# Metabolism as New Avenue for Hepatocellular Carcinoma Therapy

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Alterations in cellular metabolism are important drivers of the tumorigenesis process and the promotion of drug resistance. The balance between glycolysis and oxidative phosphorylation (OXPHOS) is a central point. The complex interplay between tumor cell metabolism and drug resistance is an important aspect, and that the balance between OXPHOS and fermentative glycolysis is critical for determining the cellular behavior during neoplastic transformation and the acquisition of drug resistance by tumor cells. Furthermore, it is worth mentioning that, modulating the balance between OXPHOS and lactic acid fermentation to trigger OXPHOS and inhibit fermentation could be considered a strategy to reduce tumor invasiveness and drug resistance.

hepatocellular carcinoma (HCC) metabolism therapeutic approaches

## 1. Cancer and Metabolism

Genetics dominated cancer research for a long time after the pioneering work of Otto Warburg and Albert Szent-Gyorgyi in the 1930s [1][2][3][4][5]. Dysregulation of cellular metabolism has now reemerged as an important driver of cancer [6]. After decades of research primarily focused on tumor genetics, tumor cell metabolism has received renewed attention [6]. Currently, the scientific community is paying great attention to cellular metabolism and metabolic plasticity in tumor cell biology and has investigated the role of specific metabolic pathways in tumorigenesis [7][8]. Glycolysis, lactic acid fermentation, and oxidative phosphorylation (OXPHOS) are key metabolic processes that are now widely studied and considered targets for inhibiting tumor growth and overcoming drug resistance. In general, normal cells rely on the metabolic axis glucose→pyruvate→TCA/OXPHOS (oxidative glycolysis), whereas tumor cells shift towards lactic fermentation, glucose→pyruvate→lactate (fermentative glycolysis). This “fermentative signature” characterizes the Warburg effect, which is aerobic glycolysis or lactic acid fermentation regardless of the presence of hypoxic conditions [9]. Tumor cells primarily rely on the Warburg effect to generate energy, although this process is energetically inefficient (36 versus 4 ATP molecules/mol glucose) [10]. The switch to fermentative glycolysis provides several advantages to tumor cells, such as fast biosynthesis of ATP molecules, an increased supply of biosynthesis intermediates [10], and reduced generation of reactive oxygen species (ROS), which protects cancer cells from apoptosis [11]. Two steps are finely tuned: the conversion of pyruvate into lactate, which is catalyzed by lactate dehydrogenase (LDH), and the activation of OXPHOS.

What triggers the Warburg effect is still being debated. Hypoxia, considered an activating stimulus, has recently been ruled out as a major factor, as some tumors develop the Warburg effect even under normoxic conditions [10].

It has been suggested that the Warburg phenotype is activated by an increased demand for $NAD^+$ that is not matched with an adequate supply of ATP [12]. In general, it is now believed that the very early activation step of the Warburg effect could be an alteration of the metabolic control necessary for tumor cells to acquire a cell-autonomous nutrient uptake and an anabolic profile. Nevertheless, this initial trigger has not been fully elucidated. The mechanisms by which the Warburg effect supports drug resistance are also still under debate. It is known that the glycolytic environment can influence the response of cancer cells to drugs [13]. The relevance of the Warburg effect in tumorigenesis and drug resistance is still under debate [11][13], and the role of OXPHOS in cancer biology remains controversial.

