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Metabolic reprogramming and its clinical implication for liver cancer

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Abstract

Cancer cells often encounter hypoxic and hypo-nutrient conditions, which force them to make adaptive changes to meet their high demands for energy and various biomaterials for biomass synthesis. As a result, enhanced catabolism (breakdown of macromolecules for energy production) and anabolism (macromolecule synthesis from bio-precursors) are induced in cancer. This phenomenon is called “metabolic reprogramming,” a cancer hallmark contributing to cancer development, metastasis, and drug resistance. HCC and cholangiocarcinoma (CCA) are 2 different liver cancers with high intertumoral heterogeneity in terms of etiologies, mutational landscapes, transcriptomes, and histological representations. In agreement, metabolism in HCC or CCA is remarkably heterogeneous, although changes in the glycolytic pathways and an increase in the generation of lactate (the Warburg effect) have been frequently detected in those tumors. For example, HCC tumors with activated β -catenin are addicted to fatty acid catabolism, whereas HCC tumors derived from fatty liver avoid using fatty acids. In this review, we describe common metabolic alterations in HCC and CCA as well as metabolic features unique for their subsets. We

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AUTHOR CONTRIBUTION

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CONFLICT OF INTEREST

Nothing to report.

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discuss metabolism of NAFLD as well, because NAFLD will likely become a leading etiology of liver cancer in the coming years due to the obesity epidemic in the Western world. Furthermore, we outline the clinical implication of liver cancer metabolism and highlight the computation and systems biology approaches, such as genome-wide metabolic models, as a valuable tool allowing us to identify therapeutic targets and develop personalized treatments for liver cancer patients.

INTRODUCTION

Uncontrolled cell proliferation and activation of invasion and metastasis pathways are 2 hallmarks of cancer, and they both require ATP as a main source of energy along with various biomaterials for high biomass synthesis.^[1–3] Due to the desmoplastic stroma, defective tumor vasculature, and rapid cell proliferation, cancer cells often encounter hypoxic and hypo-nutrient conditions, and they must make adaptive bioenergetic changes to meet high energy demands or create a favorable microenvironment for their growth. This process is termed “metabolic reprogramming,” which results in enhanced catabolic metabolism (energy production by the breakdown of macromolecules) and anabolic metabolism (synthesis of macromolecules from small metabolic intermediates using energy) in cancerous tissues compared with normal tissues.^[4,5]

In a nonmalignant cell, ATPs are mainly generated by the breakdown of energy inputs, such as glucose, fatty acids (FAs), and amino acids, through multiple biochemical processes like glycolysis, the tricarboxylic acid (TCA) cycle, oxidative phosphorylation (OXPHOS), beta-oxidation, amino acid catabolism, and so on (cell A, Figure 1). These processes constitute cellular respiration events that occur in the cytosol or mitochondria where glucose, FAs, and proteins are converted into energy stored in the form of ATPs or broken down into bio-precursors for macromolecule synthesis. Among the ATP-generating pathways, OXPHOS in mitochondria is the most efficient mechanism. However, cancer cells tend to utilize glycolytic pathways and generate lactate in the presence of sufficient oxygen, which is known as “the Warburg effect.”^[6–8] Although the Warburg effect was observed 100 years ago, the interest in cancer metabolism has been renewed and tremendous progress has been made since the last 20 years.

We now know that cancer consists of metabolically heterogeneous cells. For example, cancer stem-like cells (CSCs) possess a unique metabolic feature responsible for developing resistance to anti-cancer therapies.^[9] Stromal cells within cancer, such as cancer-associated fibroblasts (CAFs), neurons, vascular endothelial cells, and immune cells also undergo metabolic reprogramming in response to the cues in the tumor microenvironment.^[10,11] To support maximal tumor growth, cancer and stromal cells, or metabolically heterogeneous cancer cells engage in crosstalk by transferring their metabolites including lactate between each other. This phenomenon is termed as “metabolic coupling” or “metabolic symbiosis”^[9,11] (Figure 1). In addition, lactate released by cancer cells leads to an acidic microenvironment that, in turn, inhibits proliferation of the immune cells, causes apoptosis of the tumor lytic immune cells, and polarizes the macrophages towards a type 2 subpopulation favoring growth, invasion and migration of cancer cells.^[12–14] Thus, metabolic reprogramming contributes significantly to the immune evasion of cancer. It also

allows cancer cells to adapt to the changing environment and survive unfavorable conditions so that the resistance develops against anti-cancer therapy.^[9]

Primary liver cancer consists of 2 major types, HCC and cholangiocarcinoma (CCA), with HCC accounting for 75%–85% and CCA for 10%–15% of malignancies in the liver.^[15,16] Although HCC and CCA share common risk factors such as hepatitis viral infection, alcohol abuse, and exposure to environmental toxins, they are 2 distinct types of cancer with high intertumoral heterogeneity in terms of mutational landscapes, histological representations, and transcriptomes.^[16–19] Although the Warburg effect is detected in both HCC and CCA tumors,^[15,20–22] liver cancer subsets possess unique metabolic features, and their metabolic heterogeneity contributes to the development of drug resistance and treatment failure of patients. The recent exploitation of computation and systems biology approaches, such as genome-wide metabolic models (GEMs) reconstructed through integrating genomics, transcriptomics, epigenomics, proteomics, and metabolomics for individual liver cancer, may help us better understand cancer metabolism, speed up drug development, and create personalized treatment for liver cancer patients.^[15] In this review, we describe the major metabolic pathways in mammalian cells for maintaining physiological functions and summarize metabolic alterations commonly detected across various cancer types. We also review metabolic features specific for HCC or CCA subsets, followed by a discussion of their clinical implications. Since metabolic reprogramming in the immune cells is an important and very hot topic that has been reviewed elsewhere,^[23–26] it is only briefly mentioned in this review. NAFLD has been predicted as a leading etiology for liver cancer in the future due to a rising global obesity rate. Therefore, we discuss its metabolism as well.

Overview of the major metabolic pathways in mammalian cells

Fermentation (anaerobic glycolysis), the TCA cycle, and OXPHOS—Glycolysis refers to a process where glucose is broken down into pyruvate by sequential enzymatic reactions, leading to generation of 2 ATPs (cell A, Figure 1). In the absence of oxygen, pyruvate enters fermentation where it is converted into lactic acid (cell A red, Figure 1).^[27] When oxygen is present, pyruvate is converted into acetyl-CoA in the mitochondrion to enter the TCA cycle (cell A light yellow, Figure 1). The TCA cycle, also known as the Krebs or citric acid cycle, consists of a series of enzymatic reactions allowing energy to be released from acetyl-CoA oxidation.^[27] Through the TCA cycle, 1 glucose molecule results in the generation of 6 CO₂, 8 reduced NADH, 2 reduced flavin adenine dinucleotide (FADH₂), and 2 ATPs. OXPHOS contains the electron transport chain (ETC) and chemiosmosis, which is an efficient ATP generation pathway later^[28] (cell A blue, Figure 1). The ETC consists of protein complexes at the inner mitochondrial membrane, complex I, II, III, and IV.^[29] When oxidized, NADH and FADH₂, produced by the TCA cycle, release high energy electrons and the transmembrane complexes use this energy to create a proton gradient. Chemiosmosis occurs as the protons diffuse back into the matrix via the complex V, which is an ATP synthase, producing around 34 ATPs with water as a byproduct^[30] (cell A blue, Figure 1).

Pentose phosphate pathway (PPP)—PPP is a branch of the glycolysis pathway, converting glucose-6-phosphate into ribulose-5-phosphate with NADPH produced (cell A Figure 1).^[31,32] NADPH is used in FA, cholesterol synthesis, and the reduction of

glutathione. Ribulose-5-phosphate is converted into ribose-5-phosphate used for DNA and RNA synthesis or fructose-6-phosphate that enters the glycolysis cascade (cell A Figure 1).^[31,33]

Beta-oxidation—Glycerol heads and free FAs, generated by lipolysis, are in the blood. After entering a cell, the glycerol heads enter the glycolysis, and FAs enter β -oxidation for energy production (cell A purple lines, Figure 1). β -oxidation is a 4-step process that occurs in the mitochondrion and converts FAs into acetyl-CoA to feed the TCA cycle. Each β -oxidation cycle followed by the TCA cycle leads to generation of ~12–17 ATPs. FAs are either originated from dietary fats or by *de novo* lipogenesis from acetyl-CoA that is converted from pyruvate (cell A purple lines, Figure 1), and they are predominantly synthesized in the liver and adipose tissue. Cholesterol can be broken down to FAs for β -oxidation as well.^[34]

Amino acid and 1 carbon metabolism—While amino acids are usually recycled to make proteins, some can be used for ATP production. For example, glutaminolysis is the process by which glutamine is converted to glutamate by glutaminase, which is then converted into α -ketoglutarate (α -KG) to enter the TCA cycle^[35] (cell A pink lines, Figure 1). When glucose concentration is low, cells can shift from glycolysis to glutaminolysis. Serine can be synthesized by *de novo* from an intermediate of glycolysis or imported from the extracellular space (cell A green lines, Figure 1). Serine participates in the folate metabolism (one carbon metabolism) for the synthesis of 5,10-methylene tetrahydrofolate (5,10-methylene THF) required for DNA, RNA, and methionine synthesis (cell A green lines, Figure 1).^[36] *S*-adenosyl methionine is a methyl donor in methylation occurred on DNA, RNA, or histones.^[34]

Common metabolic alterations in cancer

The Warburg effect—The Warburg effect was first proposed by a German scientist Dr Otto Warburg in the 1920s when he observed that, even in the presence of sufficient oxygen, cancer cells used more glucose and produced more lactate than their surrounding normal cells, implying upregulation of fermentation in cancer cells^[6–8] (cell A red, Figure 1). Although many studies have shown that tumorigenesis still requires the ETC and OXPHOS, an increase of aerobic glycolysis has been observed across various cancerous tissues, and the Warburg effect has remained the prevailing theory in academia for tumor metabolism.^[37,38]

The Warburg effect can be explained by the following mechanisms (cell A red lines, Figure 1): (1) mitochondrial defects induced by DNA mutations. It is believed that the mitochondrial DNA in cancer cells has high mutation rates due to its weak repair capacity, the lack of the introns and protective histones, as well as the closeness to mitochondria-derived reactive oxygen species (ROS).^[39–41] (2) Hypoxia inhibits OXPHOS and stabilizes hypoxia-inducible factor 1 alpha (HIF-1 α) which, in turn, upregulates glucose transporters 1 and 3 (Glut1 and Glut3), as well as glycolytic enzymes transcriptionally.^[42] (3) Oncogenic signals such as those induced by RAS, Bcr-Abl, or PI3K/AKT make cancer cells reliant on glycolysis.^[43–45] Tumor suppressor gene p53 is mutated or deleted in 50% of all human cancers, and its key metabolic function is to suppress gene transcription of Glut1

and Glut4. Therefore, its loss of function enhances glucose uptake by cancer cells.^[46,47] (4) The increase in expression of hexokinase II (HK2) for glycolysis and transketolase-like 1 for the PPP, or the loss of fumarate hydratase and succinate dehydrogenase (SDH) in the TCA cycle, may lead to the Warburg effect in cancer.^[48–52]

