the early diagnosis, disease assessment, and treatment monitoring of tumors.[65]

In conclusion, alterations in the activity of key glycolytic enzymes play a crucial role in iCCA, and targeting these metabolic enzymes may offer new therapeutic approaches and potential treatment targets for iCCA.

Non-Coding RNA

Non-coding RNAs (ncRNAs) represent a diverse family of molecules that regulate gene expression, including long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and circular RNAs (circRNAs).[66] Dysregulated expression of ncRNAs can significantly alter gene expression, contributing to the pathogenesis of malignant tumors and inflammatory diseases. For instance, in intrahepatic cholangiocarcinoma (iCCA), circ_0000284 acts as an oncogene by sponging miR-152-3p, which in turn upregulates PDK1 expression, promoting iCCA growth, metastasis, and glycolysis.[67] Similarly, the loss of hsa_circ_0019054 suppresses iCCA cell proliferation and glycolysis by targeting the miR-340-5p/HIF1A axis, while inducing apoptosis in iCCA cells.[68] Another ncRNA, FAM66C, drives iCCA tumor progression and enhances glycolytic activity through the miR-23b-3p/KCND2 axis. FAM66C upregulates KCND2 by adsorbing miR-23b-3p, and KCND2 is highly expressed in iCCA tissues and cells. This process promotes the proliferation, migration, and invasion of iCCA cells, and the alterations in these cellular functions are often associated with enhanced glycolysis. Although there is no direct evidence indicating that KCND2 alone affects glycolysis, within the FAM66C-miR-23b-3p-KCND2 regulatory axis, as a key regulated node, the high expression of KCND2, along with FAM66C promoting cellular glycolysis and other processes, implies that KCND2 may be indirectly involved in the glycolysis process or have a synergistic relationship with it in this complex regulatory network, thereby facilitating the metabolism and proliferation of iCCA cells.[69] Through the integrated analysis of the matched miR and transcriptome data, it was found that 9925 genes were significantly correlated with the expression of miR-27a-3p. Among these targets, there were 12 known targets that were negatively correlated with the expression of miR-27a-3p. MiR-27a-3p affects cell metabolism by regulating the FoxO signaling pathway. After the knockout of miR-27a-3p in HuCCT-1 cells, the expression of FOXO1 increased, the mitochondrial function decreased, the dependence on glycolysis increased, and the glycolysis efficiency of the knockout cells was lower than that of the wild-type CCA cells.[70] Collectively, these findings underscore the crucial roles that ncRNAs play in modulating glycolysis and tumor progression in iCCA. Targeting specific ncRNAs involved in these pathways may present promising therapeutic strategies for controlling iCCA progression. Thus, the dysregulation of ncRNAs, which orchestrates key metabolic pathways such as glycolysis, emerges as a pivotal mechanism in iCCA development and offers novel insights into potential targets for clinical intervention.

Protein Post-Translational Modifications

Protein post-translational modifications (PTMs) have a profound impact on protein function and play a critical role in nearly all cellular biological processes, including phosphorylation, ubiquitination,[71] methylation, succinylation, and lactylation.[72] Ribosomal protein S6 can be phosphorylated by ribosomal protein S6 kinase (70S6K) after mTORC1 is activated, and then 70S6K phosphorylates ribosomal protein S6. Phosphorylation of S6 serves as an important indicator of mTORC1 activation. In the study, the activity of mTORC1 was reflected by detecting the phosphorylation status of S6 through immunohistochemistry.[73,74] For instance, inhibiting S6 phosphorylation can suppress the glycolytic pathway and the TCA cycle in cell lines, resulting in a significant reduction in cell proliferation.[75] Moreover, the ubiquitin-proteasome system (UPS), responsible for intracellular protein degradation, when abnormally activated, accelerates protein breakdown within cells.[72] The UPS can influence tumor cell survival by either promoting the degradation of tumor suppressor proteins or preventing the degradation of oncogenic proteins.[72] Ubiquitin-specific protease 21 (USP21) stabilizes HSP90 through deubiquitination and directly or indirectly enhances aerobic glycolysis in iCCA cells by regulating HIF1A degradation and stabilizing ENO1.[6] Additionally, methyltransferase like 3 (METTL3)-mediated transcription can activate m6A modification on nuclear factor of activated T cells 5 (NFAT5) mRNA, allowing the m6A reader insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) to bind to these m6A sites, thereby maintaining NFAT5 mRNA stability. Consequently, NFAT5 activates GLUT1 and PGK1 expression, boosting glycolytic activity in iCCA cells and promoting disease progression, leading to poor clinical outcomes.[76] Elevated METTL3 expression also promotes CCA tumor growth and glycolysis through m6A modification of Aldo-keto reductase 1B10 (AKR1B10), suggesting that METTL3 is a potential therapeutic target for inhibiting glycolysis in CCA.[77] Furthermore, HAT1-mediated succinylation of histone H3 at K122 participates in the epigenetic regulation of gene expression in tumor cells.[78] Histone AcetylTransferase1 (HAT1) also catalyzes the succinylation of PGAM1 at K99, which enhances its enzymatic activity and stimulates glycolytic flux in cancer cells.[78] In another pathway, lactylated nucleolin (NCL) binds to the primary transcript of MAP kinase-activating death domain protein (MADD), ensuring efficient translation by preventing premature termination codons. This modification promotes extracellular signal-regulated kinase (ERK) activation and tumor xenograft growth and is associated with overall survival in iCCA patients.[79]

