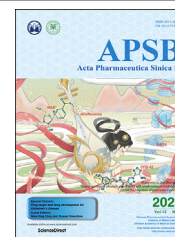




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REVIEW

Metabolic dysregulation and emerging therapeutic targets for hepatocellular carcinoma



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Abbreviations: 1,3-BPG, 1,3-bisphosphoglycerate; 2-DG, 2-deoxy-D-glucose; 3-BrPA, 3-bromopyruvic acid; ACC, acetyl-CoA carboxylase; ACLY, adenosine triphosphate (ATP) citrate lyase; ACS, acyl-CoA synthase; AKT, protein kinase B; AML, acute myeloblastic leukemia; AMPK, adenosine mono-phosphate-activated protein kinase; ASS1, argininosuccinate synthase 1; ATGL, adipose triacylglycerol lipase; CANA, canagliflozin; CPT, carnitine palmitoyl-transferase; CYP4, cytochrome P450s (CYPs) 4 family; DNL, *de novo* lipogenesis; EMT, epithelial-to-mesenchymal transition; ER, endoplasmic reticulum; ERK, extracellular-signal regulated kinase; FABP1, fatty acid binding protein 1; FASN, fatty acid synthase; FBP1, fructose-1,6-bisphosphatase 1; FFA, free fatty acid; G6PD, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLS1, renal-type glutaminase; GLS2, liver-type glutaminase; GLUT1, glucose transporter 1; GOT1, glutamate oxaloacetate transaminase 1; HCC, hepatocellular carcinoma; HIF-1 α , hypoxia-inducible factor-1 alpha; HK, hexokinase; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; HSCs, hepatic stellate cells; IDH2, isocitrate dehydrogenase 2; LCAD, long-chain acyl-CoA dehydrogenase; LDH, lactate dehydrogenase; LPL, lipid lipase; LXR, liver X receptor; MAFLD, metabolic associated fatty liver disease; MAGL, monoacylglycerol lipase; MCAD, medium-chain acyl-CoA dehydrogenase; MEs, malic enzymes; mIDH, mutant IDH; MMP9, matrix metalloproteinase 9; mTOR, mammalian target of rapamycin; NADPH, nicotinamide adenine nucleotide phosphate; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OTC, ornithine transcarbamylase; PCK1, phosphoenolpyruvate carboxykinase 1; PFK1, phosphofructokinase 1; PGAM1, phosphoglycerate mutase 1; PGK1, phosphoglycerate kinase 1; PI3K, phosphoinositide 3-kinase; PKM2, pyruvate kinase M2; PPAR α , peroxisome proliferator-activated receptor alpha; PPP, pentose phosphate pathway; ROS, reactive oxygen species; SCD1, stearoyl-CoA-desaturase 1; SGLT2, sodium-glucose cotransporter 2; *SLC1A5/ASCT2*, solute carrier family 1 member 5/alanine serine cysteine preferring transporter 2; *SLC7A5/LAT1*, solute carrier family 7 member 5/L-type amino acid transporter 1; SREBP1, sterol regulatory element-binding protein 1; TAGs, triacylglycerols; TCA cycle, tricarboxylic acid cycle; TKIs, tyrosine kinase inhibitors; TKT, transketolase; VEGFR, vascular endothelial growth factor receptor; WD-fed MC4R-KO, Western diet (WD)-fed melanocortin 4 receptor-deficient (MC4R-KO); WNT, wingless-type MMTV integration site family.

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KEY WORDS

Metabolic dysregulation;
Hepatocellular carcinoma;
Glycolysis;
Tricarboxylic acid cycle;
Pentose phosphate
pathway;
Fatty acid β -oxidation;
Glutamine metabolism;
Cancer therapy

Abstract Hepatocellular carcinoma (HCC) is an aggressive human cancer with increasing incidence worldwide. Multiple efforts have been made to explore pharmaceutical therapies to treat HCC, such as targeted tyrosine kinase inhibitors, immune based therapies and combination of chemotherapy. However, limitations exist in current strategies including chemoresistance for instance. Tumor initiation and progression is driven by reprogramming of metabolism, in particular during HCC development. Recently, metabolic associated fatty liver disease (MAFLD), a reappraisal of new nomenclature for non-alcoholic fatty liver disease (NAFLD), indicates growing appreciation of metabolism in the pathogenesis of liver disease, including HCC, thereby suggesting new strategies by targeting abnormal metabolism for HCC treatment. In this review, we introduce directions by highlighting the metabolic targets in glucose, fatty acid, amino acid and glutamine metabolism, which are suitable for HCC pharmaceutical intervention. We also summarize and discuss current pharmaceutical agents and studies targeting deregulated metabolism during HCC treatment. Furthermore, opportunities and challenges in the discovery and development of HCC therapy targeting metabolism are discussed.

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1. Introduction

Hepatocellular carcinoma (HCC) accounting for nearly 90% of primary liver cancer represents one of the most lethal and prevalent human cancers^{1,2}. Most patients are diagnosed at an advanced stage due to heterogeneity and complexity of HCC; thereby systemic therapies are usually recommended as the standard of medical care³. Yet, conventional systemic chemotherapy yields negligible clinical benefits. Several phase III trials of doxorubicin alone are compared to doxorubicin plus sorafenib, or to FOLFOX4 regimen (fluorouracil, oxaliplatin and leucovorin (folinic acid)) for HCC treatment, however, all above have negative results, in several circumstances with considerable toxicity^{4,5}.

Later, targeted small-molecule tyrosine kinase inhibitors (TKIs) or antibodies, primarily sorafenib⁶, lenvatinib⁷, regorafenib⁸, cabozantinib⁹, ramucirumab¹⁰ are approved by U.S. Food and Drug Administration (FDA) for the first/second-line choice for HCC treatment. Sorafenib shows survival benefits with a median survival of 10.7 months compared to placebo of 7.9 months⁶, and lenvatinib exerts a non-inferior median survival of 13.6 months to that of 12.3 months in sorafenib groups⁷, respectively. Even so, associated severe adverse events, such as hand-foot skin reaction and cardiac ischemia, need to be carefully managed, or else they could be an obstacle to these drugs' clinical use¹¹. Also, chemoresistance to TKIs developed during treatment limits their clinical use¹². Although the clinical benefits of immune-based therapies for HCC are emerging, a phase III trial shows an overall survival of 67.2% with atezolizumab-bevacizumab and of 54.6% with sorafenib at 12 months¹³. It is still urgent to explore effective novel strategies to combat HCC.

Recently, metabolic (dysfunction) associated fatty liver disease (MAFLD)—a reappraisal of new nomenclature for non-alcoholic fatty liver disease (NAFLD), has attracted heated attention from biologists, pharmacologists, pharmaceutical companies and clinical researchers¹⁴. Inclusion of metabolic (dysfunction) in the nomenclature indicates growing appreciation of metabolism in the initiation and progression of liver disease, including HCC¹⁵. Notably, fast-rising NAFLD and non-alcoholic steatohepatitis (NASH) have made metabolic disorders a major risk factor for the

development of HCC^{16,17}, in parallel with other identified etiological factors as chronic hepatitis B and C viral infections¹⁸, as well as excessive chemical and alcohol exposure¹⁹. As earlier in 1956, Otto Warburg discovers that cancer cells prefer to consume large amounts of glucose through glycolysis rather than favor oxidative phosphorylation, even in a sufficient supply of oxygen. Now, it is clear that tumorigenesis is driven by the rearrangement of cellular metabolism resulting from direct and indirect consequences of oncogenic mutations²⁰. And reprogramming of energy metabolism is characterized as a new hallmark to further rationalize the complexities of neoplastic disease²¹. However, whether and how the key enzymes or intermediates function in HCC initiation and progression remain to be deeply illustrated²⁰. Here, we review the critical enzymes and intermediates in glucose, fatty acids, amino acids and glutamine metabolism during HCC pathogenesis, so as to provide metabolic targets suitable for pharmaceutical intervention. We also summarize and discuss current pharmaceutical agents and studies targeting deregulated metabolism for HCC treatment. Furthermore, the opportunities and challenges in the discovery and development of HCC therapy targeting metabolism are highlighted.

2. Metabolic rearrangements in HCC**2.1. Glucose metabolism**

Carbohydrate metabolism is a complex biological process. Endless newly-found evidence reveals a well-recognized emphasis on the deregulation of glucose metabolism during the initiation and progression of HCC. HCC cells are metabolically distinct from normal hepatocytes and express different metabolic enzymes²². Glucose transporters sodium-glucose cotransporter 2 (SGLT2) and glucose transporter 1 (GLUT1) are highly expressed in HCC and functionally promote tumorigenicity. Enzymes and substrates in carbohydrate metabolism act as pathological fingerprints during the tumorigenesis of HCC, including the ones in glycolysis, pentose phosphate pathway (PPP), gluconeogenesis and tricarboxylic acid cycle (TCA cycle) (as shown in Fig. 1).

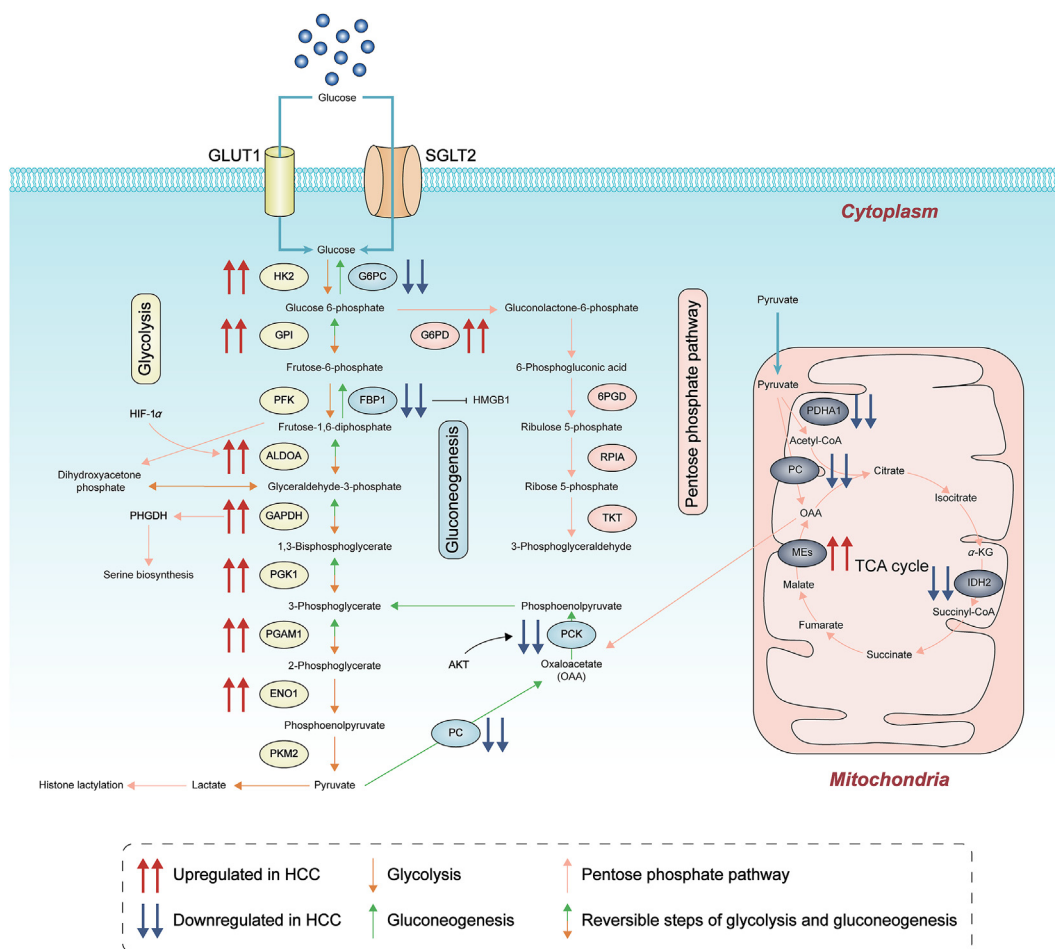


Figure 1 Deregulated alterations of glucose metabolism in HCC. Deregulation of glucose metabolism during the initiation and progression of HCC has been early and well emphasized, including glucose transporters, enzymes and substrates in glycolysis, pentose phosphate pathway, gluconeogenesis and TCA cycle. Newly discovered functions of metabolic intermediates have also been referred, as histone lactylation by lactate. G6PC, glucose-6-phosphatase alpha; GPI, glucose-6-phosphate isomerase; HMGB1, high mobility group box 1; ALDOA, fructose-bisphosphate aldolase A; ENO1, enolase 1; PC, pyruvate carboxylase; PDHA1: pyruvate dehydrogenase A1; PHGDH, phosphoglycerate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; RPIA, ribose 5-phosphate isomerase A.