PPP—Enhanced PPP provides abundant pentose phosphate and NADPH, which are required for nucleic acid and FA synthesis.^[53] The upregulation of *TP53*-induced glycolysis and apoptosis regulator directs glycolytic metabolites to enter the PPP in cooperation with HK2.^[46] Glucose-6-phosphate dehydrogenase (G6PDH) in the PPP is post-translationally upregulated by an oncogenic protein, such as ATM, PI3K/AKT, RAS, or SRC; *G6PDH* gene transcription is elevated by mammalian target of rapamycin complex 1 (mTORC1)-mediated signaling; other key enzymes in the PPP such as 6-phosphogluconate dehydrogenase, transketolase, and transaldolase, are upregulated by K-RAS, B-RAF, MYC, or the hyperactivation of the PI3K/AKT signaling in cancer cells.^[31]

Mitochondrial OXPHOS—Although the Warburg effect has been widely detected and accepted as a common feature of cancer, increased mitochondrial activity has been found in subgroups of human cancers.^[54] CSCs are known to rely on mitochondrial metabolism so cancer cells that survived a targeted therapy against a cancer driver mutation, such as *KRAS(G12D)* or *BRAF(V600E)*, depended on OXPHOS rather than glycolysis.^[55,56] Similarly, a prolonged exposure of HCC cells to chemotherapeutic agents led to elevated mitochondrial respiration, and the drug resistance developed in those cells was abrogated by targeting mitochondrial respiration.^[57]

Glutaminolysis and pyruvate carboxylation—Glutaminolysis and pyruvate carboxylation are activated in cancer cells so that the TCA cycle is refilled by intermediary metabolites.^[2] For example, glutaminolysis produces α -KG that enters the TCA cycle and OXPHOS, and ultimately, generates more ATPs and biosynthetic precursors to support cancer growth. Pyruvate carboxylase adds bicarbonate to pyruvate and converts it to oxaloacetate, which is another TCA intermediate. Glutaminolysis enables reduction of NADP⁺ to NADPH required for lipid and nucleotide metabolism and cellular redox maintenance.^[58] The mTOR signaling or c-MYC-mediate signaling regulates glutamine uptake and catabolism, making certain cancer addicted to glutamine.^[59–62]

Metabolic symbiosis in cancer—CAFs are the most abundant cancer stromal cells, and TGF β is a potent cytokine leading to myofibroblast activation.^[63,64] When human fibroblasts were manipulated to have the activation of TGF β signaling, their mitochondrial density and the level of OXPHOS complexes were reduced. In contrast, autophagy markers were increased, suggesting mitochondrial damage in the TGF β -stimulated fibroblasts.^[65] Consequently, glycolysis and lactate production were induced in those cells^[65] (cell A an orange line, Figure 1). ROS, such as H₂O₂, induces glycolytic switch by activating NF κ B and HIF-1 α in CAFs,^[66] and downregulation of caveolin-1 (Cav1) is another mechanism for the Warburg effect in CAFs^[65,66] (cell A orange lines, Figure 1).

The “Reverse Warburg Effect” describes that in a 2-part model: CAFs undergo glycolysis and their glycolytic metabolites, such as lactate, pyruvate, and ketone body, are transferred

to cancer cells to engage in mitochondrial metabolism^[11] (cell B Figure 1). This lactate shuttle occurs in metabolically heterogeneous cancer cells as well. While a hypoxic cancer cell engages in glycolysis and the release of the metabolites (cell A Figure 1), a neighboring well-oxygenated cell or CSC uptakes the metabolites for OXPHOS (cell B Figure 1). The monocarboxylate transporters (MCTs) are a family of proton-linked membrane transporters responsible for the metabolite transfer. MCT4 expressed in CAFs or cancer cells is used for the release of the metabolites, whereas MCT1 in other cancer cell subsets is used for uptake of lactate^[11,67] (cell A and cell B Figure 1).

Metabolic reprogramming in primary liver cancers and associated mechanisms

HCC and CCA are 2 different cancers with high intertumoral heterogeneity—

HCC and CCA are 2 types of primary liver cancer derived from different cell lineages (hepatocellular and biliary differentiation). Although HBV and HCV account for ~80% of HCC cases worldwide,^[68] the link of metabolic syndrome and/or the presence of NAFLD to HCC and CCA deserves great attention due to the growing global obesity epidemic. NASH is an aggressive form of NAFLD and currently the most common cause of liver cirrhosis in the Western world. Figure 2 shows a 71-year-old female presented with liver mass, and her liver needle core biopsy confirmed mixed steatohepatitic HCC and mucin-producing CCA on the background of NASH with cirrhosis. HCC tumors derived from NASH often contain cancer cells with various differentiation (Figure 3).

A total of 161 putative driver genes in a HCC cohort were identified by exome sequencing, which potentially affect 11 pathways, such as the activation of telomerase expression (60%), WNT/ β -catenin (54%), PI3K/AKT/mTOR (51%), TP53/cell cycle (49%), MAP kinase (43%), hepatic differentiation (34%), epigenetic regulation (32%), chromatin remodeling (28%), oxidative stress (12%), I κ B/JAK/STAT (9%), and TGF β (5%).^[69] Alterations in *CTNNB1*, *TERT*, *CDKN2A*, *SMARCA2*, and *HGF* were linked to alcohol-related HCC, whereas *TP53* mutations were detected in HBV-related HCC.^[69] By a similar approach, 32 driver genes in a cohort of 260 CCA patients were identified with highest mutation frequency for *TP53*, *KRAS*, *SMAD4*, *BAP1* and *YEATS4*, affecting oncogenic modules, such as kinase-RAS module (51.9%), TGF β -SWI/SNF-MYC module (40.2%), P53 module (33.9%), cell cycle module (11.7%), cyclin E mutation (9%), and epigenetic module caused, in part, by IDH1/2 mutations (29.3%).^[70] This study also revealed that *FGFR1/2* and IDH1/2 mutations occurred exclusively in intrahepatic CCA, whereas mutations in EGFR family genes (*EGFR*, *ERBB2*, and *ERBB3*), as well as alterations in p53 and cell cycle modules occurred more frequently in gallbladder cancer.

The combined activation of oncogenes, including *AKT*, *CTNNB1*, and *NRASG12V* in a different inflammatory liver microenvironment such as one induced by carbon tetrachloride or 3,5-diethoxycarbonyl-1,4-dihydrocollidine, led to different liver cancer in mice, HCC, CCA, or mixed HCC-CCA.^[71] Moreover, liver tumors induced by same oncogenes exhibited different transcriptomes, supporting that the hepatic microenvironment and inflammation contribute to liver cancer heterogeneity as well.^[71] In a patient study, intratumoral heterogeneity was indeed detected in 87% of HCC cases.^[72] In agreement, metabolic reprogramming in HCC or CCA tumors is remarkably heterogeneous. Below, we

discuss common metabolic alterations in HCC or CCA tumors as well as metabolic features unique for HCC subsets or CCA subsets.

The Warburg effect, PPP, and hexosamine biosynthetic pathway (HBP) in HCC—A meta-analysis involving 521 human HCC and 2761 metabolic genes led to the identification of 284 metabolic genes upregulated and 350 genes downregulated in the HCC cohort.^[73] While the downregulated genes were mostly involved in xenobiotic, FA, and amino acid metabolism, the upregulated genes were involved in glycolysis, PPP, nucleotide biosynthesis, TCA, OXPHOS, proton transport, membrane lipid, and glycan metabolism.^[73] Nontargeted metabolic profiling was performed with another cohort of 50 patient HCC, which revealed the principal metabolic alterations included the upregulation of glycolysis, gluconeogenesis, and β -oxidation and downregulation of the TCA cycle and ω -12 desaturase.^[74] These 2 studies supported the Warburg effect as one of the top metabolic alterations in certain HCC tumors.

TP53 mutations and the activation of the PI3K/AKT/mTOR pathway occur frequently in HCC,^[69] and they are known to promote the Warburg effect by upregulating Glut1 and Glut4 and other glycolytic enzymes.^[47,75,76] Indeed, elevated Glut1 was detected in HCC tumors with its high expression correlated with poor prognosis of HCC patients^[77–79] (Figure 4). HK2 converts glucose to glucose-6-phosphate (G6P) during the first step of glycolysis. Elevated HK2 expression was detected in HCC of patients,^[80–82] and its high expression was associated with poor overall survival of patients as well.^[82] Consistent with HK2 data, increase of G6P in patient HCC was revealed by metabolomics^[74] (Figure 4).

In 688 HCC tumors resected from 2000 and 2013, PKM2 overexpression was correlated with a high TNM stage and high level of vascular invasion^[83] (Figure 4). Tripartite motif-containing 35 (TRIM35) blocks PKM2 phosphorylation, thereby, inhibiting the Warburg effect and the tumorigenesis of HCC.^[84] Patients with HCC positive for PKM2 and negative for TRIM35 expression had shorter survival and recurrence time compared with patients negative for PKM2 and positive for TRIM35.^[83] However, PKM2 loss in mice led to spontaneous HCC development, suggesting a complex role of PKM for HCC development.^[85] Lactic dehydrogenase (LDH) converts pyruvate to lactate (Figure 4). In 200 HCC patients who received curative resection, a high preoperative LDH level in serum correlated with early HCC recurrence in patients.^[86] In addition, a high serum LDH level was associated with poor survival of HCC patients after sorafenib treatment.^[87]

G6PDH is the first rate-limiting enzyme in the PPP. qRT-PCR revealed that *G6PD* mRNA was increased in 30 patient HCC tumors compared with adjacent nontumorous liver tissues and that its level was higher in metastatic HCC than in nonmetastatic HCC^[88] (Figure 4). In addition, its high level correlated with short survival in 127 HCC patients.^[88] In a resistant-hepatocyte (RH) hepatocarcinogenesis rat model, the preneoplastic nodules displayed increased aerobic glycolytic activity, PPP activation, and decreased OXPHOS.^[89] NRF2 silencing reduced *G6PDH* expression in RH tumorigenic cells and the amount of glucose diverted into the PPP.^[89] Expression of inhibitor of differentiation 1 promoted *G6PDH* expression and PPP in HCC cells by a WNT/ β -catenin/MYC pathway.^[90]

HBP is another branch in the glycolysis cascade where fructose 6-phosphate is used for synthesis of uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc) by glutamine:fructose-6-phosphate amidotransferase (GFAT) (Figure 4). UDP-GlcNAc is a sugar donor for *O*-linked β -*N*-acetylglucosamine (*O*-GlcNAcylation), a post-translational modification on serine and threonine residues of nucleocytoplasmic proteins catalyzed by *O*-GlcNAc transferase (OGT). The global *O*-GlcNAcylation level was elevated in HCC, which associated with HCC recurrence in patients following liver transplantation^[91] (Figure 4). *In vitro*, elevated glycolysis, GFAT expression, and protein *O*-GlcNAcylation promoted migration and metastatic potential of HCC cells.^[91,92]

Because of the Warburg effect, positron emission tomography (PET) with [18F] 2-fluoro-2-deoxy-D-glucose (FDG) has been used to detect tumors in the liver of patients. The detection rate of FDG-PET for HCC, however, is only 50%–55% compare with 98% for colorectal (CRC) liver metastasis.^[93] The difference is possibly caused by differential expression of proteins in glycolysis.^[93] Izuishi et al.,^[93] found that the mRNA levels of Glut1, HK1, and HK2 were higher in CRC liver metastases than in HCC tumors, and that of glucose-6-phosphatase (G6Pase), converting G6P back to glucose to be released from cells, was lower in CRC liver metastasis than in HCC. Immunostaining confirmed that Glut1 protein level was higher in CRC liver metastasis and poorly differentiated HCC than in moderated differentiated HCC.^[93] When FDG PET and CT were applied for restaging 33 HCC lesions in 24 patients following radiofrequency ablation, the detection rate of FDG PET for HCC recurrence was 92%, higher than that of CT (75%).^[94] Thus, FDG PET is a powerful imaging modality for HCC recurrence for patients after radiofrequency ablation.