Various Carcinogenic Signaling Pathways Involved in Glycolysis

In CCA, the regulation of glycolysis involves multiple oncogenic signaling pathways, including HIF-1α/PDK1, phosphatidylinositol 3-kinase/protein kinase B/mammalian target of the rapamycin (PI3K/Akt/mTOR), nuclear factor-κB (NF-κB), wingless-related integrated site (Wnt), epidermal growth factor receptor (EGFR)/signal transducer and activator of transcription 3 (STAT3), and transforming growth factor-β (TGF-β)/Smad2/3. These pathways promote glycolysis and metabolic reprogramming, providing energy and growth advantages to tumor cells.

HIF-1α/PDK1/PDHA1

SIRT3, a mitochondrial deacetylase, is involved in regulating cellular metabolism and energy balance.[80] Its role in cancer cells through inhibition of the Warburg effect has been extensively studied. In iCCA, SIRT3 exerts anti-cancer effects by suppressing the HIF-1α/pyruvate dehydrogenase kinase 1 (PDK1)/pyruvate dehydrogenase (PDHA1) pathway.[10] Under hypoxic conditions, HIF-1α is upregulated, inducing PDK1 expression.[11] PDK1 inhibits PDHA1 activity, reducing pyruvate entry into the tricarboxylic acid (TCA) cycle, thus driving cells toward anaerobic glycolysis.[10,81] SIRT3 inhibits both HIF-1α and PDK1, restoring PDHA1 activity, promoting pyruvate oxidation, reducing glycolysis, and suppressing rapid tumor cell proliferation. Thus, SIRT3 plays a key role in suppressing the Warburg effect in CCA cells.[10]

PI3K/AKT/mTOR

The PI3K/AKT/mTOR pathway plays a crucial role in various cancers, regulating cell growth, proliferation, and survival.[82] Its dysregulation in CCA drives tumor progression and metabolic shifts.[83] In CCA, overexpression of mutant IDH2 inhibits the PI3K/AKT pathway, reducing glycolysis and increasing dependence on mitochondrial oxidative phosphorylation, thus suppressing tumor growth.[13] Additionally, protein regulator of cytokinesis 1 (PRC1) silencing inhibits tumor growth by suppressing glycolysis and mTORC1 signaling.[84]

NF-κB

The NF-κB pathway, frequently activated in cancers, regulates aerobic glycolysis and supports tumor cell proliferation.[85] In FGFR2 fusion-driven iCCA, NF-κB activation drives glycolysis, and inhibiting NF-κB impairs tumor cell growth.[12] By regulating glycolysis-related gene expression, NF-κB enables tumor cells to meet high metabolic demands.[12] Inhibition of FGFR2 or NF-κB disrupts glucose metabolism, limiting cancer cell growth and survival, positioning NF-κB as a crucial metabolic regulator and therapeutic target in iCCA.[12]

Wnt

The classical Wnt pathway plays a role in promoting aerobic glycolysis, thereby exerting an influence on the development and progression of cancer.[86] The overexpression of FEN1 plays an important role in CCA progression. It promotes cell proliferation, invasion, migration, DNA damage repair, and glycolysis, potentially via the Wnt/β-catenin signaling pathway.[14] Similarly, USP3 promotes cholangiocarcinoma proliferation and invasion through the Wnt/MYC pathway, enhancing tumor cell glycolysis, energy production, and upregulating metabolic target genes such as GLUT1, HK2, PDM2, and LDHA.[87]

Egfr/Stat3

The EGFR/STAT3 signaling pathway plays a critical role in regulating cell proliferation and survival, particularly in various cancers.[88] NNMT activates the EGFR/STAT3 axis by reducing the levels of H3K9me3 and H3K27me3 in the EGFR promoter region, driving glycolysis and promoting iCCA cell proliferation[15].

aPKC-Iota/P-Sp1/Snail

In cholangiocarcinoma, the atypical protein kinase C-iota (aPKC-i)/Ser59-phosphorylated specificity protein 1 (P-Sp1)/Snail signaling induces immunosuppressive natural T-cell regulation, contributing to immune suppression.[89] Atypical protein kinase C-iota (aPKC-iota) is highly expressed in primary and metastatic iCCA tissues and regulates EMT via the aPKC-iota/P-Sp1/Snail signaling pathway. The aPKC/Snail-mediated inhibition of fructose-1,6-bisphosphatase (FBP1) enhances the invasion and aerobic glycolysis of iCCA[90].

TGF-β/Smad2/3

The TGF-β/Smad2/3 signaling pathway plays an important role in cancer development. It is closely associated with glycolysis by enhancing tumor cell glucose metabolism, and supporting tumor growth and dissemination.[91,92] E-twenty-six-specific sequence variant 4 (ETV4) significantly promotes CCA growth, migration, invasion, and glycolysis by activating the TGF-β/Smad2/3 signaling pathway, confirming its oncogenic role in CCA[16].