# 2. Hepatocellular Carcinoma and Metabolism

Metabolic dysregulation is emerging as an important risk factor for HCC [14]. As in other tumors, the neoplastic transformation of a normal hepatocyte is characterized by a general metabolic rewiring. Transformed hepatocytes display the Warburg phenotype, which in the liver is characterized by rearrangements in the expression and phosphorylation of key glycolytic enzymes [15]. Indeed, more than fifty years ago, metabolic reprogramming to enhance glycolysis was shown to be central to transforming hepatocytes [16]. Glucose metabolism rewiring can find a mechanistic explanation in the hypoxic milieu in which HCC lies. In fact, in normal hepatic tissue, the median $O_2$ partial pressure is about 30 mmHg, whereas in the HCC intratumor region, it is roughly 6 mmHg [17][18][19]. In line with this, HIF-1α is highly expressed in HCC [20]. In this hypoxic microenvironment, LDH activity is increased [21] and the pyruvate dehydrogenase complex (PDH) is reduced [22], thus obtaining an increase in lactate production and a dampening of the conversion of pyruvate into acetyl-CoA, which contribute to the instauration of a glycolytic phenotype.

## 2.1. Glucose Metabolism Rewiring

Glucose homeostasis is rearranged in hepatocellular carcinoma (HCC), leading to a change in the expression and activity of transporters and enzymes, which in turn leads to a rewiring of the metabolite flux. The Warburg phenotype in HCC is not characterized by increased gluconeogenesis because fructose 1,6-bisphosphatase 1 (FBP1) and phosphoenolpyruvate carboxykinase 1 and 2 (PEPCK 1 and 2) expression are reduced [23]. In addition, the expression of glucose transporters GLUT1 and GLUT2 is increased in HCC, and while GLUT2 overexpression worsens the prognosis of patients, the inhibition of GLUT1 expression attenuates the malignant behavior of HCC cells [24][25][26]. The conversion of glucose into glucose-6-phosphate (G6Ph) is a highly regulated metabolic reaction catalyzed by the hexokinase (HK) enzyme, which has five isoforms: HK1 to HK4 and the hexokinase domain containing 1 (HKDC1). The expression of HK2, the prevalent isoform in HCC, is associated with reduced survival in HCC patients [27], and, accordingly, HK2 inhibition dampens glycolysis in favor of OXPHOS, relieving the tumor phenotype [28]. HKDC1 expression has also been shown to exacerbate HCC aggressiveness and prognosis [29].

## 2.2. Pentose Phosphate Pathway

The Pentose Phosphate Pathway (PPP) plays a central role in the metabolic reprogramming of tumor cells and HCC. The main outcome of the PPP is the production of ribose-5-phosphate, which is the precursor for the synthesis of nucleotides that are needed for tumor cell growth. In addition, NADPH is another product of the PPP that sustains lipid biosynthesis and keeps the tumor cell in an antioxidant environment by maintaining thioredoxin and glutathione in a reduced state. This may help tumor cells protect themselves from apoptotic cell death and develop resistance to drug therapy [30]. Although the levels of ribulose 5-phosphate (Ru5P) and ribose 5-phosphate (R5P) are decreased due to their rapid utilization by cancer cells, the transcription of most enzymes involved in the PPP is elicited in HCC [31]. Among the enzymes that are involved in PPP rewiring, glucose-6-Ph dehydrogenase (G6PDH) should be mentioned. The expression of this enzyme was shown to result in increased metastasis, drug resistance, and decreased survival in HCC.

## 2.3. TCA Cycle Rewiring

The TCA cycle produces NADH and $FADH_2$, which fuel OXPHOS activity. The TCA cycle is dysregulated at several points in HCC. Pyruvate dehydrogenase kinase 4 (PDK4), which phosphorylates and inhibits pyruvate dehydrogenase (PDH), is downregulated in HCC and associated with increased lipid biosynthesis and poor prognosis [32]. Succinate dehydrogenase (SDH) [succinate→fumarate] and fumarate hydratase (FH) [fumarate→malate] are deficient in HCC. Moreover, succinate and fumarate-mediated stabilization of HIF-α enhances glycolysis and angiogenesis [33].