The Warburg effect, PPP, and HBP in CCA—Due to the Warburg effect, FDG-PET has been used in clinics for CCA diagnosis, staging, detecting tumor recurrence, making a treatment plan, and for patient prognosis.^[95,96] FDG uptake by CCA correlated with Glut1 expression and high Glut1 expression level correlated with histological grading, HIF-1 α expression, larger tumor size, poor differentiation, lymphatic invasion and metastasis of CCA,^[97,98] as well as poor survival of CCA patients^[99] (Figure 5). In 82 CCA induced by liver fluke, elevated HK2 expression was detected in hyperplasia (80%), dysplasia (80%), and CCA (82%) although it had no correlation with any clinical feature and survival of the patients (Figure 5). Elevated HK2 expression was also detected in 40% of hamster CCA induced by *N*-nitrosodimethylamine + liver fluke.^[100] Regarding PKM2, its expression was higher in the plasma and bile of CCA patients, with an association with CCA cell proliferation, migration, angiogenesis, and poor survival of the patients^[101,102] (Figure 5). In a study involving 114 advanced CCA received first-line treatment (gemcitabine and cisplatin), a high serum LDH was associated with poor survival of the CCA patients.^[103] Moreover, 38 patients with LDH level decreased as the result of treatment had improved survival, supporting serum LDH as a prognostic marker for CCA patients received first-line chemotherapy.^[103]

As mentioned above, NRF2 promotes *G6PDH* transcription to activate PPP. Elevated NRF2 and reduced FoxO3 mRNA were detected in 28 patient CCA tumors. FoxO3 deficiency potentiated CCA formation in nude mice and rendered CCA cisplatin resistance by activating NRF2^[104] (Figure 5). QBC939 CCA cells are resistant to cisplatin. After cisplatin

treatment, glucose uptake, lactate production, G6PDH activity, and the NADPH/NADP ratio were all higher in QBC939 cells than in HepG2 cells, indicating that a more efficient PPP in QBC939 cells conferred cisplatin resistance.^[105] Activation of the HBP pathway was also detected in CCA tumors. Immunohistochemistry revealed that the level of O-GlcNAc modified proteins and OGT were higher in CCA than in normal bile ducts, and a high O-GlcNAcylation level was associated with non-papillary type CCA and poor survival of patient^[106] (Figure 5). *In vitro*, an increase of glucose concentration in culture medium promoted global O-GlcNAcylation in CCA cells as well as O-GlcNAcylation of vimentin that, in turn, enhanced migration and metastasis of CCA cells.^[92]

Changes in the TCA cycle of HCC—In 30 HCC tumors of patients, fumarate and malate were reduced and they were among 253 metabolites that significantly discriminated HCC tissues from paired non-tumor tissues^[107] (Figure 4). Downregulation of fumarate, malate, and succinate also occurred in another cohort of HCC patients.^[74] Fumarate hydratase and SDH that converts succinate to fumarate, are tumor suppressors,^[108] and their function loss results in abnormal levels of succinate or fumarate that, in turn, stabilize HIF-1 α for the Warburg effect and angiogenesis. Downregulation of SDH subunit B (SDHB) was detected in a cohort of HCC tumors, which correlated with an advanced tumor stage and poor survival rate of patient^[109] (Figure 4). *In vitro*, silencing of SDHB led to glycolysis and enhanced proliferation and motility of HCC cells.^[109]

Changes in the TCA cycle of CCA—Succinate and glycerol 3-phosphate were reduced in 10 patient CCA tumors compared with nontumor tissues, suggesting the Warburg effect and upregulation of FA catabolism in certain CCA tumors^[110] (Figure 5). In the mitochondria, pyruvate dehydrogenase (PD) converts pyruvate to acetyl-CoA that enters the TCA cycle. So the TCA cycle can be blocked and aerobic glycolysis is promoted once PD is inactivated by pyruvate dehydrogenase kinase (PDK) (Figure 5). In a cohort of CCA tumors, PDK3 expression level was 27 folds higher in CCA than in benign tissues, and the high serum PDK3 level correlated with a shorter survival of the patients.^[111] HIF-1 α is stabilized by acetylation and degraded by Sirtuin-3 deacetylase (SIRT3)-mediated deacetylation. Reduced SIRT3 and elevated HIF-1 α expression were detected in a cohort of CCA^[112] (Figure 5). Mechanistically, HIF-1 α upregulated PDK expression transcriptionally so a HIF-1 α /PDK signaling pathway suppressed the TCA cycle and promoted the Warburg effect in CCA cells^[112] (Figure 5).

About 10%–20% of CCA tumors carry mutations on isocitrate dehydrogenase genes (IDH1 and IDH2) (Figure 5).^[15,70,113–116] Wildtype IDH1 and IDH2 catalyze the formation of α -KG and the mutants generate (R)-2-hydroxyglutarate (2-HG), which is an oncometabolite. Due to the structural similarity, 2-HG acted as a competitive inhibitor for α -KG-dependent histone demethylases so hypermethylation for more than 2,000 genes was detected in CCA subsets with IDH1 or IDH2 mutations. IDH mutations are therapeutic targets for the CCA subsets.^[117,118]

Two contrasting FA catabolism in HCC subsets—About 50% of HCC cases are affected by the Wnt/ β -catenin signaling in a patient cohort.^[69] In the HCC subset with activated β -catenin, elevated FA oxidation (FAO) was detected in 20 of 26 tumors

(GSE62232), and this FAO elevation was validated in another HCC cohort.^[119] Using *Apc* knockout mice, Senni and colleagues found that hepatocytes or HCC with β -catenin activation used FAO as an energy provider instead of the Warburg effect (Figure 4, green lines). In contrast, a steatohepatic HCC subset characterized by large lipid droplets and tumor cell ballooning avoided FAO (Figure 4, purple lines). As revealed by Fujiwara and colleagues, the expression of carnitine-acylcarnitine translocase and carnitine palmitoyltransferase 2 (CPT2) directing acyl-carnitine to enter the mitochondria were all reduced in high-fat diet induced HCC,^[120] similar to those in *PIK3CA* transgenic mice and high-fat diet fed *MUP-uPA* mice^[99,120] (Figure 4, purple lines). In agreement, reduced CPT2 and elevated acylcarnitine levels were detected in the serum of HCC patients.^[99] In this HCC subset, downregulation of FAO protected HCC cells from lipotoxicity and led to accumulation of oncometabolite acylcarnitine for tumorigenesis and cisplatin chemoresistance.^[121] As the result, pyruvate was re-routed to OXPHO to compensate for the suppressed FAO.

Lipid and mitochondrial metabolism fuel CSCs responsible for metastasis and drug resistance of HCC or CCA—CSCs refer to a subset of cancer cells capable of self-renewing and responsible for tumor relapse, metastasis, and chemoresistance. Although it is still controversial for CSC existence in HCC, LO Re et al.^[122] found that loss of macroH2A1 (an epigenetic regulator of stem cell function) led to CSC-like features with metabolic changes in HCC, such as enhanced lipogenesis and acetyl-CoA production, as well as PPP activation. In 72 HCC of patients, SIRT1 overexpression was detected in 56% of cases, which correlated with microvascular invasion, advanced TNM stage, and short patient survival^[123] (Figure 4, red lines). SIRT1 depletion attenuated mitochondrial biogenesis, ATP production, cell migration and invasion of HCC, which was rescued by overexpression of peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1 α), a master regulator of mitochondrial biogenesis. Thus, SIRT1/PGC-1 α -induced mitochondrial biogenesis facilitates HCC invasion and metastasis^[123] (Figure 4, red lines). Moreover, when Hep3B cells underwent a full epithelial-to-mesenchymal transition in response to TGF β stimulation, lipolysis and free FA uptake in the cells increased, while glycolysis and lipogenesis reduced^[124] (Figure 4, green lines). Thus, lipid and mitochondrial metabolism fuel a cell subset responsible for HCC invasion and metastasis.

Overexpression of PGC-1 α promoted OXPHOS in CCA, which in turn facilitated CCA metastasis but not the growth of CCA cells.^[125] In addition, the CSC-like phenotypes in CCA were defined by PGC-1 α overexpression and mitochondrial metabolism (Figure 5, red line), and elevated expression of PGC-1 α or mitochondrial complex II was correlated with worse prognosis and early recurrence in CCA patients.^[126] Thus, the CSC subset in CCA is fueled by oxidative mitochondrial metabolism.

It has been shown that hypoxia induced by an anti-angiogenic drug stimulated free FA uptake and subsequent FAO so as to promote HCC cancer cell proliferation^[127] (Figure 4 green lines). In addition, hypoxia promoted PGC-1 α expression and mitochondrial biogenesis, allowing HCC cells to survive a harsh hypoxic condition by a high mobility group box 1 (HMGB1)-dependent mechanism.^[128] However, others revealed that PGC-1 α was a tumor suppressor and that its expression was downregulated in 25% of HCC cases

in a patient cohort.^[129] Moreover, hypoxia suppressed FAO in a HCC subset by a PGC-1 β -dependent mechanism.^[130,131] Thus, the lipid metabolic reprogramming is remarkably heterogeneous in HCC.