These signaling pathways promote glycolysis and metabolic reprogramming, providing energy and growth advantages to tumor cells. Among them, SIRT3 exerts anti-cancer effects by inhibiting the Warburg effect, while FGFR2 fusion, FEN1, and USP3 promote aerobic glycolysis through the activation of NF-κB and Wnt pathways. Inhibition of these key signaling pathways has been shown to significantly suppress iCCA growth and invasion, making them potential therapeutic targets.

The Role of Targeting Glycolysis in Improving Cholangiocarcinoma Treatment

The Role of Glycolysis in Cholangiocarcinoma Proliferation

Glycolysis plays a crucial role in the proliferation of CCA. The regulatory network of key proteins, enzymes and natural products involved in glycolysis in cancer cells (Figure 2). Studies have shown that the mRNA and protein levels of DPY30 are significantly elevated in CCA tissue.[93] This upregulation is closely associated with pathological differentiation, tumor size, and TNM staging, making it an independent unfavorable prognostic factor for overall survival (OS).[93] Notably, knocking down the DPY30 gene induces G2/M cell cycle arrest, significantly inhibiting CCA cell proliferation and reducing glycolysis.[93] In addition, enoyl-coenzyme A hydratase short chain 1 (ECHS1) inhibits the Warburg effect in CCA QBC939 cells by downregulating PKM2 protein expression, which in turn suppresses CCA proliferation.[94] Retinoic acid receptor alpha (RARα) exhibits oncogenic functions in CCA by promoting cell growth, proliferation,