2.1.1. Glycolysis

Normal cells break down glucose or glycogen into lactate while produce a small amount of ATP under anaerobic or hypoxic conditions. However, due to the energy demand for rapid cell multiplication, even under aerobic conditions, tumor cells decompose glucose into lactate through glycolysis, known as Warburg effect²³. This is one of the reasons why glycolysis is particularly important in tumor cell metabolism. Targeting an enzyme that is only over-activated in HCC cells but not in the corresponding normal liver tissue could be a selective therapeutic strategy. The enzymes included in glycolysis are highly expressed and closely related with poorer overall survival in HCC. Some enzymes have an isoform specificity during HCC tumorigenesis and progression. For example, a switch of hexokinase (HK) from low-affinity HK4 to high-affinity HK2 is made in hepatocytes, and a specific isoform of pyruvate kinase-pyruvate kinase M2 (PKM2) is highly expressed in all proliferating cells, including HCC^{24,25}.

The diversion of glycolytic flux to other pathway arouses much attention recently. Glucose-6-phosphate isomerase diverges the

glucose flow to the PPP to produce nicotinamide adenine nucleotide phosphate (NADPH) and pentose²⁶. NADPH acts as a critical power-producer to fuel the protein-based antioxidant system and combat oxidative stress. Antioxidant machinery is increased in HCC to overcome reactive oxygen species (ROS)-induced cell death²⁷. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is another knot to promote diversion from glycolysis to serine biosynthesis by elevating the transcription of phosphoglycerate dehydrogenase, a key enzyme for serine biosynthesis²⁸. The requirement of *de novo* synthesis of serine from glucose is a characteristic of cancer cells to thrive the synthesis of cellular glycine and nucleotide, and also to support the folate cycle and amino acid transport²⁹. Therefore, glycolytic enzymes fuel cell growth by tuning the HCC cells to cope with metabolic requirement for rapid proliferation.

Some enzymes have other functions apart from propelling glycolysis. Glucose-6-phosphate isomerase also acts as an extracellular cytokine to suppress apoptosis, promote tumor progression and invasiveness with the involvement of PI3K

(phosphoinositide 3-kinase)/AKT (protein kinase B) activation³⁰. GAPDH is proposed to contribute in nuclear tRNA export, DNA replication and repair, exocytosis, cytoskeletal organization, and cell death³¹. These above functions involved in other biological process boost HCC cell growth in a synergistic way.

The regulation of glycolytic enzymes is only partially understood. Hypoxia induced increases of glycolytic flux is exaggerated in cancer cells to promote cell survival and growth. Hypoxia induces nuclear GAPDH translocation in hepatic stellate cells (HSCs), which is associated with HCC patient prognosis and tumorigenesis³². Fructose-bisphosphate aldolase A is a target gene of hypoxia-inducible factor 1 alpha (HIF-1 α)^{33,34}, a critical transcription factor that controls hypoxia-induced gene expression and elicits metabolic events crucial for tumor development³⁵. Besides, autophagy suppresses glycolysis with the autophagic degradation of HK2³⁶. However, the regulation of autophagy by PKM2 in tumor cells is intriguing but still contradictory. PKM2 knockdown inhibits autophagy vesicle formation³⁷, as a participant in a positive feedback loop that promotes HIF-1 α transactivation³⁸. However, it is not consistent with the finding that AKT phosphorylation leads to mammalian target of rapamycin (mTOR) inhibition and the extrapolation of autophagy activation resulting from PKM2 knockdown³⁹. Some upstream regulators affect tumorigenesis by regulating the expression of phosphoglycerate kinase (PGK1), as long noncoding RNA MSC-AS1 and microRNA-450b-3p target PGK1 post-transcriptionally and induce opposite effect to HCC cell proliferation^{40,41}.

Lactate as the main metabolic product of glycolysis is actually the nutrient instead of waste, which is related to oxidative stress resistance and lipid biosynthesis in cancer cells. Lactate is not only a carbon source for membrane lipid biosynthesis, but also participates in the production of lipid substances. It is revealed that lactate enhances ferroptosis resistance in HCC cells and contributes to tumor growth⁴². A novel function of lactate, histone lysine lactylation, is newly proposed as a post-translational modification of histone proteins to regulate gene expression⁴³. Histone lactylation is observed with M1 macrophage polarization, associated with the increased expression of inflammatory genes and activation of glycolysis. Further investigating the role of histone lactylation on modulating the activity of liver-specific macrophages and further HCC development will lead to intriguing findings.

Targeting glycolytic enzymes in HCC therapy is speculated to be a considerable approach, as some relevant drugs are now under investigation^{44,45}. Fully addressing the favorable and unfavorable effects of those agents require further understanding the role of the enzymes in biological processes.

2.1.2. Pentose phosphate pathway

Through PPP, ribose-5-phosphate is synthesized besides NADPH. Therefore, PPP is essential for cancer cells to satisfy their demand for ribonucleotide synthesis and to maintain redox balance. The requirement of high proliferation rate and NADPH shortage is among the most important factors to affect PPP activity⁴⁶. The enzymes representative for the oxidative or non-oxidative phase of PPP are glucose-6-phosphate dehydrogenase (G6PD) or transketolase (TKT) respectively. Both of the enzymes are robustly up-regulated and positively associated with poor prognosis and aggressive clinicopathological features of HCC^{47,48}. As the rate-limiting enzyme in PPP, G6PD has been the most studied one in

HCC. Besides well-established role in tumor growth⁴⁹, G6PD also contributes to migration and invasion of HCC cells *in vitro* by inducing epithelial-to-mesenchymal transition (EMT) through the activation of signal transducer and activator of transcription 3 pathway⁵⁰. TKT is a predominant form of the three human TKT genes (TKT, TKTL1, TKTL2) in the liver tissue. As uncovered by a cohort of 292 HCC patients with complete clinical data in The Cancer Genome Atlas database, TKT is an independent biomarker to predict the survival of HCC patients. The conclusion is based on the presence of vascular invasion in patients with HCC, cell viability under oxidative stress *in vitro* and the accelerated cell growth and metastasis *in vivo*⁴⁸. TKT inhibitor oxythiamine significantly reverses the chemoresistance of human HCC cells to sorafenib treatment and suppresses tumor growth *in vivo*⁵¹. The elevated expression and exaggerated behavior of TKT is a specific feature to HCC, as TKT expression is not altered in urothelial and colorectal cancer⁵². It is suggested that the role of PPP enzymes in HCC development may be different from that in other cancer types and deserves further elucidation.

2.1.3. Gluconeogenesis

Gluconeogenesis, a reverse process of glycolysis, generates glucose from small carbohydrate substrates such as pyruvate, lactate, glycerol, and gluconeogenic amino acids⁵³. Generally, gluconeogenesis pathway is inhibited in cancers because it antagonizes glycolysis. Gluconeogenesis enzymes are therefore down-regulated in HCC tissues and cells, including fructose-1,6-bisphosphatase 1 (FBP1) and phosphoenolpyruvate carboxykinase 1 (PCK1), which are also associated with unfavorable prognosis in patients with HCC^{54,55}. The loss of these enzymes is sufficient to induce HCC tumorigenesis. As shown, hepatic glucose-6-phosphatase alpha deficiency leads to autophagy impairment, mitochondrial dysfunction, enhanced glycolysis, and augmented PPP^{56–58}, therefore contributes to hepatocarcinogenesis. Simultaneously, FBP1 loss elicits senescence and senescence-associated secretory phenotype in HSCs, partly because of the increase of high mobility group box 1 expression, to promote HCC growth⁵⁴.

As the rate-limiting enzyme in gluconeogenesis, PCK1 is elevated in other cancer types as colon cancer while decreased in HCC. It indicates that HCC cells have unique metabolic property, especially in gluconeogenesis⁵⁹. Forced PCK1 expression in glucose-starved HCC cells induces TCA cycle cataplerosis, leading to energy crisis and oxidative stress⁶⁰. In the contrary, it is highlighted recently that following the phosphorylation by AKT, PCK1 is translocated to endoplasmic reticulum (ER) and then phosphorylates INSIG1/2, leading to the activation of sterol regulatory element-binding protein 1 (SREBP1) and lipogenesis, which results in accelerated development and deteriorated prognosis of HCC⁶¹. PCK1 plays as a linker between lipogenesis and carcinogenesis, and can be a potential treatment target for patients with HCC.

2.1.4. Tricarboxylic acid cycle

The TCA cycle is the final metabolic pathway of the three major nutrients and the hub of their metabolic connections. Lactate increases and provides energy as the primary carbon source for the TCA cycle in cancer cells⁶². Cataplerosis is a process in which intermediate products of the TCA cycle are transported away from the mitochondria to participate in biosynthesis, and anaplerosis

replenishes these catalytic products to maintain the stability of the cycle⁶³. Both anaplerosis and cataplerosis affect HCC development through the regulation of TCA substrates. For example, anaplerotic enzyme pyruvate carboxylase which catalyzes pyruvate carboxylation to support anaplerosis by supplying oxaloacetate to TCA cycle. Suppression of pyruvate carboxylase decreases the flux of TCA cycle intermediates, aspartate, glutamate and glucose derivatives, which in turn reduces the viability and proliferation of HCC HepG2 cells⁶⁴.

The enzymes in TCA cycle could be major factors to regulate anaplerosis and cataplerosis, but will stay far from full illustration. As the main active subunit of pyruvate dehydrogenase complex, pyruvate dehydrogenase A1 has a lower expression in HCC tumor tissues compared with normal tissues and is positively correlated with survival rate of HCC patients. Pyruvate dehydrogenase A1 overexpression has a capability to inhibit glycolysis and boost oxidative phosphorylation, which increases cell apoptosis through a mitochondria-dependent pathway⁶⁵. In consequence, isocitrate dehydrogenase 2 (IDH2) is also significantly decreased in HCC tissues. IDH2 has a strong negative correlation with matrix metalloproteinase 9 (MMP9), which might be the reason that decreased IDH2 promotes cell migration and HCC metastasis⁶⁶. Furthermore, genetic variations in *IDH* genes (*IDH1* and *IDH2*) act as prognosis predictors in HCC patients⁶⁷. Malic enzymes (MEs) are important in the regulation of cellular energy balance and redox homeostasis by catalyzing the oxidation of TCA cycle intermediate malate to form pyruvate and CO₂, with simultaneous reduction of NAD(P)⁺ to NAD(P)H⁶⁸. MEs are considered vital for glycolysis, gluconeogenesis, fatty acid synthesis and glutamine metabolism. ME1 is known as a poor prognostic predictor of HCC, as silencing ME1 decreases HCC metastasis *via* inhibition of EMT through ROS-induced pathways⁶⁹. Recently, our data also uncovers that ME1 integrates with mitochondrial IDH2 and therefore supports tumor growth and metastasis in a variety of cancer cell types, including breast cancer and non-small cell lung cancer⁷⁰. Taken together, MEs, especially ME1, deserve deeper exploration in cancer research, including HCC.

It is noted that dysregulation of glucose metabolism acts as a definitive driving force in HCC development. Recent evidences also indicate that not only enzymes in glucose metabolism, but also many other intermediate substrates or interacting proteins intervene the pathological process of liver cancer cell through glucose metabolism. The investigation on glucose metabolism in HCC is far from over, and more in-depth exploration is expected in the future.

2.2. Fatty acid and cholesterol metabolism

As a vital organ in lipid homeostasis, deregulated hepatic lipid metabolism has been considered as a driving force to HCC. Pathological conditions associated with lipid dysregulation are risks for HCC, including obesity, diabetes and NASH⁷¹. Fatty acids are used as signal precursors in the regulation of metabolism during HCC development as well as an energy source to support rapid cell proliferation, cell survival, invasion and angiogenesis⁷². Because of the poor nutrient supply in the center of tumor due to insufficient vascularization, fatty acid oxidation is another major catabolic pathway besides glycolysis, and therefore is up-regulated to provide energy for cell growth and metabolites for anabolic processes. Cholesterol appears more important for membrane integrity and fluidity and is required for

highly proliferative cancer cells, including HCC⁷³ (fatty acid and cholesterol metabolism deregulation in HCC is summarized in Fig. 2).