De novo lipogenesis in HCC and CCA—Acetyl-CoA carboxylase (ACC) converts acetyl-CoA to malonyl-CoA during *de novo* lipogenesis. Wang et al.^[132] reported that ACC α -mediated FA synthesis was required for HCC survival by providing both FA and enzymes in FAO under metabolic stress and that ACC α upregulation was associated with poor patient survival and HCC recurrence in 267 patients after curative surgery (Figure 4). Fatty acid synthase (FASN) is another rate-limiting enzyme for lipid synthesis. FASN protein and mRNA levels were progressively increased from normal liver tissues to HCC and in a HCC subgroup with poorer survival, it was further upregulated post-transcriptionally^[133] (Figure 4). Metabolic and gene expression data revealed that elevated lipid metabolites of stearoyl-CoA-desaturase 1 (SCD1) were associated with aberrant palmitate signaling and more aggressive HCC phenotypes in patients^[107] (Figure 4). Palmitoleate (C16:1) and Oleate (C18:1) are 2 biological end products of SCD1 with HCC-promoting effects.^[127] SCD1 is also a target of β -catenin and monounsaturated fatty acids that amplifies the Wnt pathway by enhancing Wnt receptor LRP5/6 expression to promote CSC-like HCC cells.^[134] Importantly, the expression of lipogenic enzymes, such as ACC, FASN, and SCD1, is controlled by sterol regulatory element-binding protein 1c (SREBP1c) in hepatocytes and HCC (also known as sterol regulatory element-binding transcription factor 1, SREBF1c) (Figure 4). Large-scale gene expression profiling showed that SREBP1c-mediated lipogenic pathway was significantly activated in HBV-related and HCV-related HCC, and high SREBP1c expression was associated with poor prognosis of HCC patients.^[135]

In contrast to HCC, reduced FASN expression was detected in human CCA and murine CCA (Figure 5). Hydrodynamic injection of AKT/Ras led to HCC and CCA lesions in wild type mice, but only CCA lesions in FASN knockout mice.^[136] In addition, FASN gene ablation failed to influence CCA development induced by AKT/Notch intracellular domain 1. In the absence of FASN, CCA cells upregulated expression of lipoprotein lipase, CD36, and SLC27A to maintain the level of FAs (Figure 5). De Gauna et al.^[137] found that highly proliferative human CCA cells relied on lipid and lipoprotein uptake to fuel FAO *in vitro*,^[104] and a high serum CD36 level was associated with worse survival of CCA patients.^[138] In contrast, in a cohort of 155 CCA collected at the Srinagarind Hospital, Khon Kaen University, high FASN expression was associated with advanced CCA stage and poor survival of the patients. In addition, FASN knockdown inhibited growth, migration, and invasion of KKKU055 and KKKU213 CCA cells *in vitro*^[139] (Figure 5).

FAs also give rise to prostaglandins, which are bioactive lipids contributing to tumorigenesis.^[140] Sphingosine-1-phosphate (S1P) is one of the prominent bioactive lipids influencing cell survival and proliferation, and it is formed by sphingosine kinases 1 and 2 (SPHK1/2). SPHK1 was found to be overexpressed in HCC and CCA compared with normal liver tissues, which correlated with reduced patient survival^[141,142] (Figures 4 and 5). High expression of SPHK1 or SPHK2 also contributed to HCC drug resistance to oxaliplatin or regorafenib.^[141,143]

Amino acid metabolism in HCC and CCA—Serine and threonine, which can be transformed into glucose for energy production, were increased in 50 HBV or HCV-related HCC of patients compared to non-tumoral tissues^[74] (Figure 4). Glutamine, glutathione, taurine, branched-chain amino acids, including valine, leucine, isoleucine, as well as the aromatic amino acids, such as tryptophan, phenylalanine, and tyrosine, were also increased.^[74] In another cohort of HCC, taurine, arginine, N-acetylaspartate, N-acetylasparagine, N-acetylalanine, N-acetylmethionine, N-(2-furoyl)glycine, and N-acetylthreonine were increased^[107] (Figure 4). Hydroxyproline was elevated in another cohort of HCC with its high expression correlated to poor prognosis of the patients^[144] (Figure 4).

Glutamine addiction was found in a subset of HCC harboring β -catenin mutations or overexpressing glutamine synthetase (GS), an enzyme converting glutamate to glutamine.^[145] In a HCC cohort, 69.81% of HCC biopsies expressed GS, whereas only 13.64% of non-malignant liver tissues expressed GS^[146] (Figure 4). Some *CTNNB1*-mutated HCC tumors were more sensitive to sorafenib than wildtype HCC, which was caused by GS-induced autophagy.^[147] High expression of glutaminase (GLS1), an enzyme for glutaminolysis, is associated with HCC differentiation, lymphatic metastasis, TNM stage, and patients' poor outcome in another HCC cohort^[148] (Figure 4). GLS1 overexpression correlated with the activation of an AKT/GSK3 β /cyclinD1 pathway that enhanced HCC proliferation.^[148] The asparagine synthetase (ASNS) catalyzes the biosynthesis of asparagine from aspartate, which relies on glutamine as the nitrogen source. It is likely that ASNS knockdown promoted HCC by the accumulation of aspartate and glutamine that enhanced nucleotide biosynthesis and glutaminolysis^[149] (Figure 4). Although HCC with low ASNS expression had poor prognosis, it may be more sensitive to L-asparaginase, which is a drug used for the treatment of acute lymphoblastic lymphoma.^[149]

Multi-omics data also revealed that isoleucine, leucine, proline, and taurine were increased in 10 patient CCA tumors compared with nontumoral tissues^[110] (Figure 5). Since certain human CCA cell lines were highly dependent on glutamine *in vitro*, derivative cell lines independent of external glutamine were established by a means of gradual reduction of external glutamine.^[150] Comparing to the control cell lines, hypoxia-induced chemoresistance to cisplatin or gemcitabine was abolished in those CCA derivative lines, supporting the role of glutamine metabolism in the development of drug resistance in CCA.^[150]

Metabolism in NAFLD/NASH and its association with liver cancer risk

Obesity is defined as body mass index of 30 or above and severe obesity as body mass index of > 40. According to Centers for Disease Control and Prevention (CDC), the incidence of adulthood obesity has increased by 300% and severe obesity by 1000% over the past 50 years (<https://www.cdc.gov/nchs/data/hestat/obesity-adult-17-18/obesity-adult.htm#>, Table 1). In a large cohort study involving 2162 liver cancer cases in the United States, a 75% increase of liver cancer was detected for obese individuals. Obese men were more susceptible for liver cancer than obese women.^[172] The fat under the skin in humans is s.c. fat tissue, while visceral fat surrounds organs deeper in the abdomen such as the liver and pancreas.^[173,174] When visceral obesity presents, the metabolites of the visceral fat

are drained into the liver by portal vein, leading to fatty liver, altered lipid and glucose homeostasis, as well as insulin resistance.^[175] Allen and colleagues analyzed the incidence of malignancies in a historical cohort of adults diagnosed with NAFLD in the Olmsted County Minnesota between 1997 and 2016 in relation to matched adults without NAFLD. The 21 years of longitudinal study revealed that NAFLD was associated with nearly a 2-fold risk of developing cancers, predominantly liver cancer followed by gastrointestinal tract and uterus cancer.^[176] NAFLD even exhibited a stronger association with cancer risk than obesity alone.^[176] A similar association was detected in a Korean population as well.^[177]

NASH is an aggressive form of NAFLD, characterized by inflammation, steatosis, and hepatocellular damage, as well as ballooning in the presence or absence of fibrosis. NAFLD is characterized by simple steatosis due to fat accumulation. In NASH patient cohorts with cirrhosis, the cumulative HCC incidence ranged from 2.4% over 7 years to 12.8% over 3 years, whereas, in the cohorts with noncirrhotic stage of NAFLD, the cumulative HCC mortality was 0%–3% for study periods up to 20 years.^[178] Among NASH-driven HCC cases, ~1% of them developed both HCC and CCA as shown in Figure 2. NAFLD/NASH has been predicted as a leading etiology of liver cancer in the coming years due to the rising global obesity rate.

Saturated FAs and ceramides accumulated in the liver are toxic to liver cells in NASH patients. Catabolizing long-chain FAs in peroxisomes for oxidation particularly increases the level of ROS and toxic lipid species, which damage the mitochondrial respiratory chain resulting in cytochrome c release and apoptotic cell death.^[179,180] ROS and oxidative stress also disrupt endoplasmic reticulum, further potentiating hepatic cell injury and death by activating caspases 9 and 3.^[179,180] On the other hand, insulin resistance and hyperglycemia drive upregulation of insulin and insulin-like growth factor-1 to enhance liver cell proliferation and inhibit apoptosis via activating the PI3K and MAPK pathways.^[181] TNF α , IL6, and/or androgen-mediated signaling also participate in enhancing cell turnover in the liver.^[181] ROS, oxidative stress, and inflammation cause DNA mutagenesis, and the genetic instability is about 10–20 folds higher in NASH than in NAFLD patients. Indeed, mutations in genes such as *PNPLA3*, *TM6SF2*, *MBOAT7*, *GCKR*, *HSD17 β 13*, and *MARCI* were detected in NASH patients.^[181,182] Further data supported that NASH driven hepatocarcinogenesis is a multi-factorial process involving aberrant lipid metabolism, mitochondrial dysfunction, oxidative stress, inflammatory response, and even the gut microbiome.^[181,182]

Metabolism as a target for the prevention and treatment of liver cancer

Caloric restriction and fasting in liver cancer prevention—Since obesity and NAFLD are associated with liver cancer risk, proper caloric restriction, weight loss, and diabetes management are important strategies for liver cancer prevention. Short-term fasting (24–72 hours) or intermittent fasting has been proposed and tested for HCC prevention or treatment. A theory behind this is that, in response to short-term fasting, cancer cells continue uncontrolled growth due to genetic mutations leading to enhanced DNA damage and oxidative stress. Contrastingly, healthy cells shut down their growth, re-invest their energy in cellular maintenance and repair to protect them from chemotherapy-induced or

radiotherapy-induced damages.^[183] In various cancer models, short-term fasting indeed reduced the toxicity associated with chemotherapy, enhanced the efficacy of chemotherapy and radiotherapy, and improved the survival of animals.^[183]

A 52-week-long experiment was conducted to evaluate whether fasting protected rats from diethylnitrosamine (DEN)-induced liver cancer, which revealed that intermittent fasting following DEN injection reduced the number and size of premalignant liver nodules.^[184] Another study showed that short-term fasting led to a transient reduction of liver weight and loss of preneoplastic lesions in the liver of rats by (1) decreasing hepatocyte volume and proliferation and (2) inducing cell apoptosis.^[185] However, it later promoted hepatocarcinogenesis in response to refeeding possibly due to enhanced cell proliferation compensatory to the fasting.^[185] Consistently, 3 cycles of 3 days fasting added to a liver tumor promotion protocol led to 2 folds higher HCC incidence compared with the control rats.^[186] Fasting-refeeding also potentiated hepatocarcinogenesis induced by a sub necrogenic dose of DEN (20 mg/kg).^[187] Thus, fasting-refeeding appeared to promote hepatic cell turnover and HCC incidence different from what happened in other organs.

Since metabolic reprogramming represents a target for anti-cancer therapy, numerous drugs targeting metabolites or metabolic pathways have been developed and approved by FDA for cancer treatment. For example, methotrexate, a folate antagonist, is able to cure 90% of acute lymphoblastic lymphoma when combined with L-asparaginase targeting arginine.^[188] 5-fluorouracil, gemcitabine, fludarabine, and hydroxyurea that are used to treat various cancers target nucleic acid synthesis, as well as ivosidenib and enasidenib target 2-HG synthesis.^[188,189] In order to identify metabolic targets for liver cancer treatment, *in vitro*, preclinical, and clinical studies have been conducted and the potential metabolic targets under investigation are summarized as below (Table 1).