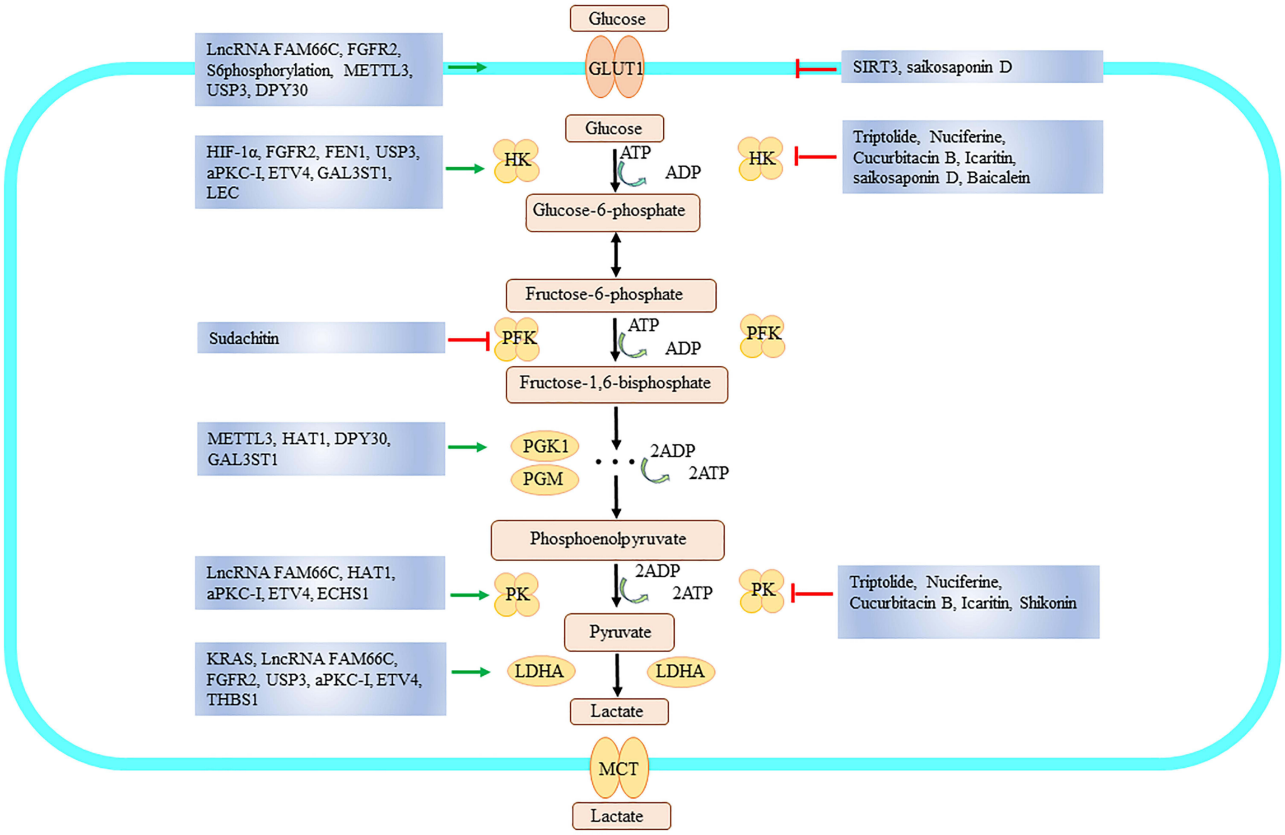

Figure 2 Regulatory network of glycolysis in cancer cells: involvement of key proteins, enzymes, and natural products. Glucose gains access to the cell via GLUT1 and is subsequently phosphorylated by hexokinase (HK) to form glucose-6-phosphate. Subsequent steps, mediated by phosphofructokinase (PFK) and pyruvate kinase (PK), drive the conversion of glucose to pyruvate, which is then metabolized to lactate by lactate dehydrogenase A (LDHA). Multiple molecules modulate glycolysis at various stages, thereby promoting cancer cell proliferation and survival. Additionally, natural products including triptolide, nuciferine, and baicalein have been demonstrated to act as inhibitors of critical glycolytic enzymes, thus presenting potential therapeutic approaches for targeting cancer metabolism. The lactate produced is transported out of the cell via the monocarboxylate transporter (MCT), contributing to the formation of an acidic tumor microenvironment. This diagram effectively highlights the interplay between metabolic regulation and oncogenic signaling, providing valuable insights into targeted therapies aimed at disrupting the glycolytic flux within cancer cells.

and aerobic glycolysis, as well as reducing drug sensitivity.[95] On the other hand, RARγ regulates the target protein AKR1C1, a member of the aldo-keto reductase family. Inhibiting AKR1C1 activity suppresses aerobic glycolysis and proliferation of CCA cells.[96] Furthermore, mitochondrial transplantation has been shown to inhibit CCA cell growth by suppressing aerobic glycolysis.[97] In CCA cells treated with metformin, branched-chain amino acids (BCAAs) are significantly enhanced. This is because metformin can inhibit the expression or activity of BCAAs catabolic enzymes[98] and significantly promote the inhibition of mTOR,[99] directly leading to autophagy activation.[100] The levels of BCAAs remained basically unchanged in HUVEC cells but were significantly enhanced in CCA cells treated with metformin. Since BCAAs are essential amino acids that cannot be endogenously synthesized, and their metabolic enzymes are inhibited by metformin, along with metformin's ability to inhibit mTOR, directly leading to autophagy activation, the accumulation of BCAAs in CCA cells may be due to autophagy-induced protein degradation.[98–102] Additionally, various compounds exert antitumor effects by inhibiting glycolysis through the Akt/mTOR signaling pathway. For instance, Sudachitin, a polymethoxylated flavonoid found in citrus peels, shows significant biological activity. It has been demonstrated to inhibit the proliferation of various tumor cells in vitro, and by reducing glycolytic activity in cancer-associated fibroblasts (CAFs), it impedes tumor progression.[103,104] Moreover, Triptolide, an active compound isolated from *Tripterygium wilfordii Hook F.*, exhibits antitumor and anti-inflammatory properties.[105] It suppresses iCCA growth by inhibiting glycolysis through the Akt/mTOR pathway, offering a potential therapeutic target for iCCA.[106] Similarly, nuciferine, an aporphine alkaloid derived from lotus leaves, has various pharmacological activities. It potentially inhibits HuCCT1 cell proliferation by modulating glycolysis through the Akt/mTOR/4EBP1

signaling cascade.[107,108] Furthermore, Cucurbitacin B, a natural compound extracted from the gourd family and the main active ingredient in the traditional Chinese medicine "Hulusupi", has been widely used in clinical cancer treatments.[109] It likely inhibits glycolysis in HuCCT1 cells via the Akt/mTOR pathway, thereby impacting cell proliferation.[110] Finally, Icaritin, an active component from the traditional Chinese herb Epimedium, exhibits antitumor effects by inhibiting proliferation and promoting apoptosis.[111] It may suppress intrahepatic cholangiocarcinoma cell proliferation by blocking glycolysis via the Akt/mTOR pathway, depleting energy supplies, and arresting cells in the G1 phase.[112] Shikonin significantly inhibits PKM2, suppresses the growth and migration of CCA cells, and induces their apoptosis[113].