2.2.1. Fatty acid uptake and transport

Fatty acids are transported across cell membrane actively by several transmembrane transporters, such as fatty acid translocase/CD36, fatty acid transport proteins. Also, some specialized small proteins like fatty acid binding proteins which bind free fatty acids (FFAs) and facilitate FFAs intracellular transport⁷⁴. To satisfy the metabolic need of HCC cells, these fatty acid transportation machineries are increased significantly in malignant tissues compared with adjacent tissues and normal liver cells. Apart from cell growth, other pathological properties of HCC cells are also involved. The up-regulation of CD36 is associated with the induction of EMT process⁷⁵ by the potential activation of wntless-type MMTV integration site family (WNT) and transforming growth factor beta signaling pathways. Both fatty acid binding protein 1 (FABP1) and vascular endothelial growth factor receptor (VEGFR) expression are up-regulated in HCC, and positive correlation exists between the two. FABP1 induces HCC cell migration through VEGFR2/SRC proto-oncogene tyrosine-protein kinase signaling and focal adhesion kinase/cell division cycle 42 pathway⁷⁶. The above findings support a role of FABP1 to promote angiogenesis, tumorigenesis and metastasis. FABP5 overexpression is also closely related to the occurrence, invasion and metastasis in HCC tumor tissues⁷⁷. Treatment with oleate activates the FABP5/HIF-1 α axis, thereby promoting lipid accumulation and cell proliferation in HCC cells⁷⁸. The expression of FABP5 and HIF-1 α are associated with poor prognosis in HCC. Patients with high expression of FABP5 deteriorate rapidly and have a high recurrence rate⁷⁹.

Lipid lipase (LPL) hydrolyzes triacylglycerols (TAGs) into FFAs and represents another group of proteins that up-regulated in HCC samples to increase the absorption of lipoproteins by cells⁸⁰. Inhibition of LPL restrains the uptake of exogenous lipids, resulting in the hindrance of HCC cell proliferation. In HCC patients, the expression level of LPL in stage III/IV is higher than that in stage I/II, positively related with poor prognosis⁸¹.

2.2.2. Fatty acid synthesis

Abnormally activated new fat formation is essential for the development and progression of HCC. Enhanced lipogenesis is characterized by increased activity and expression of various lipogenic enzymes, such as adenosine triphosphate (ATP) citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), acyl-CoA synthase (ACS) and stearoyl-CoA-desaturase 1 (SCD1). These enzymes are highly expressed in various cancers, including HCC.

Cancer cells use cytoplasmic acetyl-CoA as a substrate for fatty acid synthesis. Citrate is transported from the mitochondria to the cytoplasm, and then converted into acetyl-CoA and oxaloacetate through ACLY⁸². ACLY plays a key role in cancer metabolism by depriving cytosolic citrate to enhance glycolysis, as shown by the increased activity of phosphofructokinase1 (PFK1) and PFK2. Oncogenic drivers (such as PI3K/AKT) activate ACLY and Warburg effect in a feedback way⁸². As the rate-limiting enzyme for fatty acid synthesis, ACC is key to increase liver *de novo* lipogenesis (DNL) during HCC development. In the cytoplasm, ACC promotes the conversion of acetyl-CoA into the intermediate product malonyl-CoA. Phosphorylation of ACC decreases liver malonyl-CoA, suppresses fatty acid synthesis,

alleviates early signs of NAFLD and fibrosis development in liver⁸³. Liver-specific ACC inhibitor ND-654 inhibits liver DNL, inflammation and development of HCC⁸⁴. Then, malonyl-CoA and acetyl-CoA are condensed by FASN to form palmitate and other fatty acid synthesis products. Elevated FASN heralds the poor prognosis of HCC patients. Artificial inhibition or suppression of FASN is very unfavorable for the growth of HCC cells. FASN also participates in the occurrence and development of HCC tumors. Targeted inhibition of FASN and its related lipogenesis is a potentially relevant therapy for the treatment of HCC⁸⁵.

SCD catalyzes the conversion of saturated fatty acids into monounsaturated fatty acids⁸⁶. SCD1 interfering effectively inhibits HCC progression, while its overexpression is related to the shortening of tumor-free survival. Multi-omics studies find that the increase in monounsaturated fatty acids contributes to the increased *de novo* synthesis of fatty acids in HCC cells. The effect of SCD1 in HCC is associated with the regulation of P53⁸⁷, WNT/ β -catenin⁸⁸, epidermal growth factor receptor⁸⁹ and autophagy⁹⁰. Suppression of SCD1 sensitizes HCC cells to sorafenib by forcing the liver tumor-initiating cells to differentiate *via* the induction of ER stress. Agents targeting SCD1 in combination with sorafenib is a promising treatment strategy against HCC⁹¹.

ACS long chain family member 4 is one member of ACS family that convert fatty acid to fatty acyl-CoA esters with a substrate preference for arachidonic acid. Its knockout results in slower cell growth, while its overexpression promotes tumor formation *in vivo* and *in vitro*. ACS long chain family member 4 stabilizes c-MYC through extracellular-signal regulated kinase (ERK)/F-box and WD repeat domain-containing 7/c-MYC axis, and represents a valuable biomarker and potential therapeutic target for predicting the prognosis of liver cancer⁹².

The transcription factor SREBP1, a central player to boost DNL, is significantly higher in HCC tumor than adjacent tissues. Downregulation of SREBP1 inhibits proliferation of HCC HepG2 and MHCC97L cells and induces cell apoptosis. Knockout of SREBP1 robustly represses the migration and invasion of HCC cells. SREBP1 serves as a prognostic marker for HCC and aggravates tumor progression by promoting cell growth and metastasis⁹³.

Liver X receptor (LXR) belongs to a member of ligand-activated nuclear receptor superfamily of transcription factors⁹⁴. LXR (LXR α and LXR β) functions to regulate cholesterol homeostasis and lipogenesis by transactivation of metabolic players as SREBP1 and FASN. Divergently, LXR α expression is down-regulated in HCC tumor tissues compared with normal liver tissues. LXR agonist TO901317 up-regulates LXR α , down-regulates

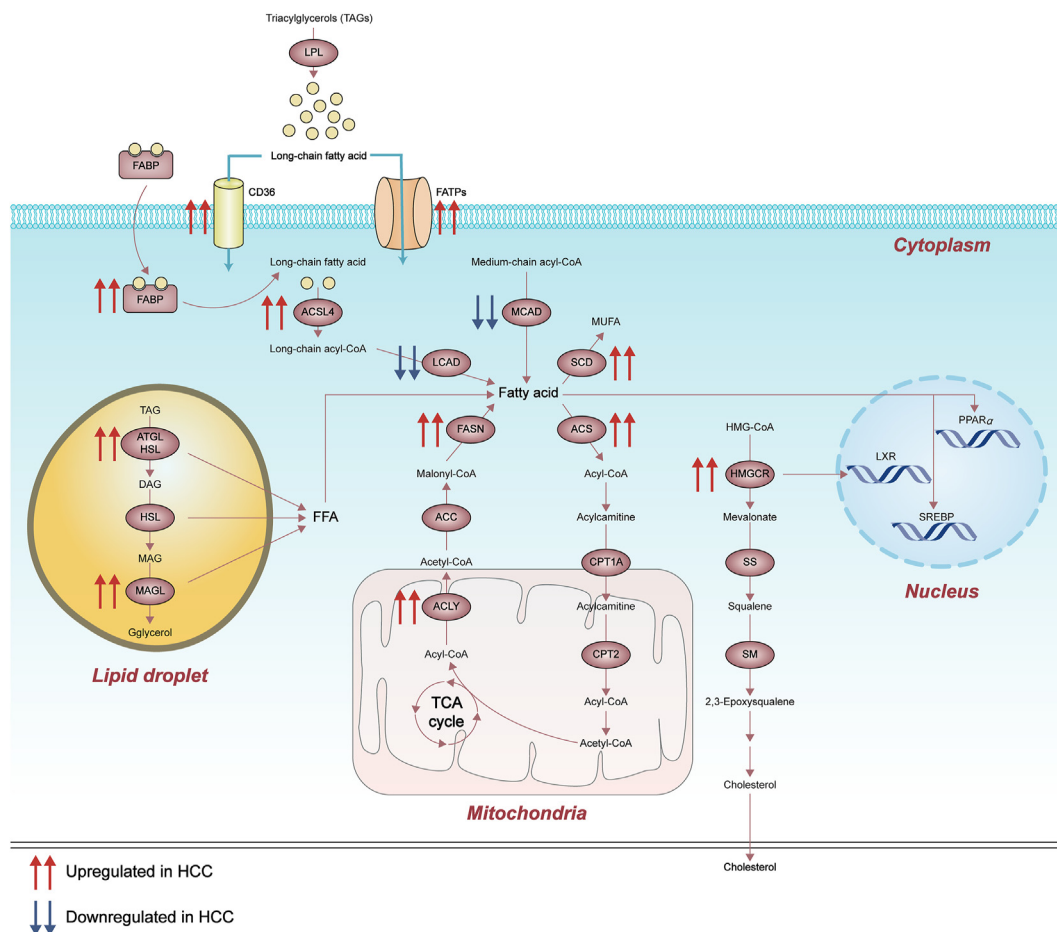


Figure 2 Deregulated alterations of fatty acid and cholesterol metabolism in HCC. Fatty acid and cholesterol metabolism function as other major catabolic pathways in the regulation of metabolism during HCC development. FATPs, fatty acid transport proteins; ACSL4, acyl-CoA synthetase long chain family member 4; HSL, hormone-sensitive lipase; SS, squalene synthase; SM, squalene monoxygenase; MUFA, mono-unsaturated fatty acid; DAG, diacylglycerol; MAG, monoacylglycerol.

GLUT1 and MMP9, and reduces intracellular glucose content, thereby inhibiting HCC progression⁹⁵. In addition, LXR expression is correlated with liver fat deposition, liver inflammation and fibrosis in patients⁹⁶. Treatment with long-term LXR agonist, oxidative stress, and high-fat diet together replicates liver conditions in mice similar to those patients with NASH and progression to HCC⁹⁷. The above split findings indicate the complexity in the role of LXR in HCC development.

2.2.3. Fatty acid β -oxidation

Compatible with elevated efficiency of fatty acid β -oxidation pathway, enzymes involved in the oxidative decomposition of fatty acids have been dysregulated in HCC. Carnitine palmitoyl-transferase (CPT) is divided into two types: CPT1 and CPT2. As the rate-limiting enzyme, CPT1 locates in the outer mitochondrial membrane and transfers long-chain fatty acyl-CoA to carnitine for transport into mitochondria for further oxidation. It is worth noting that an increase in saturated long-chain acylcarnitine and a decrease in short-chain and medium-chain acylcarnitine are simultaneously observed in HCC samples⁹⁸. Fatty acid β -oxidation and formation coordinate when ACC forms a complex with CPT1A to prevent its mitochondrial distribution under adequate glucose conditions. The formation of the complex between ACC and CPT1A is weakened with glucose starvation. The released free CPT1A molecules are relocated to the mitochondrial membrane, which enhances fatty acid β -oxidation. This provides a new mechanism for ACC and CPT1A to jointly protect HCC cells from metabolic stress⁹⁹.

Medium-chain acyl-CoA dehydrogenase (MCAD) and long-chain acyl-CoA dehydrogenase (LCAD) are key enzymes by catalyzing the first step of mitochondrial fatty acid β -oxidation. They are downregulated by hypoxic stress, leading to the inhibition of fatty acid catabolism and promotion of HCC cells proliferation¹⁰⁰. Consistently, forced expression of both MCAD and LCAD diminishes hypoxia-induced lipid accumulation in HCC cells. However, knockdown of LCAD, but not MCAD, facilitates tumor growth *in vivo* through the inhibition of tumor suppressor phosphatase and tensin homolog-mediated signaling pathway, which needs a further exploration¹⁰¹.