Targets in the Warburg effect—Targeting Glut1 has been investigated for HCC and CCA treatment (Table 1). For example, downregulating Glut1 expression by long non-coding RNA (lncRNA) *SLC2A1-AS1* suppressed the proliferation and metastasis of HCC,^[190] and targeting Glut1 by siRNA suppressed CCA cell migration and invasion.^[97] BAY-876 is a Glut1 antagonist, and single injection of microcrystalline BAY-876 into HCC tumor tissues led to inhibition of glucose uptake, proliferation, and epithelial-to-mesenchymal transition of HCC in mice.^[191] Liu et al.^[152] reported that aspirin reduced glucose uptake and hepatoma proliferation *in vitro* and in mice by downregulating Glut1. Everolimus is a mTOR inhibitor, and it suppresses glucose uptake and tumor angiogenesis by reducing HIF-1 α expression (Table 1). For HK2, HK2 silencing led to death of HCC cells and depleting hepatic HK2 reduced HCC incidence and sensitized HCC to sorafenib and metformin chemotherapy in mice.^[80] 2-Deoxy-D-glucose (2-DG), a glucose analog targeting HK2 activity, reduced HCC migration and proliferation *in vitro* and sensitized HCC to sorafenib in mice^[155,156] (Table 1). Resveratrol was able to potentiate sorafenib chemotherapy for HCC as well by downregulating HK2 expression.^[157] Similarly, LDH-A knockdown resulted in HCC apoptosis and a significant reduction in metastatic potential in a xenograft HCC mouse model.^[192]

Although the bi-phasic metabolic coupling has not been investigated for liver cancer, elevated MCT4 expression in HCC correlated with larger tumor size in patients, and MCT4 was an independent prognostic factor for HCC patients received trans-arterial chemoembolization.^[193] MCT4 knockdown *in vitro* suppressed HCC cell proliferation, migration, and invasion.^[193] In addition, AZD-3965, a small molecule targeting MCT1, is currently being tested in a clinical trial for the treatment of advanced cancers^[188] (Table 1).

Targets in the TCA cycle—As mentioned above, PD controls the amount of pyruvate entering the TCA cycle with its activity inhibited by PDK. Shen et al.^[159] tested the hypothesis that targeting PDK may direct more pyruvate into the TCA cycle so as to inhibit the Warburg effect and overcome HCC drug resistance. This hypothesis was tested by a PDK inhibitor dichloroacetate (DCA), and the results showed that DCA indeed activated OXPHOS and sensitized HCC to sorafenib treatment *in vitro* and in mice^[159] (Table 1). Metformin, widely used in diabetic patients for blood glucose control, activates AMP-activated protein kinase and inhibits OXPHOS by binding to the mitochondrial complex I in the ETC.^[2] Type 2 diabetic patients received metformin exhibited a decreased HCC incidence.^[20] Metformin inhibited the proliferation, migration, invasion of HCC *in vitro*, and suppressed HCC xenografts in mice.^[194,195] Metformin is now in 3 clinical trials for HCC treatment (NCT03184493, NCT04033107, NCT04114136) (Table 1).

Metformin is also a promising strategy for CCA prevention and treatment.^[196] It suppressed CCA expressing markers related to CSC phenotypes and reduced CCA growth in mice.^[126] *IDH1/2*-mutated cancer cells produce the oncometabolite 2-HG, and they are metabolically vulnerable to the treatment with metformin and an oral antimalarial drug chloroquine.^[197] A phase IB/II clinical trial is currently ongoing to assess the effect of metformin/chloroquine combination for CCA (NCT02496741) (Table 1). Approximately 20% of CCA carry mutations in *IDH1* and *IDH2*. Ivosidenib, targeting *IDH1* mutations in the TCA cycle, has already been approved for CCA subsets with *IDH1* mutations.

Targets in lipid metabolism—In a study, suppression of SREBP-1c induced cell cycle arrest and apoptosis of HCC, whereas overexpression of SREBP-1c enhanced HCC proliferation, suggesting that SREBP-1c may be a therapeutic target for HCC subsets, depending on *de novo* lipogenesis.^[127] Fatostatin, a small molecule targeting the activation of SREBPs, blocked the increases of body weight, blood glucose, hepatic fat accumulation in obese ob/ob mice,^[160] and inhibited prostate cancer growth and metastasis in a mouse model.^[161] A series of fatostatin derivatives have been developed, and FGH10019 or compound 24 is the most potent one with high aqueous solubility and membrane permeability (Table 1). FGH10019 may serve as a seed molecule for further development of anti-SREBP drugs.^[162] FASN inhibitor TVB2640 reduced liver fat content, inflammation, and fibrosis in NASH patients (NCT03938246). TVB3664 was able to ameliorate the fatty liver disease phenotype of mice.^[164] When TVB3664 was administered into mice at the early tumor stage, it prevented HCC development induced by PTEN inactivation/c-MET or AKT/NRAS, and improved the efficacy of sorafenib or cabozantinib on c-MYC driven HCC in combination therapies^[164] (Table 1). However, neither TVB3664 nor FASN gene deletion

influenced HCC induced by β -catenin 90/c-MET.^[164,198] Thus, FASN inhibitors may be used to treat the HCC subsets with FASN dependence.

ACC is another limiting enzyme in *de novo* lipogenesis. Targeting ACC by ND-654 suppressed lipogenesis, inflammation, and HCC development in rats^[165] (Table 1). However, liver-specific ACC knockout enhanced HCC development in mice in response to DEN due to a marked increase in antioxidants, including NADPH, and reduced glutathione.^[199] Targeting SCD1 by siRNA reduced HCC cell migration, colony formation, and HCC tumorigenicity in mice.^[107] Targeting myofibroblast SCD1 by a genetic approach also inhibited liver tumor development and growth in mice.^[134] SCD1 inhibitors A939572, CAY10566, MF-438, and BZ36 have been developed (Table 1) and their effect had been tested in other cancers, such as lung, prostate, ovarian, and colorectal cancer.^[166,200–203]

A HCC subset with activated β -catenin is addicted to FAO, so PPAR α gene deletion or a CPT inhibitor etomoxir was able to inhibit FAO and the HCC tumors in mice^[119] (Table 1). Statins, known as HMG-CoA reductase inhibitors, are lipid-lowering drugs approved for patients with high LDL cholesterol who has high risk for cardiovascular diseases. Simvastatin or atorvastatin reduced the proliferation and migration of cultured CCA cells and induced CCA apoptosis by activating p21 and caspase 3^[167] (Table 1). Pravastatin prolonged survival of HCC patients who received transcatheter arterial embolization and 5-fluorouracil treatment^[168] and HCC patients who received chemoembolization.^[204] However, the sorafenib/pravastatin combination failed to improve overall survival of HCC patients in a randomized-controlled trial (NCT01075555).^[169]

Conserved lipid kinases SPHK1 and SPHK2 lead to S1P production that, in turn, enhances survival and proliferation of cancer cells. A SPHK2 inhibitor, ABC294640 (3-(4-chlorophenyl)-adamantane-1-carboxylic acid (pyridin-4-ylmethyl)amide, reduced intra-tumoral S1P level, and induced tumor cell apoptosis in mice.^[205] In a phase I clinical trial for advanced solid tumors, a partial response to ABC294640 treatment was a patient with advanced CCA^[206] (Table 1). A phase I/IIA study is ongoing with ABC294640 alone as well as in combination with hydroxychloroquine to treat CCA patients (NCT03377179). In addition, ABC294640 had been tested as the second-line monotherapy for advanced HCC in a phase II clinical trial (NCT02939807) (Table 1).

Targets in amino acid metabolism—Anti-leukemic drug crisantaspase depletes glutamine, and methionine-L-sulfoximine inhibits the activity of GS. The combination of crisantaspase and methionine-L-sulfoximine reduced glutamine supply so as to suppress xenografts of the *CTNNB1*-mutated HCC in mice^[171] (Table 1). GLS1 converts glutamine to glutamate to feed the TCA cycle. Although the GLS1 inhibitor CB-839 had limited effect on HCC, it induced apoptosis of HCC and inhibited HCC xenografts in mice when combined with V-9302, a novel inhibitor for glutamine transporter.^[170] Downregulation of glutamine transporter suppressed CCA cell migration and invasion as well.^[207] Autophagy provides amino acids including glutamine to fuel the TCA cycle, contributing to the drug resistance of cancer. Hydroxychloroquine, approved for rheumatoid arthritis treatment, inhibits cell autophagy. In a phase II trial, sorafenib/hydroxychloroquine combination led to a much better response rate (25%) than that of sorafenib alone (2%) in

patients with advanced HCC (NCT03037437) (Table 1). Trametinib (a MEK inhibitor)/hydroxychloroquine combination is currently in a clinical trial for the treatment of KRAS mutated refractory CCA (NCT04566133) (Table 1).

Identifying metabolic targets using systems biological approaches

GEMs, constructed by integrating genomics, transcriptomics, proteomics, and metabolomics data are valuable tools allowing us to study liver diseases at the systems biology level. HepatoNet1 was reconstructed based on more than 1500 original scientific research publications in 2010. It provides a comprehensive metabolic network of human hepatocytes and a structural platform for computational studies on liver function.^[208] iHepatocytes2322 was reconstructed based on Human Metabolic Reaction 2.0 database and proteomics data in Human Protein Atlas in 2013, and it can be utilized to identify potential biomarkers and therapeutic targets for NAFLD.^[209] iHCC2578 was constructed based on the gene expression profiles of 361 HCC tissues, 49 noncancerous samples, and proteome data of HCC, leading to the prediction that mitochondrial acetate is an emerging substrate for FA synthesis catalyzed by the mitochondrial acetyl-CoA synthetase.^[131] An association between acetyl-CoA synthetase 1 expression and HCC growth was indeed confirmed by data of patients.^[131] Personalized GEMs for HCC patients were constructed and used for screening potential anti-cancer metabolites and genes, as well as *in silico* assessing the toxicity of targeted therapy.^[210,211] Despite the high heterogeneity of HCC, GEMs stratified HCC patients into different subtypes with distinct features in metabolism, signaling pathways, and clinical survival.^[212] GEMs can also be used to study the metabolic characteristics of CSCs and search for potential cures for drug resistance associated with liver cancer therapy.^[213]

CONCLUSION AND FUTURE PERSPECTIVE

Since liver is a main metabolic organ, it is not surprising that liver cancers are driven by metabolic alterations and can be targeted by compounds that block specific metabolic changes of liver cancer. However, metabolic reprogramming in HCC and CCA is remarkably heterogeneous due to heterogeneous driver gene mutations and oncogenic signals in liver cancer subsets. For example, steatohepatic HCC subset and another one with activated β -catenin exhibited contrasting FAO. The aberrant oncogenic signals make certain HCC subsets addicted to glutamine. In addition, CSCs in HCC or CCA rely on mitochondrial metabolism to maintain their phenotypes, and metastasis and drug resistance of HCC or CCA are largely mediated by FAO in the mitochondria.