The Role of Glycolysis in Cholangiocarcinoma Migration and Metastasis

Glycolysis plays a crucial role in the metabolic reprogramming of CCA during the process of metastasis. Prolonged lactic acidosis (LA) induces a shift in the metabolic phenotype of CCA cells from glycolysis to oxidative metabolism, thereby augmenting their migratory capacity.[114] It was demonstrated that long-term LA (LLA) reprograms the metabolic phenotype of CCA cells from glycolysis to oxidation and enhances their migratory activity. Coincidentally, LLA enhanced respiratory capacity along with an increase in mitochondrial mass. Inhibition of mitochondrial function abolished LLA-induced cell motility, indicating that metabolic remodeling affects phenotypic outcomes. RNA sequencing analysis revealed that LLA upregulated genes related to cell migration and epithelial-mesenchymal transition (EMT), including thrombospondin-1 (THBS1) that encodes a pro-EMT secreted protein. Moreover, high THBS1 expression was associated with poor survival rates in CCA patients.[114] Studies have shown that CCA cells lacking GAL3ST1 exhibit lower levels of EMT and tumorigenic ability, despite generating comparable amounts of ATP. However, their oxygen consumption and glycolytic capacity are significantly reduced.[115] Moreover, the overexpression of peroxisome proliferator-activated receptor γ coactivator-1α (PGC1α) promotes metastasis both in vitro and in vivo in CCA cells.[116] PGC1α reverses the Warburg effect by upregulating the expression of pyruvate dehydrogenase E1 alpha 1 subunit and mitochondrial pyruvate carrier 1, increasing the oxidative flux of pyruvate into mitochondria, and enhancing mitochondrial biogenesis and fusion, thereby shifting metabolism toward oxidative phosphorylation.[116] This metabolic reprogramming provides crucial energy support for CCA metastasis.[116] Inflamed lymphatic endothelial cells (LECs) produce high levels of the chemokine CXC motif chemokine ligand 5 (CXCL5), which signals through its receptor CXCR2 on CCA cells.[117] Subsequently, the CXCR2-CXCL5 signaling axis activates EMT, facilitating CCA migration and invasion.[117] ETV4 is upregulated in CCA epithelial cells. Its high expression is associated with poor prognosis in CCA patients. Overexpression of ETV4 enhances the proliferation, migration, invasion and glycolysis of CCA cells; while ETV4 silencing has the opposite effects. Mechanistically, ETV4 activates the TGF-β/Smad2/3 signaling pathway.[16] Additionally, inflammation in LECs and CXCL5 activation significantly upregulate mitochondrial respiration and glycolytic rates in CCA cells, indicating metabolic reprogramming.[117] Baicalein, a primary flavonoid extracted from *Scutellaria baicalensis*, exhibits anticancer effects against various malignancies.[118] Baicalein inhibits cell proliferation and migration and reduces glycolysis by suppressing the activation of the AKT/NF-κB and STAT3 signaling pathways, demonstrating potent anticancer activity[119].

Glycolysis in Cholangiocarcinoma Drug Resistance

The regulation of glycolysis plays a critical role in the drug resistance of CCA. Studies have shown that uncoupling protein 2 (UCP2) is upregulated in human CCA and is associated with poor prognosis. Inhibition of UCP2 suppresses glycolysis, cell proliferation, migration, and spheroid growth, reverses EMT, and reduces drug resistance in CCA cells.[120] Blocking placenta growth factor (PIGF) can reduce desmoplasia, decrease tissue stiffness, restore tumor vessel permeability, improve blood perfusion, reduce intratumoral hypoxia, inhibit metastatic spread, and ultimately enhance chemosensitivity and prolong survival in ICC-bearing mice. Single-cell RNA sequencing revealed that the major effect of PlGF blockade in mice was the enrichment of quiescent CAFs, which were characterized by high gene transcription levels related to the Akt pathway, glycolysis, and hypoxia signaling.[121] High glycolysis levels may be related to the chemoresistance of cholangiocarcinoma cells. Dichloroacetate (DCA), a specific inhibitor of PDK, can promote the mitochondrial aerobic oxidation process by activating PDH. DCA changes the metabolic pattern of cholangiocarcinoma cells from glycolysis to aerobic oxidation under cisplatin stress. This metabolic reprogramming increases the level of

mitochondrial reactive oxygen species (mtROS), thus promoting cell cycle arrest, increasing the expression of antioxidant genes and activating autophagy. Inhibiting autophagy further enhances the synergistic effect of DCA and cisplatin.[122] Saikosaponin D enhances the antitumor effect of gemcitabine by inhibiting ADRB2 signaling, regulating glucose metabolism, and drug efflux.[123] Furthermore, in a chemically-induced cholangiocarcinoma rat model, treatment with gemcitabine plus the mTOR inhibitor rad001 effectively suppressed tumor glycolysis.[124] These findings suggest that the regulation of glycolysis holds significant therapeutic potential in overcoming drug resistance in CCA.

Bioinformatic Analysis

In order to explore and verify the target of glycolysis in CCA, we performed bioinformatics analysis. We use GEO (https://www.ncbi.nlm.nih.gov/geo/)[125] database to screen CCA targets. The GEO2R was used to identify DEGs. |log FC| ≥ 1 and adjust *P*-value < 0.05 were considered statistically significant. Gene expression profiles of CCA tissues were obtained from GSE26566, GSE45001, and GSE107943. The data of each gene expression profile was visualized by a volcano plot (Figure 3A). Then we apply The Molecular Signatures Database (MSigDB, https://www.gsea-msigdb.org/gsea/msigdb)[126] to further screen targets for glycolysis. The results showed that the BIOCARTA_GLYCOLYSIS_PATHWAY had 3 targets, HALLMARK_GLYCOLYSIS had 200 targets, KEGG_GLYCOLYSIS_GLUCONEOGENESIS had 62 targets, REACTOME_GLYCOLYSIS had 72 targets, GOBP_GLYCOLYTIC_PROCESS_THROUGH_FRUCTOSE_6_PHOSPHATE had 19 targets, REACTO ME_REGULATION_OF_GLYCOLYSIS_BY_FRU WP_GLYCOLYSIS_AND_GLUCONEOGENESIS had 45 targets. Subsequently, 188 overlapping genes were screened using Venn online (Figure 3B). Initially, the genes were uploaded to the STRING (https://cn.string-db.org/)[127] to generate a protein-protein interaction (PPI) network. We set