In addition, lipolysis also plays a role in fatty acid β -oxidation. Adipose triacylglycerol lipase (ATGL), hormone-sensitive lipase and monoacylglycerol lipase (MAGL) are three major enzymes involved in lipolysis and release of fatty acids¹⁰². MAGL catalyzes the conversion of monoacylglycerol esters into FFA and glycerol. Elevated expression of MAGL in cancer promotes the proliferation and invasion of HCC cells by generating signal lipids including monoacylglycerol, FFA and secondary lipid metabolites. NF- κ B signaling pathway is involved in the MAGL-mediated EMT of HCC cells¹⁰³. ATGL initiates the process of TAG hydrolysis into diacylglycerol and FFA. High levels of diacylglycerol and FFA are observed because of high expression of ATGL in HCC tissues, with an indication of poor prognosis. lncRNA-NEAT1 induces abnormal lipolysis in HCC cells through the up-regulation of ATGL, which is a driving force for the growth of HCC cells¹⁰⁴.

2.2.4. Cholesterol metabolism

Dysregulated cholesterol biosynthesis is another metabolic event frequently observed in HCCs. Studies have revealed a new biochemical crosstalk between DNL and cholesterol biosynthetic pathways in the process of liver cancer¹⁰⁵. 3-Hydroxy-3-methylglutaryl-CoA reductase (HMGCR) is a rate-limiting

enzyme in mevalonate pathway for cholesterol synthesis that can be blocked by statins¹⁰⁶. In fact, this inhibition has an anti-tumor effect in many tumor types. Up-regulation of HMGCR expression is reported previously in human HCC samples along with elevated mitochondrial cholesterol levels. Cholesterol depletion by inhibiting HMGCR or squalene synthase, another enzyme catalyzing the first step in cholesterol biosynthesis, enhances the sensitivity of HCC cells to chemotherapy *via* mitochondria¹⁰⁷.

2.2.5. Others

Peroxisome proliferator-activated receptors are ligand-activated nuclear receptors of the steroid/thyroid hormone receptor superfamily. There are three isoforms, α , β/δ , and γ , related to lipid homeostasis¹⁰⁸. Peroxisome proliferator-activated receptor α (PPAR α) regulates the constitutive transcription of genes encoding enzymes involving in fatty acid transport and TAG homeostasis. The long-term administration of PPAR α ligand in rodents causes accelerated hepatocyte proliferation, increased ROS production, and HCC development. Oncogene *MYC* directly acts as a transcription amplifier for specific PPAR α target genes including *KRT23* which promotes hepatocyte proliferation and potential HCC¹⁰⁹.

The cytochrome P450s (CYPs) 4 family (CYP4) consists of a group of ω -hydroxylase that functions in the conversion of fatty acids that then transport to mitochondria to produce energy, thereby yielding elimination of excess FFA and synthesis of bioactive fatty acid molecules¹¹⁰. CYP4B, CYP4A and CYP4V, together with CYP4F metabolize short-chain fatty acids (approximately 7–10 carbon fatty acids), intermediate-chain fatty acids (C10 to 16), long-chain fatty acids (C16 to 26), respectively. Notably, decreased expression of CYP4 is associated with liver fat accumulation and NASH pathogenesis¹¹¹. *CYP4Z1* and its pseudogene *CYP4Z2P*, first identified by Rieger¹¹², are highly expressed in breast cancer. *CYP4Z1* promotes breast cancer angiogenesis and growth¹¹³. Our previous study discovers that *CYP4Z2P* 3'UTR also promotes breast cancer angiogenesis through the VEGF/VEGFR2 pathway¹¹⁴. Our later findings further explore that CYP4Z2P- and CYP4Z1-3'UTRs share miRNA-binding sites, including miR-211 and miR-197, thereby promoting tumor angiogenesis in breast cancer partly *via* miRNA-dependent activation of PI3K/AKT and ERK1/2 pathway¹¹⁵. However, whether the CYP4Z2P- and CYP4Z1-3'UTRs functions in fatty acid metabolism in cancer, especially HCC, still needs to be further revealed.

2.3. Amino acid metabolism and glutamine metabolism

Liver is responsible for more than 80% of protein synthesis such as albumin, growth factors, and many other functionally important peptides. The carbon skeleton formed by hepatic protein decomposition is oxidized into CO₂ and H₂O to produce ATP, and also provides a carbon framework for the synthesis of new proteins, sugars and fatty acids. Furthermore, urea cycle unique in liver deals with nitrogenous wastes produced by amino acid metabolism that impair cellular activity. This part focuses on the understanding of targets related to amino acid and glutamine metabolism in HCC (Fig. 3).

2.3.1. Glutamine metabolism and transportation

Glutamine is a nitrogen and carbon donor as its skeleton is incorporated versatily when synthesizing proteins for different

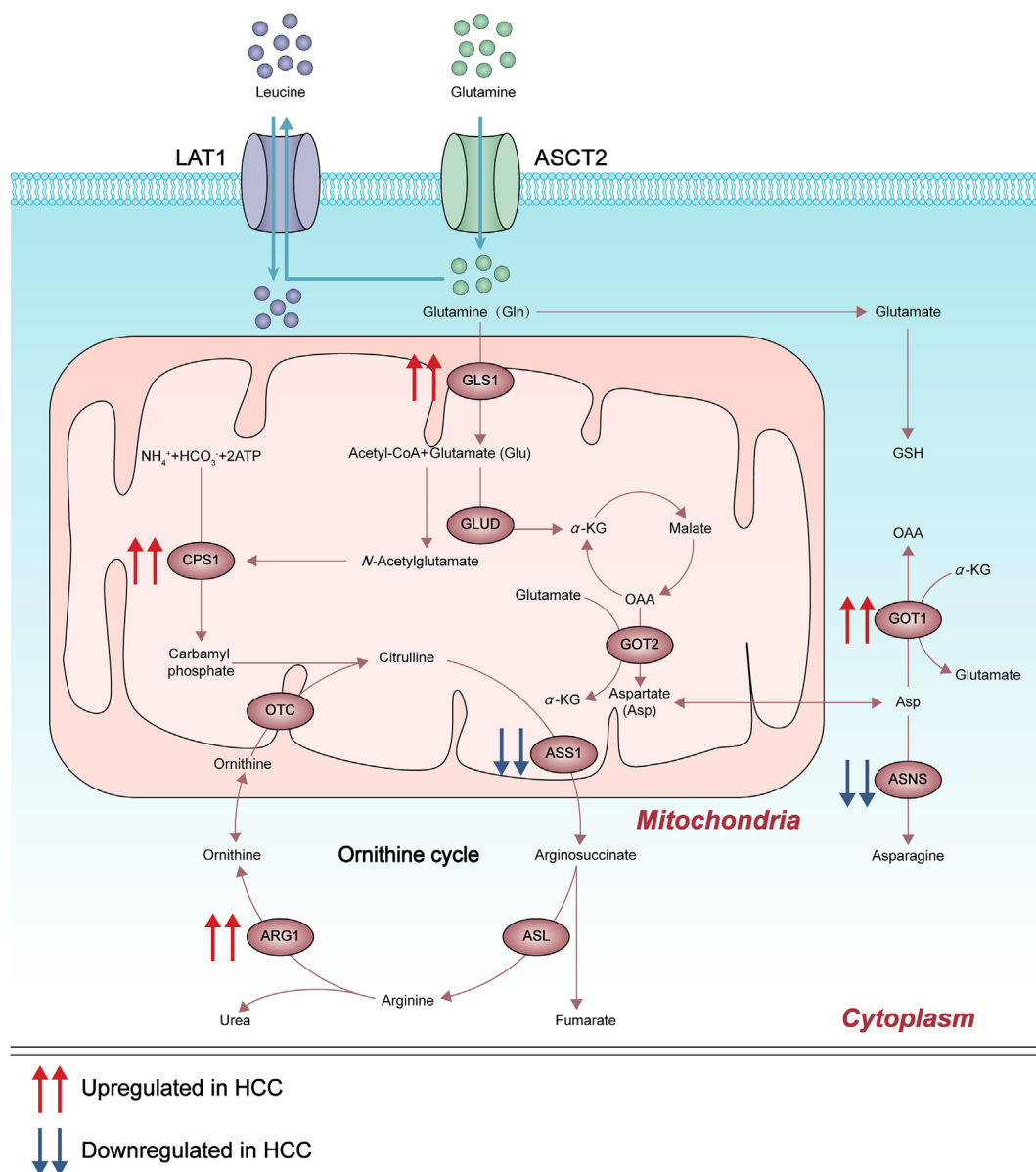


Figure 3 Deregulated alterations of amino acid metabolism and glutamine metabolism in HCC. Genes and metabolic intermediates of the amino acid and glutamine metabolism are dysregulated in HCC, some of which can be regulated by aberrantly activated oncogenes and loss of tumor suppressors, such as mutant Kirsten rat sarcoma 2 viral oncogene homolog, aflatoxin B1 as well as noncoding RNAs. CPS1, carbamyl phosphate synthase 1; ARG1, arginase-1; GLUD, glutamate dehydrogenase; GSH, glutathione; ASNS, asparagine synthetase; ASL, arginino-succinate lyase.

cell compartments¹¹⁶. Since proliferative tumor cells have increased glutamine demand, its catabolism, anabolism and transportation are essential for the survival and development of HCC¹¹⁷. Increased glutamine catabolism, also known as glutaminolysis, is one of the critical metabolic features for cancer cells. Glutamine-based therapy has been proposed as a potential strategy for cancer treatment¹¹⁸. Mammalian cells contain two distinct but related genes encoding glutaminase (enzyme for glutaminolysis), one of which is found in the kidney and a number of other tissues, including many cancer cells (called renal-type glutaminase or GLS1). Another is exclusively expressed in the liver (called liver-type glutaminase or GLS2). GLS1 overexpression boosts colony formation and cell proliferation of HCC cells with the

involvement of AKT/glycogen synthase kinase 3 beta/cyclin D1 axis¹¹⁹. Its upregulation is positively correlated with late-stage clinicopathological features and stem cell phenotype. GLS1 regulates the identity of cancer stem cells through ROS/WNT/ β -catenin pathway. Knockout of GLS1 inhibits tumorigenicity *in vivo*, suggesting that it act as a therapeutic target by eliminating cancer stem cells¹²⁰. In the contrast, GLS2 negatively regulates PI3K/AKT signaling which is often activated in HCC. Blocking the PI3K/AKT signaling pathway destroys the inhibitory effect of GLS2 on anchor dependent cell growth and xenograft growth of HCC cells¹²¹. In fibrotic livers, GLS2 level is decreased and glutamine synthase is absent, but solute carrier family 1 member 5 (*SLC1A5*) and GLS1 are up-regulated. The latter is accumulated in

Table 1 Targets for metabolic dysfunction in HCC.

Metabolism	Target
Targets in glucose metabolism in HCC	
Glycolysis	HK2 ²⁴ , PKM2 ^{25,37,38} , GAPDH ^{28,31,32} , GPI ³⁰ , ALDOA ^{33,34} , PGK1 ^{40,41}
Pentose phosphate pathway	G6PD ^{47,49,50} , TKT ^{48,51}
Gluconeogenesis	FBP1 ⁵⁴ , PCK1 ^{55,59–61} , G6PC ^{56–58}
Tricarboxylic acid cycle	PC ⁶⁴ , PDHA1 ⁶⁵ , IDH2 ^{66,67} , ME1 ^{69,70}
Targets in fatty acid and cholesterol metabolism in HCC	
Fatty acid uptake and transport	CD36 ⁷⁵ , FABPs ^{76–79} , LPL ^{80,81}
Fatty acid synthesis	ACLY ⁸² , ACC ⁸³ , FASN ⁸⁵ , SCD1 ^{87–91} , ACSL4 ⁹² , SREBP1 ⁹³ , LXR ⁹⁵
Fatty acid β -oxidation	CPT1A ⁹⁹ , MCAD ^{100,101} , LCAD ^{100,101} , MAGL ¹⁰³ , ATGL ¹⁰⁴
Cholesterol metabolism	HMGCR ¹⁰⁷ , SS ¹⁰⁷
Others	PPAR α ¹⁰⁹
Targets in amino acid metabolism and glutamine metabolism in HCC	
Glutamine metabolism and transportation	GLS1 ^{119,120} , GLS2 ¹²¹ , ASCT2 ¹²⁵ , LAT1 ^{128–131} , GOT1 ^{133,134}
Ornithine cycle	CPS1 ^{136,137} , ASS1 ^{138,139} , ARG1 ¹⁴⁰ , OTC ^{141,142}

fibrotic septa and does not compensate for the loss of GLS2 in hepatocytes. The restriction of glutamine inhibits the growth and fibrogenic activity of HSCs during NASH pathogenesis¹²².