There are numerous challenges associated with targeting metabolism for cancer treatment. Ability for dynamic metabolic changes is a surviving mechanism for cancer cells and they can utilize alternative pathways or energy resources when one metabolic pathway is blocked or eliminated. Since metabolic pathways are needed to fuel CD8⁺ T cells, natural killer cells, or type 1 macrophages and allow these cells to exhibit tumor-killing activities, agents designed to block metabolism supporting pathways may impair proliferation, activation, and cytotoxicity of the immune cells. They may also target the healthy proliferating cells in human body, such as bone marrow stem cells and normal intestinal epithelial cells, leading

to intolerable side-effects. A solution to these challenges may be achieved by performing studies on animals with intact immune system such as allografting tumor cells obtained from mice with same genetic strain than the host, utilizing genetically engineered tumor mouse models or performing patient-derived xenografts to mice with humanized immune system. This allows proper assessment of the impact of the targeted therapy on both cancer and immune cells. Targeting multiple metabolic pathways by a single chemoagent or a combination therapy that targets metabolism and cancer driving mutations or immune checkpoints may be considered to enhance therapeutic efficacy and overcome the drug resistance. In this regard, GEMs may be particularly useful for *in silico* assessment of the treatment effect and the toxicity of a targeted therapy using computational and systems biological approaches. GEMs have potential to transform cancer research and patient care for the novel and unique approach in identifying anti-cancer targets and treatment strategies personalized for liver cancer patients.

Abbreviations:

2-HG	(R)-2-hydroxyglutarate
5,10-methylene THF	5,10-methylene tetrahydrofolate
ACC	acetyl-CoA carboxylase
Acetyl-CoA	acetyl coenzyme A
AKT	protein kinase B (PKB)
ASNS	asparagine synthetase
ATM	ATM serine/threonine kinase
BAP1	BRCA1 associated protein-1 gene
Bcr-Abl	Bcr and Abl gene fusion mutation
BRAF	proto-oncogene B-Raf
CAF	cancer-associated fibroblast
Cav1	caveolin-1
CCA	cholangiocarcinoma
CD36	platelet glycoprotein 4
CDKN2A	cyclin-dependent kinase inhibitor 2A gene
CPT1B	carnitine palmitoyltransferase 1B
CPT2	carnitine palmitoyltransferase 2
CSC	cancer stem-like cell
CTNNB1	catenin beta-1 gene

EGFR	epidermal growth factor receptor
ETC complex I	NADH-coenzyme Q reductase
ETC complex II	succinate coenzyme Q reductase
ETC complex III	coenzyme Q cytochrome c reductase
ETC complex IV	cytochrome c oxidase
ETC complex V	ATP synthase
ETC	electron transport chain
FA	fatty acid
FAD	flavin adenine dinucleotide
FADH₂	reduced flavin adenine dinucleotide
FAO	fatty acid oxidation
FASN	fatty acid synthase
FGFR	fibroblast growth factor receptors gene
FoxO3	forkhead box protein O3
G6P	glucose-6-phosphate
G6PDH	glucose-6-phosphate dehydrogenase
GCKR	glucokinase (hexokinase 4) regulator gene
GEMs	genome-wide metabolic models
GFAT	glutamine:fructose-6-phosphate amidotransferase
GLS1	glutaminase kidney isoform, mitochondrial
Glut1	glucose transporter 1
GS	glutamine synthetase
HBP	hexosamine biosynthetic pathway
HIF-1α	hypoxia-inducible factor 1-alpha
HK2	hexokinase II
HMG-CoA	β -hydroxy β -methylglutaryl-CoA
HSD17β13	17 β -hydroxysteroid dehydrogenase type 13 gene
IDH	isocitrate dehydrogenase
JAK	janus kinases

KRAS	GTPase KRas gene
LDH	lactic dehydrogenase
MAPK	mitogen-activated protein kinase
MARCI	mitochondrial amidoxime-reducing component 1 gene
MBOAT7	lysophospholipid acyltransferase 7 gene
MCT1	monocarboxylate transporter 1
MCT4	monocarboxylate transporter 4
miR	microRNA
mTOR	the mammalian target of rapamycin
mTORC1	mammalian target of rapamycin complex 1
MYC	MYC proto-oncogene
NRASG12V	neuroblastoma RAS viral oncogene homolog (G12V)
NRF2	nuclear factor erythroid 2-related factor
O-GlcNAcylation	O-linked β -N-acetylglucosamine
OGT	UDP-N-acetylglucosamine peptide N-acetylglucosaminyltransferase
OXPHOS	oxidative phosphorylation
PD	pyruvate dehydrogenase
PDK3	pyruvate dehydrogenase kinase
PGC1α	the peroxisome proliferator-activated receptor γ coactivator-1 α
PI3K	phosphoinositide 3-kinase
PI3K	Phosphoinositide 3-kinases
PKM	pyruvate kinase M
PM	plasma membrane
PNPLA3	Patatin-like phospholipase domain-containing protein 3 gene
PPP	pentose phosphate pathway
RAS	Ras protein superfamily of small GTPases
ROS	reactive oxygen species

S1P	sphingosine-1-phosphate
SCD1	stearoyl-CoA-desaturase 1
SDH	succinate dehydrogenase
SDHB	succinate dehydrogenase subunit B
SHMT	serine hydroxymethyltransferase
SIRT3	Sirtuin-3 deacetylase
SLC27A1	solute carrier family 27 member 1
SLC2A1-AS1	solute carrier family 2 member 1-antisense 1
SMAD4	mothers against decapentaplegic homolog 4 gene
SMARCA2	probable global transcription activator SNF2L2 gene
SPHK1 and SPHK2	sphingosine kinase 1 and 2
SREBPs	sterol regulatory element-binding proteins
STAT	signal transducer and activator of transcription
SWI/SNF	SWItch/Sucrose Non-Fermentable
TAG	triglyceride
TCA	tricarboxylic acid
TERT	telomerase reverse transcriptase gene
THF	tetrahydrofolate
TM6SF2	transmembrane 6 superfamily 2 gene
TRIM35	tripartite motif-containing 35
UDP-GlcNAc	uridine diphosphate <i>N</i> -acetylglucosamine
WNT	Wingless/Integrated
YEATS4	YEATS domain containing 4
α-KG	α -ketoglutarate

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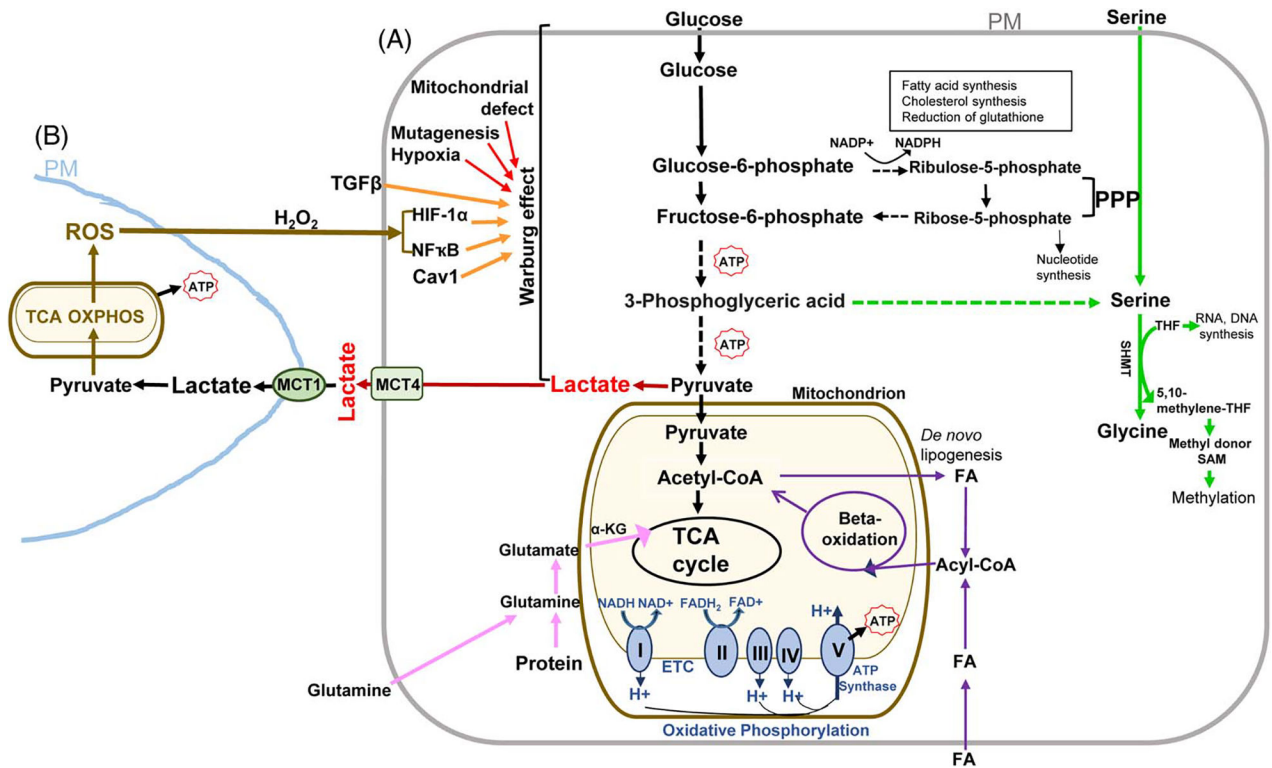


FIGURE 1.

Major metabolic pathways and alterations in cancer. Upon entering a cell, glucose undergo glycolysis to form pyruvate. In the absence of oxygen, pyruvate engages in fermentation to form lactate (cell A, red). If the cell has a sufficient oxygen supply, pyruvate is converted into acetyl-CoA in the mitochondria where the TCA cycle and OXPHOS occur. The TCA cycle plays a central role in the breakdown of organic molecules and in generation of NADH and FADH₂ for OXPHOS, a process that produces the most amount of ATP from the transfer of electrons through the ETC (cell A, blue). Through β-oxidation, FAs are broken down to form acetyl-CoA to enter the TCA cycle (cell A, purple lines). FAs are either imported from the extracellular space or by *de novo* lipogenesis (cell A, purple lines). Amino acids can enter different pathways of cellular respiration. For example, glutamine is converted to α-KG to refills the TCA cycle (cell A, pink lines). PPP is a branch of the glycolysis that forms ribulose-5-phosphate, NADPH, and ribose-5-phosphate used for synthesis of cholesterol, FAs, nucleotides, as well as for reduction of glutathione. The serine synthesis pathway is another branch of the glycolysis pathway (cell A, green lines). SHMT converts serine to glycine and the metabolites THF and 5,10-methylene THF are used for DNA and RNA synthesis or converted into a methyl donor S-adenosyl methionine (SAM). Mitochondrial defects, oncogenic mutations and signals, or hypoxia lead to the Warburg effect in cancer cells (cell A, red lines). TGFβ is in the extracellular space and ROS can be released from a neighboring cell that engages in mitochondrial metabolism (cell B, brown lines). TGFβ or ROS is able to reprogram a CAF or cancer cell to the Warburg effect by a HIF-1α-, NFκB, or Cav1-dependent mechanism (cell A, orange lines). MCT4 at the plasma membrane is responsible for lactate export (cell A, green) and MCT1 is responsible for lactate uptake (cell B, green). A dashed line indicates multiple steps in between.