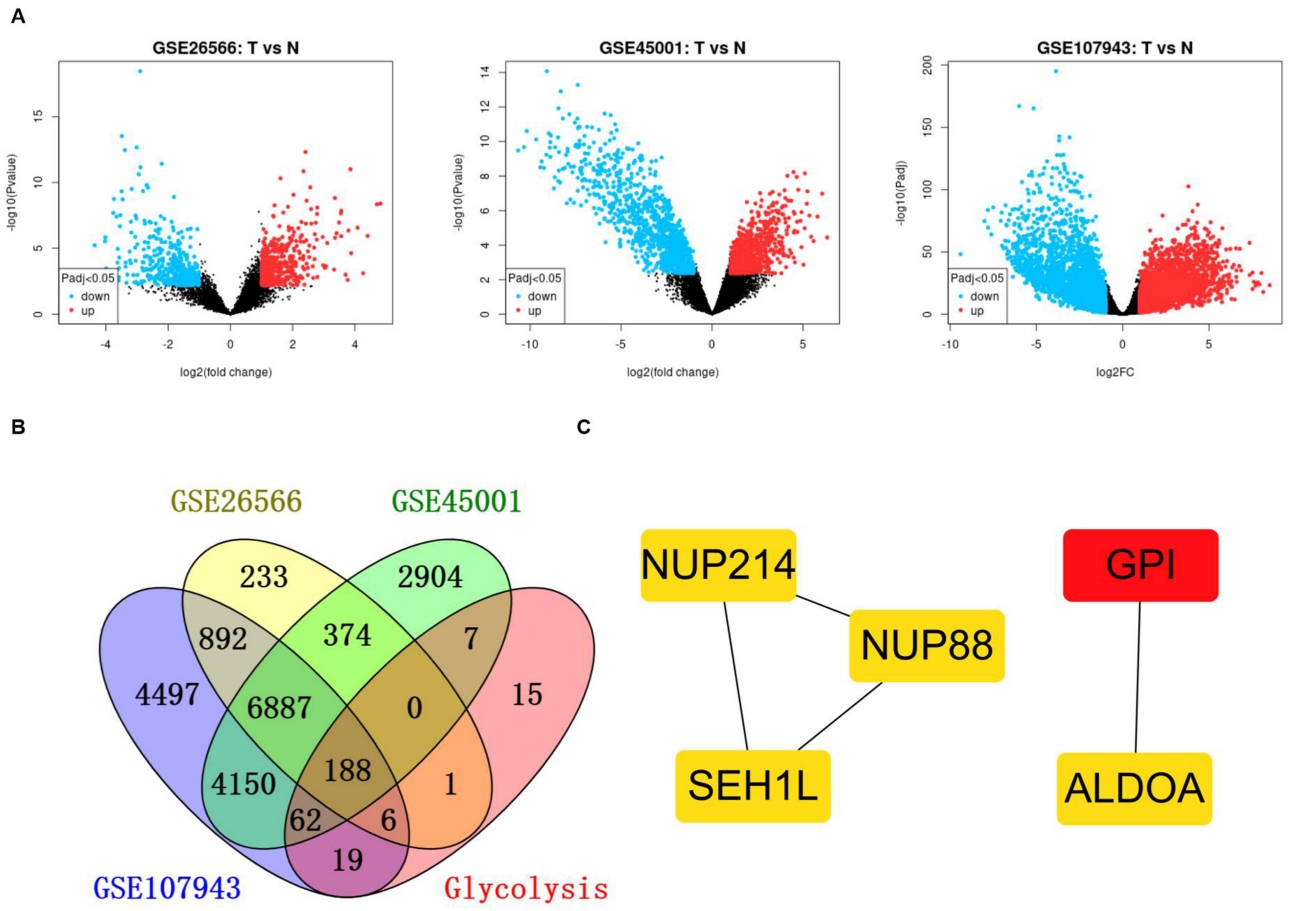

Figure 3 Volcano plots and Venn diagrams of DEGs. (**A**). Volcano map of DEGs. (**B**). Venn diagram of intersecting targets. (**C**). The 5 key genes were screened.