Due to the increased request of glutamine, cancer cells absorb more from extracellular area than normal cells. Solute carrier transporters are highly expressed in the liver and act as metabolic gateways of cells, mediating the transport of a variety of essential nutrients and metabolites, such as glucose, amino acids, vitamins, neurotransmitters and inorganic/metal ions¹²³. Solute carrier transporters play an important role in the etiology of many metabolic diseases, as well as cancer. *SLCIA5*, known as alanine serine cysteine-preferring transporter 2 (ASCT2), transports glutamine in a Na⁺-dependent manner¹²⁴. *SLCIA5* expression in tumor tissues is significantly up-regulated compared with adjacent non-tumor tissues and positively correlated with tumor size, suggesting that *SLCIA5* could be a promising prognostic indicator for HCC patients¹²⁵. *SLCIA5* promoter activity and protein expression are both dependent on the presence of glutamine¹²⁶.

SLC7A5, also known as L-Type amino acid transporter 1 (LAT1), belongs to the amino acid–polyamine–organocation superfamily and forms a heterodimer amino acid transporter through the conserved disulfide bond interaction with 4F2 cell-surface antigen heavy chain (4F2hc; also known as *SLC3A2* or CD98)—a type II membrane glycoprotein that is indispensable for the protein stability of LAT1 and for its localization to the plasma membrane¹²⁷. *SLC7A5* protein in HCC tumor lesions is significantly higher than that in non-tumor areas, indicating the association of *SLC7A5* expression with tumor growth¹²⁸. Downstream oncogenic effector Yes-associated protein 1 and transcriptional coactivator with PDZ-binding motif of Hippo pathway increases the uptake of amino acids, activates cell growth regulator mammalian target of rapamycin complex 1 and promotes cell proliferation by up-regulating the expression of *SLC7A5*¹²⁹. Patients with higher *SLC7A5* expression have significantly shortened survival rate, suggesting that *SLC7A5* is a potential therapeutic target for HCC^{130,131}. Notably, the structure of human LAT1-4F2hc heteromeric amino acid transporter complex has been clearly resolved in recent years¹³², thereby providing deep insights into the function and mechanisms by which they influence human diseases, including HCC.

The coordination between the use of carbohydrates and amino acids to meet nutritional requirements is essential for physiological maintenance as well as cell growth, as the enzyme glutamate oxaloacetate transaminase 1 (GOT1) is one of the typical examples to regulate cell metabolism¹³³. Kirsten rat sarcoma 2 viral oncogene homolog is the most frequently mutated RAS isoform in cancer cells, its mutation has recently been shown to rely on GOT1 to support long-term cell proliferation¹³⁴. lncRNA TMPO-AS1 accelerates the progression of HCC by targeting the miR-429/*GOT1* axis, which provides a new way of thinking for the treatment of HCC¹³³.

2.3.2. Ornithine cycle

The toxic final product of amino acid metabolism, ammonia, is synthesized into urea through the ornithine cycle and excreted in urine as a detoxification pathway. Carbamyl phosphate synthase 1 is the first rate-limiting enzyme in the ornithine cycle and has been reported as an HCC-hypermethylated gene^{135,136}. It is down-regulated by aflatoxin B1 and thus inhibits cell proliferation and induces cell apoptosis in HCC¹³⁷. Argininosuccinate synthase 1 (ASS1) is another rate-limiting enzyme in the ornithine cycle, and tumor ASS1 deficiency is both a biomarker of prognosis and a predictor of susceptibility to arginine deprivation therapy¹³⁸. The expression of ASS1 in HCC patients is significantly reduced due to DNA methylation. Stable silencing of ASS1 promotes HCC cell migration and invasion. In addition, ASS1 knockdown increases phosphorylation of signal transducer and activator of transcription 3 in Ser727 and boosts HCC metastasis by upregulating differentiation inhibitor 1. It is suggested that ASS1 inhibits HCC metastasis and is a potential target for HCC diagnosis and therapy¹³⁹. Arginase-1, expressed in the liver cytoplasm, plays an important role in the ornithine cycle and is involved in anti-inflammatory effects, tumor immunity and immunosuppression-related diseases. Arginase-1 overexpression enhances HCC Huh7 cell viability, migration, and invasion, leading to a significant increase in protein and mRNA expression of key factors during EMT, such as vimentin, N-cadherin, and β -catenin¹⁴⁰. Ornithine transcarbamylase (OTC) localizes in mitochondrial matrix and catalyzes the conversion of ornithine and carbamyl-phosphate to citrulline. OTC deficiency disrupts the metabolic pathways of urea, resulting in the accumulation of ammonia in the

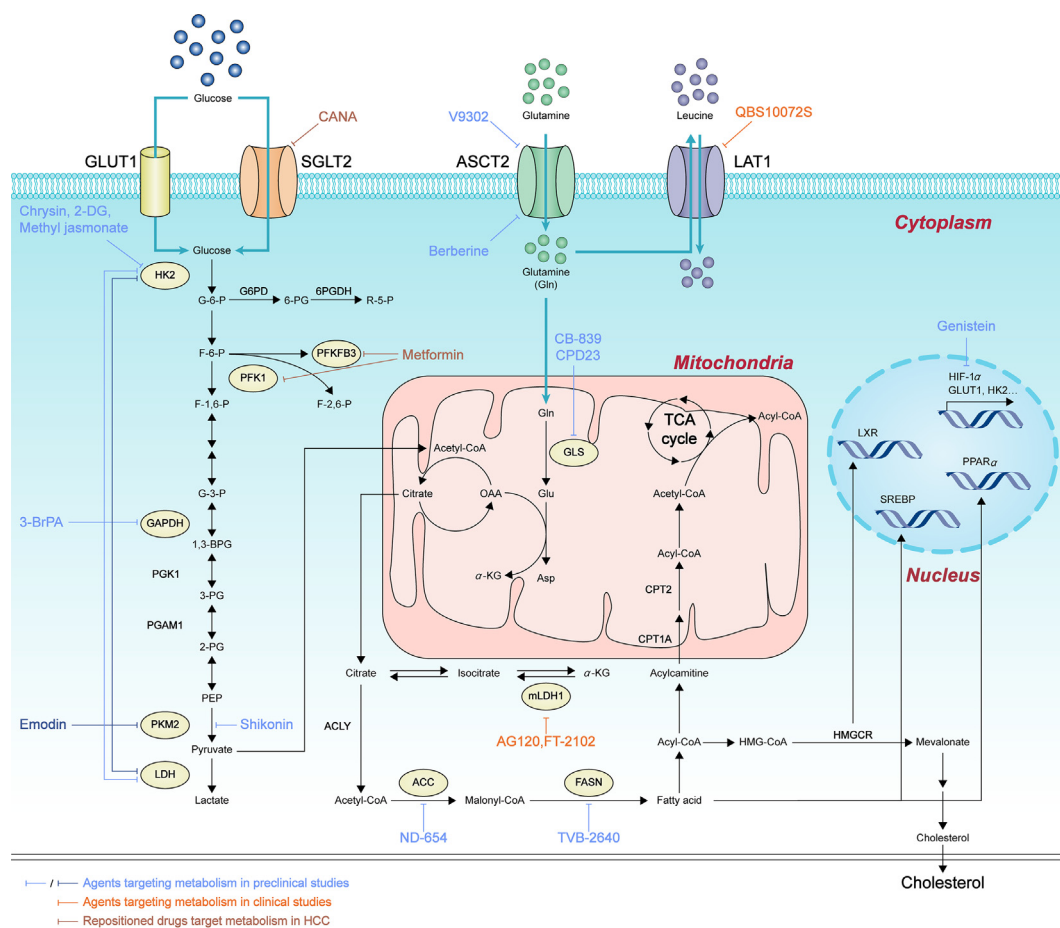


Figure 4 Agents targeting metabolism in HCC. This figure lists all drugs or chemicals developed as potentials to treat HCC by targeting metabolism. Light blue and dark blue lines depict agents targeting metabolism in preclinical studies. Orange lines depict agents targeting metabolism in clinical studies. Brown lines depict repurposed drugs targeting metabolism in HCC.

blood which is life-threatening in severe cases¹⁴¹. OTC silencing promotes proliferation of HCC SK-HEP-1 and Huh-7 cells. Clinical data indicate that patients with low level of OTC have a shorter overall survival time¹⁴². Above targets for metabolic dysfunction in HCC have been summarized in Table 1.

3. Emerging therapeutical strategies for the treatment of HCC

Given that metabolic rearrangements exist in HCC, the key or rate-limiting metabolic enzymes, metabolites, even drivers among metabolic changes, have attracted much attention. So far, a variety of drugs or chemicals have been developed as potentials to treat HCC. Whereas many agents are still in preclinical studies, some drugs have approached to clinical trials. Besides, some old drugs are repurposed to combat HCC by targeting metabolism (Fig. 4).

3.1. Preclinical studies

3.1.1. Glycolysis inhibitors

As one of the hallmarks in cancer, glucose metabolism has been targeted to combat against cancer challenges with the agents mainly inhibiting glycolysis in HCC therapy. 2-Deoxy-D-glucose (2-DG), an analog of glucose, is catalyzed by HK2 into 2-deoxy-D-glucose-6-phosphate, which is distinct from glucose

6-phosphate to noncompetitively inhibit the activity of HK2¹⁴³. 2-DG alone suppresses cell growth, metastasis and invasion in HCC cells, while enhances the anti-cancer effects synergistically with sorafenib^{144,145}. These results provide some insights into the design of combined strategies of 2-DG and other chemotherapeutic drugs for HCC treatment.

3-Bromopyruvic acid (3-BrPA) functions as a potential clinical chemosensitizer to optimize the therapeutic index of chloroethylnitrosoureas, a bifunctional antitumor alkylating agent¹⁴⁶. Besides the inhibition of HK2, the inhibition of GAPDH, PGK1, lactate dehydrogenase (LDH) and succinate dehydrogenase are also reported to be a part of anti-cancer activities of 3-BrPA^{147,148}. In all, GAPDH is characterized to be the primary intracellular target of 3-BrPA *via* mass spectrometry, immunoprecipitation techniques and *in vitro* enzyme kinetic studies¹⁴⁹. 3-BrPA could be a promising agent targeting glycolysis, or made into a fine combination strategy with other chemotherapeutic drugs for HCC treatment. Using a quantitative approach of metabolic control analysis, the central role of GAPDH in Warburg effect is established. A docking analysis indicates that koningic acid, a natural product, binds to the active site of GAPDH as a specific GAPDH inhibitor. The response to koningic acid covers NCI60 cell lines (a collection of 60 cancer cell lines from diverse tissue and genetic origins) *in vitro* shows broad but heterogeneous effects. Notably, only glucose uptake and lactate excretion are predictive of

koningic acid response, independent of the extent of enzyme inhibition and tissue type¹⁵⁰. However, whether koningic acid functions in HCC needs to be fully explored.

Inhibitors of some other glycolytic enzymes exert anti-cancer effects on cancer types other than HCC in preclinical studies, including PGK1 and phosphoglycerate mutase 1 (PGAM1). PGK1 catalyzes the conversion of 1,3-diphosphoglycerate (1,3-BPG) and ADP to 3-phosphoglycerate and ATP, which requires the binding of PGK1 to its substrates (1,3-BPG and ADP). Therefore, the design of PGK1 inhibitors has commonly focused on analogues of its natural substrate 1,3-BPG. Although several PGK1 inhibitors (CBR-470-1, bisphosphonates, terazosin or their derivatives) have been designed, the effects on cancer cells have not been reported yet¹⁵¹. GQQ-792, a thiodiketopiperazine derivative from the marine nature products, is characterized as a non-ATP-competitive PGK1 inhibitor, which leads to decreased glucose uptake and lactate production, and subsequent apoptosis of glioblastoma cells¹⁵². Moreover, a known yeast cell cycle-regulating kinase inhibitor, NG52, dose-dependently suppresses the proliferation of glioma cells *in vitro* and alleviates glioma tumor *in vivo* through inhibiting kinase activity of PGK1¹⁵³. A potent PGAM1 allosterical inhibitor, PGMI-004A, decreases 2-phosphoglycerate and increases 3-phosphoglycerate levels in lung cancer cells. Treatment of PGMI-004A also results in decreased lactate production, suppressed oxidative PPP flux and NADPH/NADP⁺ ratio, reduced biosynthesis of lipids and RNA, and attenuated cell proliferation, which are rescued by methyl-2-PG treatment. PGMI-004A treatment also suppresses cell proliferation in diverse human cancer and leukemia cells, but not in human dermal fibroblasts, human foreskin fibroblasts, human keratinocyte HaCaT cells and human melanocyte PIG1 cells, suggesting specific toxicity of PGMI-004A to the malignantly proliferating human cells¹⁵⁴. Given these findings, PGAM1 inhibitor PGMI-004A is implied to be a hopeful compound in cancer treatment. Targeting PGK1 and PGAM1 kinase activity could be a potential strategy for some types of cancer including glioma and lung cancer; however, how these inhibitors function in HCC needs further exploration.