Acetyl-CoA indicates acetyl coenzyme A; α -KG: α -ketoglutarate; CAF, cancer-associated fibroblast; Cav1, caveolin-1; ETC, electron transport chain; FA, fatty acid; FADH₂, reduced flavin adenine dinucleotide; FAD, flavin adenine dinucleotide; HIF-1 α , hypoxia-induced factor 1 alpha; OXPHOS, oxidative phosphorylation; MCT1, monocarboxylate transporter 1; MCT4, monocarboxylate transporter 4; PM, plasma membrane; PPP: the pentose phosphate pathway; ROS, reactive oxygen species; SAM, S-adenosyl methionine; SHMT, serine hydroxymethyltransferase; TCA: tricarboxylic acid; THF, tetrahydrofolate; 5,10-methylene THF, 5,10-methylene tetrahydrofolate; I: NADH-coenzyme Q reductase; II: succinate coenzyme Q reductase; III: coenzyme Q cytochrome c reductase; IV: cytochrome c oxidase; V: ATP synthase.;

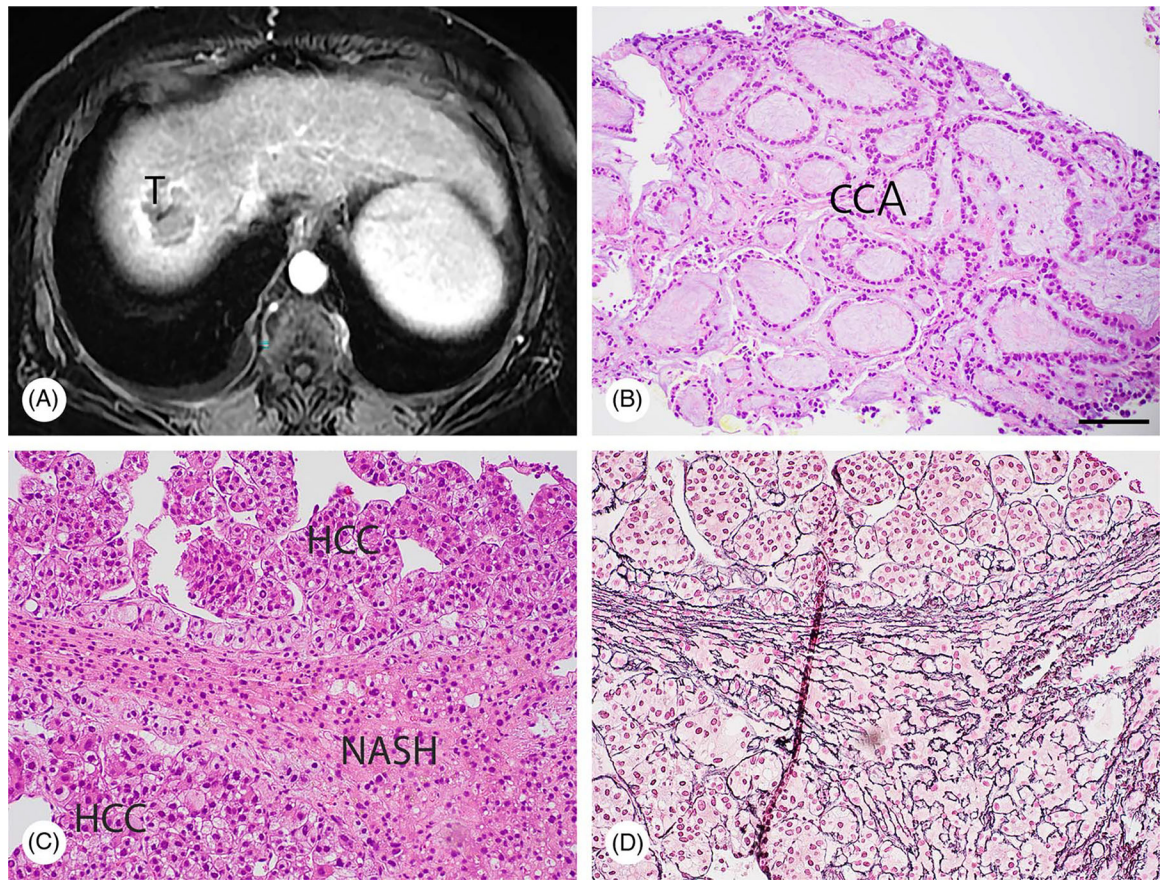


FIGURE 2.

NASH leads to both HCC and CCA in a 71-year-old female patient. (A) MRI (axial, water Ph4/GAD) revealing a 4.7 cm liver segment VIII mass (T) not specific for HCC. (B and C) Needle core biopsies were subjected to H&E staining, demonstrating that mucin-producing CCA (B) and HCC (C) co-existed on the background of NASH. (D) The result of reticulin staining with an adjacent section is shown. Bar, 50 μ m.

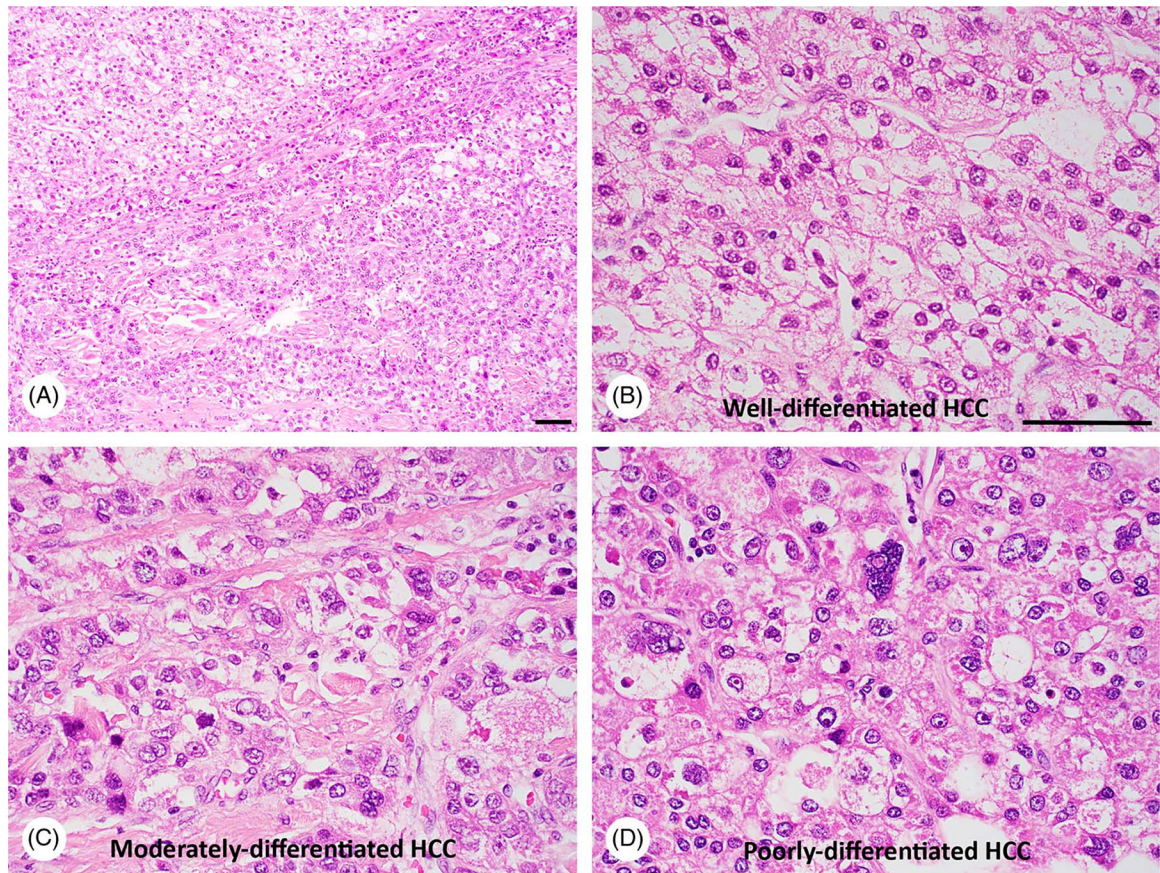


FIGURE 3.

Intratumoral heterogeneity in HCC of a 68-year-old male NASH patient. The liver tumor mass was subjected to H&E staining, revealing well-differentiated HCC (A and B), moderately-differentiated HCC (A and C), as well as poorly-differentiated HCC (A and D). Bar, 50 μ m.

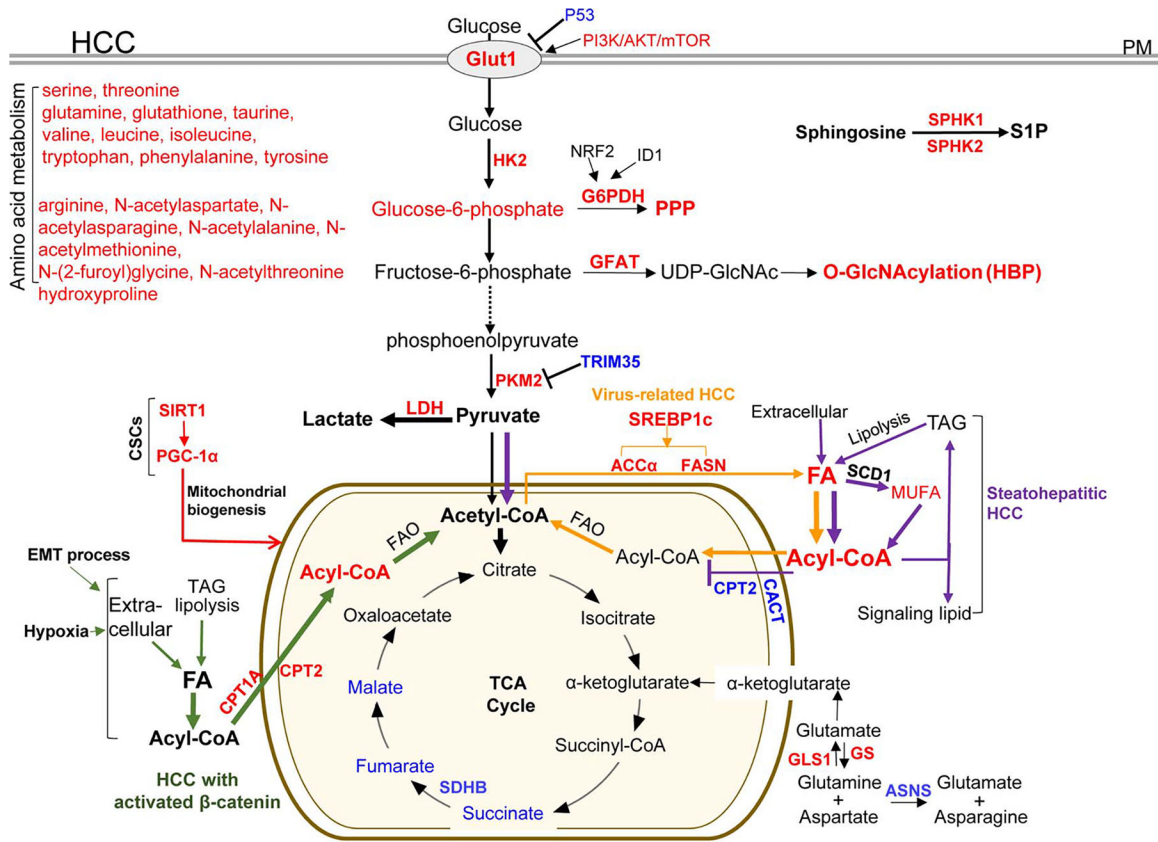


FIGURE 4.