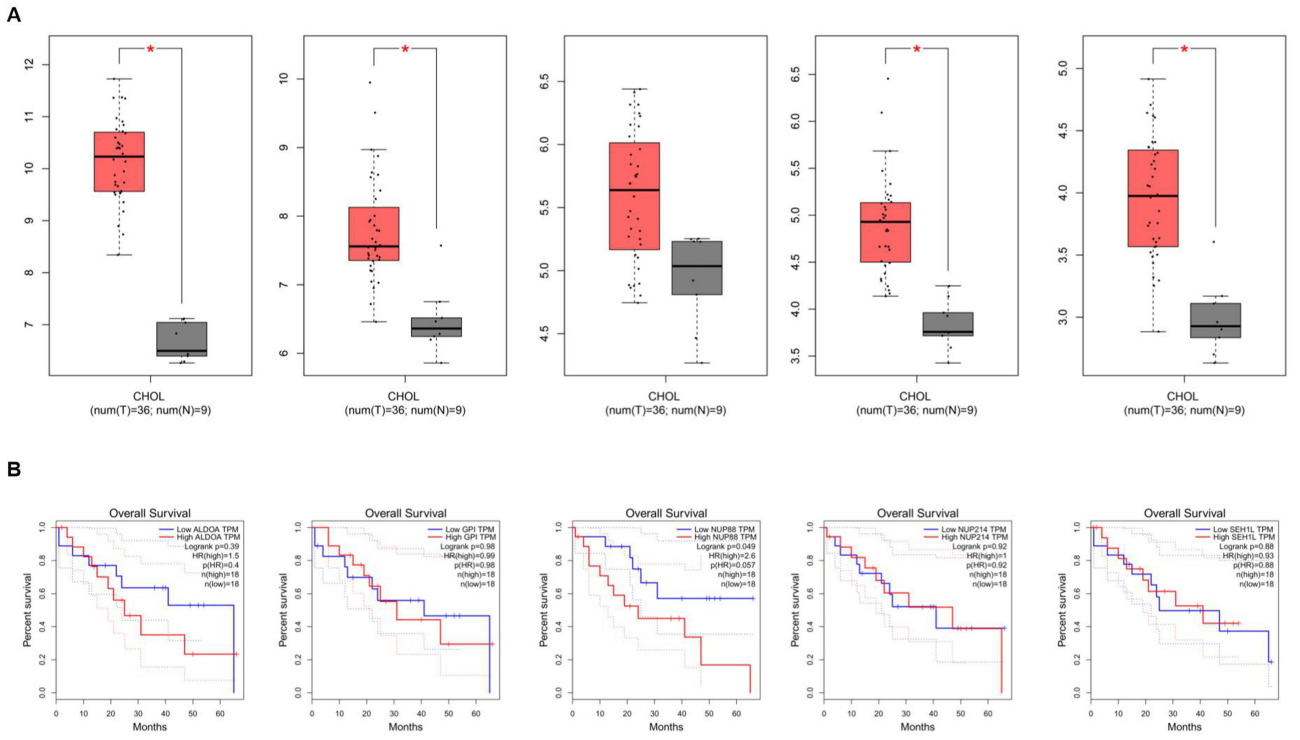

Figure 4 Abnormal expression and survival analysis of 5 key genes. (**A**). Expression level of 5 key targets (ALDOA, GPI, NUP88, NUP214, SEH1L). (**B**). Survival analysis of 5 key genes (ALDOA, GPI, NUP88, NUP214, SEH1L). A red asterisk indicates that $P<0.05$.

species as "human" and used a composite score of >0.9 as the threshold for inclusion in the network. The results were visually analyzed using Cytoscape 3.8.2. Five key genes with a high degree in CCA, namely Aldolase A (ALDOA), glycosylphosphatidylinositol (GPI), nucleoporin 88 (NUP88), NUP214 and SEH1L were identified (Figure 3C). The results showed that these 5 key genes expression in CCA tissues was higher than that in normal tissues (Figure 4A). Similar studies have also found that the expression of these five key targets is significantly increased in cancer, promoting cancer progression.[128–132] To further illustrate whether 5 key genes were potentially prognostic markers for CCA, overall survival was analyzed using GEPIA (https://www.ncbi.nlm.nih.gov/geo/).[133] High levels of NUP88 were associated with poor overall survival (Figure 4B). Due to the limited database of CCA, the remaining four targets had no significant effect on overall survival.

Conclusion and Perspectives

Potential efficacy advantages of glycolytic target therapy. Some drugs targeting glycolytic targets have shown certain anti-tumor activities in preclinical studies and early clinical trials. For example, HK, a key enzyme in glycolysis, inhibiting HK can reduce the uptake and utilization of glucose by tumor cells. Studies have found that HK inhibitors can reduce the viability of tumor cells and induce apoptosis in cholangiocarcinoma models. There are also PKM2 inhibitors, which can regulate the metabolic reprogramming of tumor cells, change the energy metabolism state of cells, and thus exert anti-tumor effects. In animal experiments, PKM2 inhibitors can inhibit the growth of cholangiocarcinoma xenograft tumors.

Potential safety advantages of glycolytic target therapy. Since glycolytic target therapy intervenes in the metabolic pathways specific to tumor cells, it theoretically has a relatively small impact on normal cells. Normal cells mainly obtain energy through mitochondrial oxidative phosphorylation and have a lower dependence on glycolysis. In contrast, traditional chemotherapy and radiotherapy can cause significant damage to normal tissue cells while killing tumor cells.

CCA metabolic reprogramming, especially the reprogramming of glycolysis, provides a crucial mechanism for tumor growth and progression. Glycolysis, serving as the primary energy source for cancer cells, is closely related to cell

proliferation, migration, invasion, and drug resistance.[134] Numerous studies have shown that targeting key glycolytic enzymes, signaling pathways, and regulatory factors can significantly inhibit CCA's tumor biological behaviors.[135] However, further exploration of these mechanisms can reveal the specific roles of glycolysis in CCA and offer new insights for future therapeutic strategies.

In terms of drug resistance, glycolysis regulation is also crucial. Inhibiting UCP2 can reverse EMT and improve drug sensitivity, offering a new approach to address common resistance issues in CCA therapy.[120] Additionally, mTOR inhibitor rad001 in combination with gemcitabine showed a significant synergistic effect, further suggesting that targeting glycolysis may be an effective strategy to improve the efficacy of CCA therapy and effectively inhibit tumor glycolysis[124].