Some natural products also exhibit promising anti-HCC effects, at least partly through inhibiting glycolysis genes or proteins expression, including GLUT1, HK2, PKM2 and LDHA. Inhibition of HK2 expression by chrysin, a natural flavone found in plant extracts which are widely used in traditional medicine in China, results in decreased glucose uptake and lactate production in HCC cells. The reduced expression of HK2 combines with voltage dependent anion channel-1 on mitochondria, which results in the translocation of BAX from the cytosol to the mitochondria and induction of cell apoptosis. And these effects of chrysin are markedly impaired in HK2 exogenously overexpressed HCC cells. Moreover, chrysin treatment restrains tumor growth in a HCCLM3 xenograft model and significantly decreases HK2 expression in chrysin-treated tumor tissue¹⁵⁵. Methyl jasmonate, a plant stress hormone, detaches HK2 from voltage dependent anion channel to cause a reduction in mitochondrial transmembrane potential. It leads to the release of cytochrome *c* and apoptosis inducing factor, thereby promoting cell apoptosis and exerting a prominent inhibitory effect on the growth of HCC LM3 and BEL-7402 cells, with little effect on normal liver cells¹⁵⁶. Shikonin, a natural naphthoquinone isolated from *Lithospermum erythrorhizon*, has therapeutic effects on various diseases, including cancer^{157,158}. Shikonin, suppresses cell proliferation and glycolysis, and thus leads to cell apoptosis in HCCLM3 and SMMC-7721 cells.

Artificial manipulation of PKM2 abolishes the effect of shikonin on cell proliferation, apoptosis, and glycolysis. Moreover, shikonin enhances the drug sensitivity of HCC cells to sorafenib¹⁵⁹. Genistein, a natural isoflavone with many therapeutic effects, including anti-tumor effects. Genistein inhibits glycolysis and induces mitochondrial apoptosis in HCC cells by directly down-regulating HIF-1 α , therefore inactivating GLUT1 and HK2. Genistein also reverses chemoresistance in sorafenib-resistant HCC cells and HCC-bearing mice¹⁶⁰. Emodin, another natural product, decreases the expression of glycolytic enzymes (HK2, PKM2, and LDHA), thus inhibits glycolysis, limits energy supply and attenuates the growth of HCC HepG2 cells¹⁶¹. Moreover, our previous study also showed that saponin monomer 13 of dwarf lilyturf tuber (DT-13), the main steroidal saponin from *Liriodops Radix*, inhibits glucose uptake and ATP generation, reduces lactate production of colorectal cancer cells. DT-13 also remarkably inhibits GLUT1 expression in both mRNA and protein levels. Knockdown of GLUT1 decreases inhibitory ratio of DT-13 on colorectal cancer cell growth *in vitro* and in an orthotopic implantation mouse model¹⁶². However, whether and how DT-13 functions in HCC still need to be illustrated. Taken together, natural products serve as a rich resource to show potential to be applied in future HCC clinical treatment as glycolysis inhibitors.

3.1.2. Pentose phosphate pathway inhibitors

In addition to glycolysis, blocking PPP in HCC therapy has been illustrated. TKT inhibitor oxythiamine, a thiamine antagonist, synergizes with sorafenib to halt HCC cell growth both *in vitro* and *in vivo*, mechanically by raising the ROS levels⁵¹. These findings suggest that oxythiamine could be used in a combination therapy together with sorafenib.

3.1.3. Chemical inhibitors targeting gluconeogenesis

FBP1 loss is frequently displayed in HCC, as epigenetic regulation^{163,164} and ubiquitin-mediated degradation¹⁶⁵ lead to the suppression of FBP1. Thus, chemical inhibitors targeting histone deacetylase, promoter methylation, and other upstream regulators have been explored to restore the expression of FBP1 for HCC treatment. Treatment with histone deacetylase inhibitors sodium butyrate, SAHA or LBH589 upregulates FBP1 expression in HCC, leading to a switch from glycolysis to gluconeogenesis. The therapy inhibits HCC HepG2 and SK-HEP-1 cells growth *in vitro* and restrains tumor growth in a SK-HEP-1 xenograft model *in vivo*¹⁶³. Besides, treatment with lysine specific histone demethylase 1 inhibitor tranlycypromine robustly increases FBP1 expression in HCC HepG2 cells¹⁶⁶. However, these epigenetic drugs for HCC treatment are still far away from clinical application, in particular, due to their low specificity and associated pleiotropic effects¹⁶⁷. In HCC cells, tripartite motif-containing protein 28 directly binds to FBP1 and leads to its ubiquitination and degradation. Meanwhile, 26S proteasome inhibitor bortezomib abolishes FBP1 loss, thus inhibits cell growth, decreases glucose consumption and lactate production¹⁶⁵. Significant activity of bortezomib monotherapy in HCC patients is still absent, but a combination therapy is suggested¹⁶⁸. Furthermore, dexamethasone, a synthesized glucocorticoid, is reported to restore the expression of gluconeogenesis genes, including FBP1, thereby antagonizing the Warburg effect and showing efficacy in HCC treatment¹⁶⁹. Nevertheless, clinical relevance is limited due to adverse effects caused by poor specificity.

3.1.4. Agents targeting fatty acid metabolism

Given that ACC functions as rate-limiting enzymes in *de novo* fatty acid synthesis, it is of special interest to establish ACC as a therapeutic target in multiple cancer types, including HCC¹⁷⁰. While ACC inhibition has been profiled in preclinical analysis of various other cancer types, little evidence exists in HCC models exclusively¹⁷¹. ND-654, a hepatoselective (about 3000:1 liver to muscle exposure), allosteric inhibitor of ACC1 and ACC2, is explored for efficacy in rat models of liver cirrhosis and HCC. Interestingly, dual inhibition of ACC1 and ACC2 by ND-654 significantly reduces HCC incidence by 41%, which is comparable to sorafenib alone (57% reduction). The combination of ND-654 and sorafenib further decreases HCC incidence by 81%¹⁷². Numerous FASN inhibitors, such as C75, C93, orlistat, GSK2194069 and GSK837149A have been examined for pre-clinical anti-tumor activities^{173,174}. Unfortunately, agents above have shown unfavorable toxicity, preventing them from moving into the clinic as viable treatment options for patients. To date, another small molecule TVB-2640 is the only FASN inhibitor moving into the phase I human clinical trial. It is initiated to investigate safety and to determine the recommended phase II dose of TVB-2640 as monotherapy and in combination with paclitaxel or docetaxel in patients with advanced or metastatic solid tumor cancers¹⁷⁵. Although no objective responses are observed, an interesting trend of longer median time to progression is observed among patients with Kirsten rat sarcoma 2 viral oncogene homolog-mutant non-small cell lung cancer compared to wild-type. TVB-2640 is also being studied for the treatment of NASH, as well as other cancer types, including ovarian, and breast cancer¹⁷⁶. ACLY inhibitors have been reported as cholesterol-lowering drugs in human clinical trials^{177,178}. Several natural and synthetic ACLY inhibitors alone or in combination with other chemotherapeutics have demonstrated anti-proliferative effects on several cancer cell lines *in vitro* and *in vivo*, including lung cancer, ovarian cancer, and leukemia^{179,180}. However, further human clinical trials dedicated to evaluate ACLY inhibitors as anti-tumor therapeutics and in the treatment of HCC are still needed. A939572, an SCD1 inhibitor, suppresses the number and size of spheres formation, migration and invasion, in both SCD1-high expressing HCC Huh7 and PLC/PRF/5 cells. Moreover, A939572 sensitizes those cells to sorafenib *via* induction of ER stress-induced differentiation⁹¹. Thus, the deep exploration for A939572 in HCC therapy deserves to be conducted.

In addition to target the enzymes (ACC, FASN, ACLY, and SCD1) of fatty acid synthesis for HCC therapy, transcriptional factors like LXR has also been emphasized. TO901317, a specific LXR agonist, inhibits HCC progression by upregulating LXR α , downregulating GLUT1 and MMP9 expression, and decreasing glucose uptake in HCC SMMC-7721 and HepG2 cells⁹⁵.

YM-53601, a squalene synthase inhibitor of cholesterol biosynthesis pathway, synergizes doxorubicin-mediated HCC growth arrest and cell death *in vivo*¹⁰⁷. It is suggested that targeting enzymes in cholesterol synthesis pathways in combination with other drugs could be an approach to HCC therapy.

3.1.5. Agents targeting amino acid and glutamine metabolism

Targeting GLS1 is expected to selectively halt the growth of tumor cells but not normal cells¹⁸¹. Various GLS1 inhibitors have been developed for cancer; however, some of which show good anti-tumor activity but serious toxicity *in vivo*, *i.e.*, acivicin and 6-diazo-5-oxo-L-norleucine, perhaps due to the lack of selectivity as GLS2 is important for liver function¹⁸². Bis-2-(5-

phenylacetamido-1,2,4-thiadiazol) ethyl sulfide (BPTES) is a GLS1 allosteric inhibitor but with poor solubility (0.01 $\mu\text{mol/L}$)¹⁸³, thus a series of BPTES derivatives including CB-839 have been explored to improve solubility¹⁸⁴. As another potent GLS1 allosteric inhibitor, CB-839 inhibits triple-negative breast cancer cells growth *in vitro*, but results only 61% tumor inhibition in patient-derived triple-negative breast cancer xenograft model and 54% tumor inhibition in JIMT-1 xenograft model¹⁸⁴. Later, CPD23 (a selenium analogue of CB-839) is designed to improve the anti-cancer activity *in vivo*, but still shows partial tumor growth inhibition in a glutamine-dependent mouse H22 liver cancer xenograft model. Despite of this, CPD23 causes tumor tissue damage and prolongs survival in this model¹⁸². After all, effective and selective GLS1 inhibitors for HCC treatment need deep exploration. V-9302 is a competitive small molecule antagonist of transmembrane glutamine flux which selectively and potently inhibits *SLC1A5* (ASCT2)-mediated glutamine uptake in a concentration-dependent manner. It exhibits a 100-fold improvement in potency over gamma-L-glutamyl-*p*-nitroanilide, and results in attenuated cancer cell growth and proliferation, increased cell death, and increased oxidative stress. V-9302 prevents tumor growth in xenograft mouse models *in vivo*¹⁸⁵ and sensitizes glutamine-dependent HCC cells to glutaminase inhibitor CB-839 treatment by inducing ROS and promoting apoptosis¹⁸⁶. Dual inhibition of glutamine metabolism by targeting both glutaminase and *SLC1A5* represents a potential novel treatment strategy for glutamine addicted HCC. Our recent data reveal a novel anti-cancer mechanism of a Topo I inhibitor topotecan as inhibition of glutamine uptake *via* downregulation of *SLC1A5* in gastric cancer cells¹⁸⁷. Some natural products also exhibit promising anti-cancer effects *via* affecting genes or proteins related to amino acid and glutamine metabolism. For example, berberine, a natural compound isolated from herbal medicines, suppresses glutamine intake and the growth of HCC tumor xenografts, as well as the proliferation of HCC cells *in vitro*¹⁸⁸. Agents targeting deregulated metabolism for the treatment of HCC in preclinical studies has been summarized in Table 2.