Metabolic enzymes and pathways deregulated in HCC tumors or HCC subsets. Red: the expression level of the enzymes or metabolites is upregulated in HCC tumors compared with nontumoral tissues; blue: the expression level is downregulated. Green lines: a HCC subset with activated β-catenin exclusively uses FA catabolism; purple lines: a steatohepatic HCC subset shuts down FA catabolism and pyruvate is re-routed into the TCA cycle; orange lines: enhanced lipogenesis in virus-related HCC; and red lines: metabolic changes in CSC cell subset. Acetyl-CoA indicates acetyl coenzyme A; ACCα, acetyl-CoA carboxylase alpha; ASNS, asparagine synthetase; CACT, carnitine acylcarnitine translocase; CPT1A, carnitine palmitoyltransferase 1A; CPT2, carnitine palmitoyltransferase 2; CSC: cancer stem-cell like; EMT, epithelial-to-mesenchymal transition; FAO, fatty acid oxidation; FASN, fatty acid synthase; HK2, hexokinase II; Glut1, glucose transporter 1; GLS1, glutaminase kidney isoform, mitochondrial; GS, glutamine synthetase; G6PDH, glucose-6-phosphate dehydrogenase; GFAT, glutamine:fructose-6-phosphate amidotransferase; HBP, hexosamine biosynthetic pathway; ID1, inhibitor of differentiation 1; LDH, lactic dehydrogenase; MUFA, monounsaturated fatty acids; mTOR, the mammalian target of rapamycin; NRF2, nuclear factor erythroid 2-related factor; O-GlcNAcylation, O-linked β-N-acetylglucosamine; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator-1alpha; PKM2, pyruvate kinase M; PM, plasma membrane; PPP, the pentose phosphate pathway; SCD1: stearoyl-CoA-desaturase 1; SDHB, succinate dehydrogenase subunit B; SREBP1c, sterol regulatory element-binding protein 1c; SPHK1/2: sphingosine

kinase 1 and 2; S1P, sphingosine-1-phosphate; SIRT1, Sirtuin 1; TAG, triglyceride; TRIM35, tripartite motif-containing 35.

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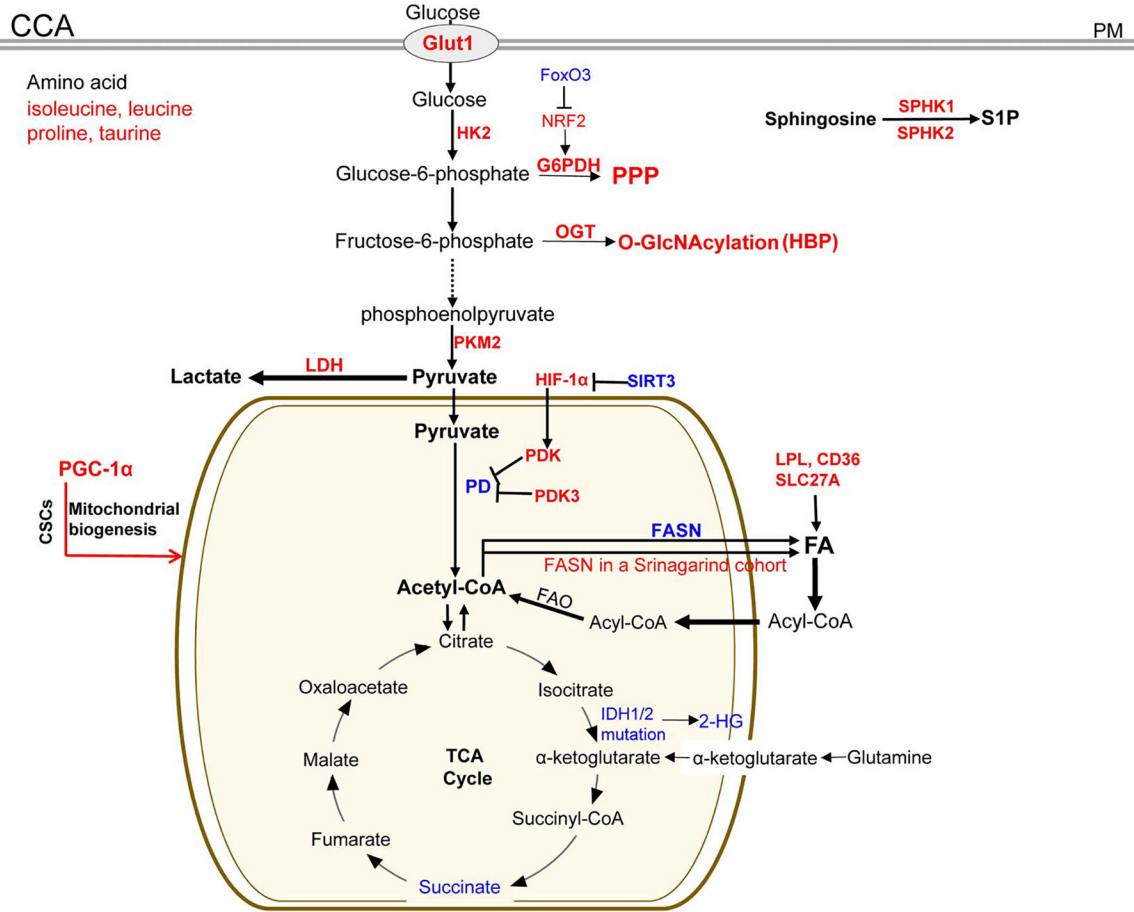


FIGURE 5.

Metabolic enzymes and pathways deregulated in CCA tumors or CCA subsets. Red: the expression level of the enzymes or metabolites is upregulated in CCA tumors compared with nontumoral tissues; blue: the expression level is downregulated. CCA indicates cholangiocarcinoma; CD36, platelet glycoprotein 4; CSC, cancer stem-cell like; FAO, fatty acid oxidation; FoxO3, forkhead box protein O3; Glut1, glucose transporter 1; G6PDH, glucose-6-phosphate dehydrogenase; HIF-1α, hypoxia-induced factor 1 alpha; HK2, hexokinase II; HBP, hexosamine biosynthetic pathway; IDH, isocitrate dehydrogenase; NRF2, nuclear factor erythroid 2-related factor; LDH, lactic dehydrogenase; LPL, lipoprotein lipase; OGT, UDP-N-acetylglucosamine peptide *N*-acetylglucosaminyltransferase; O-GlcNAcylation, O-linked β-*N*-acetylglucosamine; PD, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PKM2, pyruvate kinase M; PM, plasma membrane; PPP, the pentose phosphate pathway; SIRT3, Sirtuin 3; PGC-1α: peroxisome proliferator-activated receptor-gamma coactivator-1alpha; SPHK1/2, sphingosine kinase 1 and 2; S1P, sphingosine-1-phosphate; SLC27A1, solute carrier family 27 member 1; 2-HG: D-2-hydroxyglutarate.

TABLE 1

Metabolic targets and inhibitors under investigation for the treatment of liver cancer

Metabolic Pathway	Metabolic Target	Inhibitor (Cancer Type)	Clinical Trial or Approved Drug	References
Glycolysis (the Warburg effect)	Glut1	BAY-876 (HCC)		[151]
	HK2	Aspirin (HCC) 2-DG (HCC) Resveratrol (potentiates efficacy of sorafenib on HCC)	FDA approved drug Dietary supplement NCT01791595 (advanced cancer) FDA approved cancer drug	[152–154] [155,156] [157]
TCA Cycle	MCT1	AZD-3965		
	mTOR (Glut1 and glycolytic enzymes)	Everolimus (CCA)		[158]
	PDK Mitochondrial complex I	Dichloroacetate (inhibit glycolysis & promote OXPHOS) Metformin		[159]
De novo lipogenesis (FA synthesis)	IDH1/2	Metformin + chloroquine Ivosidenib	NCT03184493 (HCC) NCT04033107 (HCC) NCT04114136 (HCC) NCT02496741 (CCA) Approved for CCA subsets with IDH1 mutations	[160–163]
	SREBP-1c	Fatostatin, FGH10019 (compound 24) (HCC)		
FAO and cholesterol	FASN	TVB2640	NCT03938246 (NASH)	[164]
	ACC	TVB 3664 (HCC induced by PTEN loss/c-MET or AKT/ NRAS)		[165]
	SCD1	ND-654 (HCC)		[166]
	CPT	A939572, CAY10566, MF-438, BZ36		[119]
	HMG-CoA reductase	Etomoxir (HCC with activated β -catenin) Simvastatin, Atorvastatin (CCA)		[167]
Amino acid metabolism	SPHK2	Pravastatin(potentiates chemoembolization on HCC) ABC294640	NCT01075555 (Pravastatin + sorafenib for HCC) NCT01488513 (advanced solid tumor) NCT02939807 (HCC) NCT03377179 (ABC294640 alone or in combination with hydroxychloroquine for advanced CCA)	[168,169]
	Glutaminase (GLS1)	CB-839 (HCC)		[170]
Glutamine transporter	Glutamine transporter	V-9302 (HCC, CCA)		
	Glutamine	Crisantaspase		[171]
	GS	Methionine-L-sulfoximine	FDA approved cancer drug	

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Metabolic Pathway	Metabolic Target	Inhibitor (Cancer Type)	Clinical Trial or Approved Drug	References
Autophagy		Hydroxychloroquine	NCT03037437 (Hydroxychloroquine +sorafenib for HCC) NCT04566133 (Hydroxychloroquine +trametinib for CCA)	

ACC indicates acetyl-CoA carboxylase; CCA, cholangiocarcinoma; HK2, hexokinase II; FASN, fatty acid synthase; GLS1, glutaminase kidney isoform, mitochondrial; Glut1, glucose transporter 1; GS, glutamine synthetase; OXPHOS, oxidative phosphorylation; PDK, pyruvate dehydrogenase kinase; SCD1, stearoyl-CoA-desaturase 1.