Despite the recognized importance of glycolysis regulation in CCA, several unresolved issues remain. Firstly, the interaction mechanisms between glycolysis and other metabolic pathways such as oxidative phosphorylation, lipid metabolism, and amino acid metabolism are not fully understood. Understanding the coordination between these metabolic pathways will help identify more specific metabolic targets. Secondly, the complex interactions among ncRNAs, post-translational modifications, and metabolic pathway regulation require further study, particularly their roles in glycolysis regulation in CCA. Finally, clinical translation remains a challenge, as most studies on glycolysis regulation are still in vitro or animal models. Translating these findings into practical clinical applications will be a key direction for future research.

Many studies have investigated the relationship between CCA and glycolysis. For example, using CCA cell lines like HuCCT-1 and RBE, cells were treated with glycolysis inhibitors to observe their metabolic changes, proliferation ability, and apoptosis status.[10,136] It was found that when key enzymes in the glycolysis process, such as HK2, were inhibited, the glucose uptake and lactate production of CCA cells significantly decreased.[45,137] Meanwhile, cell proliferation was inhibited, and obvious apoptosis features like cell shrinkage and nuclear fragmentation appeared. This indicates that interfering with the glycolysis process can have a negative impact on the growth of CCA cells at the cell level. Studies on intracellular signaling pathways have also revealed the potential mechanisms of glycolysis inhibition. In CCA cells, glycolysis inhibition usually causes changes in the PI3K-AKT-mTOR pathway. For example, some glycolysis enzyme inhibitors can reduce the phosphorylation level of AKT protein, thereby blocking the cell's proliferation signal, which further illustrates the rationality of targeting glycolysis for treating CCA.[106] Experiments using CCA xenograft models have also provided strong evidence. Human CCA cells were inoculated into immunodeficient mice to construct a CCA xenograft tumor model. When glycolysis-targeted drugs were administered to the mice, a significant slowdown in tumor growth rate and a reduction in tumor volume were observed. For example, in mice treated with PKM2 inhibitor, the PKM2 activity in the tumor tissue decreased, glycolysis metabolism was inhibited, the viability of tumor cells decreased, and the survival period of the mice was prolonged to a certain extent.[138] These animal experiment results provide an important preliminary basis for the transition of glycolysis-targeted treatment of CCA from the basic to the clinic.

Target complexity: The glycolysis process involves multiple enzymes and regulators, like HK, PFK-1, PK, etc. Identifying truly crucial and effective targets in cholangiocarcinoma is challenging. For example, although HK plays a key role in the initial stage of glycolysis in many tumors including cholangiocarcinoma, it has multiple isoenzymes (such as HK1, HK2, etc)., and the specific functions and importance of different isoenzymes in cholangiocarcinoma may vary, requiring in-depth research to precisely locate the most therapeutically valuable targets. Drug screening and design: It's difficult to develop drugs that can effectively inhibit glycolytic targets. Due to the structural and functional characteristics of glycolytic enzymes, designing small molecule compounds or biologics that can bind to targets with high affinity and specificity is a complex process. For example, the active site of the enzyme may be relatively concealed, or there may be multiple allosteric regulatory mechanisms, making it hard for drugs to precisely bind to and exert inhibitory effects. Difficulty in monitoring metabolic changes: Assessing the efficacy of glycolytic target therapy requires accurately monitoring the metabolic changes of cholangiocarcinoma cells. However, currently, the clinical techniques for monitoring tumor metabolism are limited. Traditional imaging examinations (such as CT, MRI) mainly focus on the morphological changes of tumors and can hardly directly reflect the inhibition of glycolysis. Although there are some emerging technologies, such as positron emission tomography (PET) combined with specific metabolic tracers that can be used to monitor glycolysis-related metabolites, these technologies still have deficiencies in terms of accuracy and

popularity. Tumor heterogeneity: Cell lines or animal models commonly used in preclinical studies cannot fully simulate the complex situation of cholangiocarcinoma in the human body. Cholangiocarcinoma is a highly heterogeneous tumor, and tumor cells from different patients vary greatly in gene expression, metabolic state, etc. Microenvironmental factors: The growth and metabolism of tumors in vivo are affected by the surrounding microenvironment, including angiogenesis, immune cell infiltration, extracellular matrix composition, etc. Preclinical models often fail to fully reflect the interference of these microenvironmental factors on glycolytic target therapy. In the actual clinical setting, the tumor microenvironment may change the sensitivity of cholangiocarcinoma cells to glycolytic target drugs or provide alternative energy acquisition pathways for tumor cells, thus reducing the treatment effect.

In conclusion, glycolysis regulation plays multifaceted roles in CCA growth, migration, and drug resistance. Targeting glycolysis holds promise as an innovative strategy to improve CCA treatment outcomes. By further elucidating the mechanisms of glycolysis-related molecules and developing new targeted drugs, future CCA treatment will advance towards more precise and effective approaches.

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Disclosure

The authors declare that they have no competing interests in this work.

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