3.2. Clinical trials

3.2.1. Mutant IDH inhibitors

A number of mutant IDH (mIDH) inhibitors have been developed to directly inhibit the neomorphic activity of mIDH enzymes in an effort to reduce oncometabolite D-2-hydroxyglutarate production. The inhibitors exert anti-cancer effects especially in IDH-mutant acute myeloblastic leukemia (AML), gliomas and other cancers. Small molecule ivosidenib (AG-120) is an orally available inhibitor of mIDH1 that has been developed for the treatment of cancer in patients with IDH1 mutations¹⁸⁹. Meanwhile, relevant clinical trials for AML, cholangiocarcinoma, glioma, myelodysplastic syndromes and solid tumors are ongoing worldwide (NCT04056910 and NCT02073994). Olutasidenib (FT-2102) is another highly potent, orally active, brain penetrant and selective inhibitor of mIDH1, with IC₅₀ values of 21.2 and 114 nmol/L for IDH1- R132H and IDH1- R132C, respectively. FT-2102 is under study in the treatment of AML or myelodysplastic syndrome¹⁹⁰. Another phase Ib/II study of FT-2102 in participants with advanced solid tumors (including HCC, bile duct carcinoma, intrahepatic cholangiocarcinoma, other hepatobiliary carcinomas), and gliomas with an IDH1 mutation is ongoing (NCT03684811). To sum up, although pharmaceutical agents targeting mIDH have been approved for cancers with IDH mutation, efficacy of these

Table 2 Agents targeting deregulated metabolism for the treatment of HCC in preclinical studies.

Compd.	Highest phase	Mechanism of action	Functions in HCC	Ref.
Agents targeted glucose metabolism				
<i>Glycolysis inhibitors</i>				
2-DG	Pre-clinical	An analog of glucose to inhibit HK2 activity	Suppresses cell growth, metastasis and invasion in HCC HLF and PLC/PRF/5 cells; synergistically enhances the effect of sorafenib; 2-DG and sorafenib increase HCC Hep3B and Huh7 persister cells apoptosis and inhibit colony formation, significantly inhibit tumor growth in a Hep3B persister cell xenograft model	144,145
3-BrPA	Pre-clinical	Targets GAPDH, decreases GAPDH, LDH and SDH expression at protein levels; Targets HK2 and dissociates HK2 from mitochondrial complex	—	146–148
Chrysin	Pre-clinical	Inhibits HK2	A potential clinical chemosensitizer to optimize the therapeutic index of CENUs Decreases glucose uptake and lactate production in HCC cells, reduces HK2 which combined with VDAC1 on mitochondria, thus induction of cell apoptosis; Restrains tumor growth in a HCCLM3 xenograft model and significantly decreases HK2 expression	155
Methyl jasmonate	Pre-clinical	A plant stress hormone to detach HK2 from VDAC	Causes a reduction in mitochondrial transmembrane potential that leads to the release of cytochrome <i>c</i> and apoptosis inducing factor, results in intrinsic apoptosis, thereby exerting a prominent inhibitory effect on the growth of HCC cells	156
Shikonin	Pre-clinical	Inhibits PKM2	Suppresses cell proliferation and glycolysis and thus leads to cell apoptosis in HCC LM3 and SMMC-7721 cells, enhances the drug sensitivity of HCC cells to sorafenib	159
Genistein	Pre-clinical	Directly downregulates HIF-1 α , therefore inactivating GLUT1 and HK2	Inhibits glycolysis and induces mitochondrial apoptosis in HCC cells, and enhances the antitumor effect of sorafenib in sorafenib-resistant HCC cells and HCC-bearing mice	160
Emodin	Pre-clinical	Decreases glycolytic enzymes expression (HK2, PKM2, and LDHA)	Inhibits glycolysis, limits energy supply and attenuates the growth of HCC HepG2 cells	161
<i>Pentose phosphate pathway inhibitors</i>				
Oxythiamine	Pre-clinical	A TKT inhibitor	Synergizes sorafenib to halt HCC cell growth both <i>in vitro</i> and <i>in vivo</i>	51
<i>Chemical inhibitors targeting gluconeogenesis</i>				
Sodium butyrate, SAHA, LBH589	Pre-clinical	HDAC inhibitors	Upregulates FBP1 expression in HCC, leading to a switch from glycolysis to gluconeogenesis and inhibits HCC HepG2 and SK-HEP-1 cells growth <i>in vitro</i> and restrains tumor growth in a SK-HEP-1 xenograft model <i>in vivo</i>	163
Tranylcypromine	Pre-clinical	An LSD1 inhibitor	Increases FBP1 expression in HCC HepG2 cells	166
Bortezomib	Pre-clinical	A 26S proteasome inhibitor	Abolishes FBP1 loss, thus inhibits cell growth, decreases glucose consumption and lactate production	165
Dexamethasone	Pre-clinical	A synthesized glucocorticoid	Restores the expression of gluconeogenesis genes, including FBP1, thereby antagonizing the Warburg effect and showing efficacy in HCC treatment	169
Agents targeted for fatty acid metabolism				
ND-654	Pre-clinical	An allosteric inhibitor of ACC1 and ACC2	Reduces HCC incidence by 41%, which is comparable to the results with sorafenib alone (57%); ND-654 and sorafenib significantly reduce HCC incidence by 81%	172

Table 2 (continued)

Compd.	Highest phase	Mechanism of action	Functions in HCC	Ref.
A939572	Pre-clinical	An SCD1 inhibitor	Suppresses the number and size of spheres formation, migration and invasion, in both SCD1-high expressing HCC Huh7 and PLC/PRF/5 cells; sensitizes those cells to sorafenib <i>via</i> induction of ER stress-induced differentiation	91
TO901317	Pre-clinical	A specific LXR agonist	Inhibits HCC progression by upregulating LXR α , downregulating GLUT1 and MMP9 expression, and decreasing glucose uptake in HCC SMMC-7721 and HepG2 cells	95
YM-53601	Pre-clinical	An SS inhibitor	Synergizes doxorubicin-mediated HCC growth arrest and cell death <i>in vivo</i>	107
Agents targeted for amino acid and glutamine metabolism				
CPD23	Pre-clinical	A selenium analogue of CB839 that targets GLS1	Shows partial tumor growth inhibition in a GD mouse H22 liver cancer xenograft model; Causes tumor tissue damage and prolongs survival	182
V-9302	Pre-clinical	Selectively and potently targets ASCT2 (<i>SLC1A5</i>)	Inhibits ASCT2-mediated glutamine uptake in human cells in a concentration-dependent manner, attenuates cancer cell growth and proliferation, increases cell death, and increases oxidative stress Sensitizes GD HCC cells to glutaminase inhibitor CB-839, by inducing ROS, thus results in apoptosis of GD cells; Shows tumor inhibition in HCC xenograft mouse models <i>in vivo</i>	185,186

–, not applicable. CENUs, chloroethylnitrosoureas; GD, glutamine-dependent; HDAC, histone deacetylase; LSD1, lysine specific histone demethylase 1; SDH, succinate dehydrogenase; VDAC, voltage dependent anion channel.

agents in HCC patients with mIDH is still under expectation in above trials.

3.2.2. Agents targeting fatty acid metabolism

In terms of therapeutically targeting dysregulated fatty acid metabolism in cancer, FASN has arguably received the most widespread interest. It is not surprising considering its multifaceted roles in supporting both anabolic metabolism and oncogenic signaling. However, the transition of FASN inhibitors from bench to bedside has largely been elusive, and marked with several challenges and shortcomings. The first-generation FASN-targeting drugs, such as C75, orlistat and cerulenin, initially show obvious anti-cancer effects in preclinical studies^{191–193}. Systemic side effects as drastic weight loss and anorexia are found in clinical study¹⁹⁴, which highlights the involvement of fatty acid metabolic enzymes in both disease pathology and normal whole-body metabolic homeostasis. More recently, next-generation FASN inhibitors, TVB-3166 (an orally-available, reversible, and selective FASN inhibitor) and TVB-2640 (the first inhibitor of FASN to enter the clinic), have shown tremendous anti-tumor effects in preclinical breast and colorectal cancer models, as well as excellent tolerability and limited systemic toxicity in early-phase clinical trials^{195,196}. One explanation for the improved tolerance of the next-generation inhibitors is that TVB-3166 and TVB-2640 do not contribute to the indirect activation of CPT1 in peripheral tissues, in contrast with C75 and cerulenin¹⁹⁵. The induction of β -oxidation peripherally following C75 or cerulenin treatment contributes to increased energy expenditure, loss of adipose tissue and significant weight loss, whereas next-generation inhibitors display higher specificity for FASN with limited off-target effects^{195,197}. Although there is not any FASN inhibitors applied in clinical trials for now for HCC treatment, the application of FASN

inhibitors in other cancer types provides guidance to HCC treatment.

Statins, known as HMGCR inhibitors, exert an anti-tumor effect in many cancer types, including breast cancer, AML, and have a high therapeutic index to target tumors *in vivo*, despite the ubiquitous expression of the mevalonate pathway¹⁰⁶. However, how statins function in HCC is obscure. A combined treatment of pravastatin and chemoembolization improves survival of patients with advanced HCC compared to patients receiving chemoembolization alone¹⁹⁸. Still, deep exploration for statins in HCC therapy needs to be conducted in clinical trials.

3.2.3. SLC7A5 inhibitors

Since *SLC7A5* is overexpressed in a variety of cancers, efforts are undertaken in order to synthesize and characterize potent inhibitors of *SLC7A5*-mediated amino-acids transport for cancer intervention. JPH203 is recently evaluated in a first phase I clinical trial as a *SLC7A5* selective inhibitor. Six out of 17 patients diagnosed with advanced solid tumors achieves partial response or stable stage. Four out of the 6 responders are diagnosed with biliary tract cancer, in which plasma levels of *SLC7A5* substrates remains high¹⁹⁹. High plasma levels of *SLC7A5* substrate amino acids could be an important factor that determines the efficacy of JPH203 in biliary tract cancer. However, since only 17 patients are enrolled in this phase I clinical trial, these findings need to be validated in a larger cohort of patients. Importantly, based on the promising results of the phase I clinical trial, JPH203 is currently being evaluated in a phase II clinical trial in patients with advanced biliary tract cancers (UMIN Clinical Trials Registry UMIN000034080). Besides, QBS10072S, another novel small-molecule inhibitor targeting *SLC7A5*, is now under a dose escalation phase I study to assess its safety, tolerability,

Table 3 Agents targeting metabolism for the treatment of HCC in clinical trials.

Compd.	Highest phase	Mechanism of action	Types of cancer	Ref./NCT No.
AG-120 (Ivosidenib)	I	Targets IDH1 and prevents production of D-2-hydroxyglutarate	IDH1-mutant advanced cholangiocarcinoma; IDH1-mutant advanced solid tumors	NCT04056910, NCT02073994
	II			
FT-2102 (Olutasidenib)	Ib/II	Targets IDH1 and reduces production of D-2-hydroxyglutarate	Advanced solid tumors (including HCC, bile duct carcinoma, intrahepatic cholangiocarcinoma, other hepatobiliary carcinomas), and gliomas with an IDH1 mutation	NCT03684811
Pravastatin	—	A HMGCR inhibitor	Pravastatin and chemoembolization improve survival of patients with advanced HCC compared to patients receiving chemoembolization alone	198
QBS10072S	I	Inhibits LAT1 (<i>SLC7A5</i>)	Patients with advanced or metastatic cancers with high LAT1 (<i>SLC7A5</i>) expression including liver cancer	NCT04430842

—Not applicable.

pharmacokinetics, and pharmacodynamics (NCT04430842), and the applicable cancer types includes HCC. The response of the test is still expecting. The agents used to target metabolism for HCC treatment in clinical trials are summarized in Table 3.

3.3. Repositioned drugs target metabolism

Drug repositioning, also known as drug rediscovery or drug repurposing, is well defined to explore new anti-cancer indications for existing drugs due to their long clinical history and established safety records^{200,201}. For instance, Repurposing Drugs in Oncology project (ReDO project) (<http://www.redo-project.org>) has identified more than 270 licensed non-cancer drugs with evidence of anticancer activity. Among them, some drugs are explored to exert anti-cancer properties at least in part by targeting metabolism (Table 4).

3.3.1. Metformin

Metformin, a first-line therapy for the treatment of type 2 diabetes, is found to have potentials as an anti-cancer agent or an adjuvant chemotherapy sensitizer *in vitro* and *in vivo*^{206,207}. Mechanistically, metformin affects mitochondrial metabolism in ovarian cancer cells²⁰⁸ through the liver kinase B1/adenosine monophosphate-activated protein kinase (AMPK) signaling pathway^{209,210} or AMPK-independent signaling²¹¹. Besides, metformin also induces breast cancer cell apoptosis and inhibits cell proliferation in nutrient-poor conditions through down-regulating PKM2 expression, which is mediated by AMPK activation²¹². In a recent study, metformin alleviates HCC cell proliferation by decreasing glycolytic flux through inhibition of PFK1 *in vitro*. Besides, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3, a potent allosteric activator of PFK1, is also significantly inhibited by decreased HIF-1 α accumulation induced by metformin²⁰³. Moreover, metformin diminishes the increase in respiration upon HK2 depletion in HCC cells, and thus induces

cell death both *in vitro* and *in vivo*²⁰². It is partly because that loss of HK2 causes a reduction in glycolysis and thus a compensatory upregulation of oxidative phosphorylation. Collectively, these results provide new insights into the mechanisms of metformin in combatting HCC.

Since the side effects and safety records have been well-established for metformin, its anti-cancer effect is promisingly applicable in a variety of cancer types mentioned above. Till now, about 361 clinical trials using metformin with/without other drugs as treatment conducted in cancer patients are ongoing (<https://clinicaltrials.gov>)²¹³. Although there is no clinical trial conducted using metformin as a single treatment for HCC for now, its combination with other drugs for HCC treatment are included. A phase II clinical trial is aimed to evaluate the efficacy and safety of high-dose vitamin C combined with metformin in the treatment of malignant tumors, including HCC (NCT04033107). Besides, a phase III clinical trial is conducted to compare the role of celebrex (a selective cyclic oxidase-2 inhibitor) alone, metformin alone, and celebrex plus metformin in preventing HCC recurrence after hepatic resection (NCT03184493), since some observational studies with small sample size have found that metformin plus celebrex reduce the recurrence rate of HCC after surgery²¹⁴. Yet, the clinical efficacy needs to be monitored and underlying mechanisms need to be illustrated thoroughly.

3.3.2. Canagliflozin

Canagliflozin (CANA) is a SGLT2 inhibitor, which decreases circulating glucose levels in type 2 diabetes patients by inhibiting SGLT2-dependent reabsorption of glucose in the kidney. A newly research has found that CANA exerts anti-cancer effects in pancreatic cancer and prostate cancer *in vitro* and *in vivo*, potentially through inhibition of SGLT2-mediated glucose uptake²¹⁵. Besides, several other studies about CANA actions in HCC are also carried out.

Table 4 Repositioned drugs targeting metabolism in HCC treatment.

Drug	Highest phase	Mechanism of action	Functions in cancer/types of cancer	Ref./NCT No.
Metformin	[1] [2] Pre-clinical	[1] Mitochondrial complex I inhibitor which diminishes the increase in respiration upon HK2 depletion	[1] Induces HCC Huh7 and HepG2 cell death <i>in vitro</i> and inhibits Huh7 subcutaneous tumor growth <i>in vivo</i> upon HK2 depletion	[1] ²⁰²
		[2] Decreases glycolytic flux through the HIF-1 α /PFKFB3/PFK1 pathway	[2] Alleviates HCC Huh7 and HepG2 cell proliferation	[2] ²⁰³
	[3] [4] Phase 2	[3] & [4] Needs to be monitored and illustrated	[3] Evaluates the efficacy and safety of high-dose vitamin C combined with metformin in the treatment of malignant tumors, including HCC	[3] NCT04033107
			[4] Compares the role of celebrex alone, metformin alone, and celebrex plus metformin in preventing HCC recurrence after hepatic resection	[4] NCT03184493
Canagliflozin (CANA)	[1] [2] Pre-clinical	[1] Inhibits glucose uptake presumably in a SGLT2-dependent way, induces G2/M arrest and facilitates apoptosis with inhibited phosphorylation of ERK, AKT, P38 and cleavage of caspase3	[1] Suppresses SGLT2-expressing HCC Huh7 and HepG2 cells proliferation and xenograft tumors growth both <i>in vitro</i> and <i>in vivo</i> , but not SGLT2-null HCC HLE cells, or PHH and LX-2 cells	[1] ²⁰⁴
		[2] Inhibits ectopic fat accumulation in the liver and suppresses the ratio of GSSG/GSH to reduce oxidative stress in adipose tissue	[2] One year CANA treatment significantly reduces the number of liver tumors in WD-fed MC4R-KO mice compared to vehicle-treated groups	[2] ²⁰⁵

GSSG/GSH, oxidized/reduced glutathione; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3.

CANA has an inhibitory effect on the glucose uptake of SGLT2-expressing HCC Huh7 and HepG2 cells, but not SGLT2-null HCC HLE cells, or primary human hepatocytes and HSCs (LX-2). As three above HCC cell lines all overexpresses GLUT1²¹⁶, it suggests that the glucose uptake inhibitory effect of CANA on HCC cells is presumably SGLT2-dependent. Similarly, CANA inhibits cell proliferation and subcutaneous xenograft tumors growth in HCC Huh7 and HepG2 cells, but not SGLT2-null HCC HLE cells. In addition, CANA also induces G2/M arrest and cell apoptosis with suppressed phosphorylation of ERK, AKT, P38 and cleavage of caspase3 in Huh7 and HepG2 cells²⁰⁴. Inconsistently, HCC cell growth is not directly inhibited by tofogliflozin, another SGLT2 inhibitor, even under insulin resistance-mimicking and hyperglycemic conditions²¹⁷. One possible explanation for this discrepancy is at least partially the differential effect on SGLT1 and SGLT2 between CANA and tofogliflozin²¹⁸. Above all, CANA is a potential new strategy for the treatment of HCC.

In a study investigating the effect of CANA on NASH and NASH-associated HCC, a mouse model of human NASH is established using Western diet (WD)-fed melanocortin 4 receptor-deficient (MC4R-KO) mice. CANA treatment for 8 or 20 weeks alleviates hepatic steatosis and hepatic fibrosis in WD-fed MC4R-KO mice, separately. Not surprisingly, CANA-treatment for 1 year

significantly reduces the number of liver tumors in WD-fed MC4R-KO mice compared to vehicle-treated groups. Mechanically, CANA induces adipose expansion without deteriorating inflammation or fibrosis, and markedly suppresses the ratio of oxidized/reduced glutathione to reduce oxidative stress in adipose tissue. It implies an inhibitory effect of CANA on ectopic fat accumulation in the liver and therefore NASH and NASH-associated HCC *via* promoting healthy adipose expansion²⁰⁵. Nevertheless, clinical trials to investigate CANA as monotherapy or in combination with other drugs in cancer treatment is still rare. For now, there is only one project conducted to check whether controlling the glucose/insulin feedback enhances the efficacy of serabelisib, a PI3K alpha isoform inhibitor, in treating solid tumors without the inclusion of HCC patients though (NCT04073680). Thus, CANA for HCC treatment needs further exploration.

4. Challenges and opportunities

Currently, primarily two targeted small-molecule TKIs, sorafenib⁶ and lenvatinib⁷, are approved by FDA as the first line choice for HCC treatment. Although their targets are mainly cancer-related protein kinases (*i.e.*, RAF/MEK/ERK, VEGFR, PDGFR), it is

recently revealed that sorafenib disrupts lipogenesis and triggers liver cancer cell death by targeting SCD1 through the ATP/AMPK/mTOR/SREBP1 pathway²¹⁹. Moreover, it uncovers that decreased mitochondrial biogenesis is found in sorafenib-resistant cells under sorafenib treatment, mechanically by accelerated degradation of peroxisome proliferator-activated receptor γ coactivator 1 β ²²⁰. The above studies reveal cell metabolism-associated signaling pathways mediated by sorafenib, which potentially allow strategies to augment the efficacy and specificity of sorafenib in HCC cells. Taken together, metabolic targeting therapy could also be a supplemental choice in combination with sorafenib or lenvatinib to treat HCC in the future.

Whether metabolic dysregulation is the causative factor of HCC or a consequence of HCC development still remains inconclusive; however, sufficient evidence suggests that metabolic dysregulation acts as a critical contributor in the initiation and progression of HCC. For instance, although overexpression of GAPDH fails to develop HCC without carcinogen presence in mouse models, it does aggravate tumor development by using GAPDH transgenic mouse in a diethylnitrosamine-induced HCC mouse model. Mechanically, overexpression of GAPDH promotes diversion from glycolysis to serine biosynthesis, by elevating phosphoglycerate dehydrogenase and promoting histone methylation independent of its catalytic function, thereby accelerates cell proliferation and liver tumorigenesis²⁸. Moreover, hepatocyte-specific loss of the gluconeogenic enzyme FBP1 disrupts liver metabolic homeostasis which induces steatosis with the activation and senescence of HSCs, and therefore aggravates tumor progression⁵⁴. Moreover, preclinical mouse models for studying NASH-driven HCC²²¹ also supports the causative role of metabolic dysregulation in HCC. A recent review article also echoes that increasing evidence points to the involvement of metabolic stress in the initiation of primary liver cancers, over 90% among which are HCC²²². Also, we are not able to deny that the consequence of HCC development results in metabolic alterations. The rapid cell growth surely requires more metabolic supply. As shown, genomic deviations in HCC such as mutations in *TP53* (in 31% of patients), *MYC* (in 15%), fuels from pentose phosphate pathway as well as serine metabolism^{223,224}. Although a range of preclinical studies targeting metabolism have shown tremendous anti-tumor effects in HCC, only AG120 and FT2102 (both target mIDH1) [NCT02073994] or QBS10072S (targets *SLC7A5*) [NCT04430842] are now tested in phase I/II clinical trials for HCC treatment. Thus, mIDH1 or *SLC7A5* are possibly most promising targets to intervene metabolic dysfunction in HCC to date, but still needs further supportive evidence.

In the past decade, the application of integrative high-throughput omics approaches, including genomics, transcriptomics, proteomics and metabolomics, in parallel with informatics and computational analysis studies, has uncovered many novel genes or deregulated metabolites and therefore built up a metabolic network during HCC development. This information contributes greatly to the discovery and development of new agents targeting metabolism. However, the use of HCC cell lines in the *in vitro* screening studies has significant limitations, since these cells have at least partially different metabolic properties and therefore fails to recapitulate the key features of HCC cells in cancer tissues *in vivo*. Mouse models and patient-derived xenograft models with full pictures of metabolic dysregulation instead have a seat at the table of drug discovery in HCC therapy. Even though some genetically engineered murine models are validated to investigate the potential as a drug target of a certain

gene or abnormally altered metabolic pathway, the limitations as high-cost and time-consuming set an obstacle as an efficient tool for *in vivo* drug screening against this life-threatening malignancy. In addition, due to the genetic heterogeneity of HCC in clinic, the available genetically modified animal models are still far from full coverage. Alternatively, long-term organoid culture derived from tumor biopsies of HCC patients is a good choice to screen the emerging drug agents before clinical phase and to develop tailored therapies individually²²⁵. The approach to recapitulate the histological properties of the original HCC tumors will at least partially cover the shortage of current models; however, new strategies to copy the metabolic properties *in vivo* is still a challenge in the field.

5. Conclusions

A recent nomenclatural alteration from NAFLD to MAFLD highlights the importance of deregulated metabolism during HCC tumorigenesis and development. As one of the hallmarks of cancer, metabolic reprogramming in HCC could be of significance to combat the existing limitations in current systemic therapies of HCC. Herein, a comprehensive summary of key enzymes, and intermediates in nutrients metabolism contributes to deep understanding of potential targets for HCC treatment (Table 1). A range of preclinical and clinical studies targeting deregulated metabolic enzymes in HCC are carried out, requiring fully and carefully monitoring and evaluation though (Tables 2 and 3). Besides, drug repositioning provides a new direction for the treatment of neoplastic diseases, including HCC (Table 4). Fully utilizing the unique metabolic variations in HCC therapy remains a huge challenge for biologists, pharmacologists, and clinicians.

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Author contributions

Jing Xiong contributed to the conception and preparation of the manuscript; Danyu Du, Chan Liu, Mengyao Qin and Xiao Zhang jointly drafted the manuscript *via* an intense literature survey and made the figures; Danyu Du made the tables; Tao Xi and Shengtao Yuan reviewed and edited the manuscript; Haiping Hao and Jing Xiong supervised the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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