## 2.4. OXPHOS Rewiring

In normal cells, OXPHOS utilizes NADH and $FADH_2$ from the TCA cycle for ATP production. Several pieces of evidence associate defective OXPHOS with enhanced HCC development through different mechanisms, for example, mitoribosome defects or by TGFβ-mediated reduction of OXPHOS without the involvement of the glycolytic pathway [34][35]. HCV-mediated reduced expression of OXPHOS protein subunits leads to a Warburg-like phenotype, eventually promoting HCC development [36]. Membrane trafficking-involved Rab3A GTPase is overexpressed and also post-translationally modified by the addition of N-acetylglucosamine (O-GlcNAcylated) in HCC. Usually, unmodified Rab3 inhibits the metastatic process in HCC by boosting OXPHOS, but O-GlcNAcylation impedes this function [37].

## 2.5. Lipid Metabolism Rewiring

Lipid metabolism is one of the important metabolic tasks accomplished by the liver. Altered lipid metabolism homeostasis is responsible for many pathologic conditions. Dyslipidemias may lead to diabetes, obesity, and liver steatosis, and all these conditions have been correlated with HCC onset [38][39]. De novo lipogenesis (DNL) is very well regulated, and several hindrances have been reported for DNL in HCC. In general, DNL follows this route: pyruvate→acetyl-CoA+oxaloacetate (OAA)→citrate [inside mitochondria]. In conditions of carbohydrate abundance, citrate is exported into the cytoplasm where ATP-citrate lyase (ACLY) converts citrate into acetyl-CoA+OAA. Then, lipid biosynthesis takes place in the cytoplasm, mainly by acetyl-CoA carboxylase (ACC) [acetyl-CoA→malonyl-CoA] and fatty acid synthase (FASN) [malonyl-CoA→palmitoyl-CoA] [40]. DNL works in close

association with the PPP to produce the necessary NADPH. DNL deregulations have been reported in HCC-predisposing conditions, such as NAFLD [41].

HCC uses DNL and exogenous fatty acids to meet its growth requirements [42], and increased free fatty acid uptake through the fatty acid translocase CD36 correlates with the initiation and progression of HCC [43].

The liver β-oxidizes fatty acids in either peroxisomes or mitochondria, where they are transported by carnitine palmitoyltransferase-I (CPT-I), which condenses carnitine with very long Acyl-CoA [44]. Interestingly, NASH-induced rats show reduced CPT-I activity as well as defective complexes I and II of the mitochondrial electron transport chain (ETC) [45][46]. Furthermore, HCC patients showed defective mitochondrial β-oxidation due to decreased expression of several enzymes involved [23]. Thus, impaired mitochondrial functions can be considered predisposing conditions for HCC development.

## 2.6. Nucleotide Metabolism Rewiring

Nucleotide metabolism is essential for nucleic acid biosynthesis, and this is particularly true for tumor cells that have an increased proliferative rate compared to healthy cells. In this regard, the PPP provides R5Ph, which is needed along with amino acids for nucleic acid biosynthesis. In HCC, the expression of several genes involved in this biosynthetic pathway is increased. In particular, three enzymes constitute the enzyme complex that catalyzes the initial reaction in pyrimidine biosynthesis: carbamoyl phosphate synthase 2, aspartate transcarbamylase, and dihydroorotase [47][48]. Furthermore, other rate-limiting enzymes, such as thymidylate synthase (TYMS), thymidine kinase 1 (TK1), and deoxythymidylate kinase (DTYMK), are upregulated in HCC and are associated with poor prognosis and cancer stemness [49][50][51].

## 2.7. Protein and Amino Acid Metabolism Rewiring

The liver is also a central hub for protein and amino acid homeostasis, so this imbalance due to pathological conditions reflects changes in protein and amino acid levels. For example, negative correlations between serum albumin levels and tumor diameter, tumor multifocality, portal vein thrombosis, and α-fetoprotein, have been reported [52]. The liver is also responsible for the biosynthesis of non-essential amino acids (NEAAs), which are involved in many biochemical pathways. In many tumors, NEAA production is increased due to the rewiring of glucose metabolism, which increases the demand for oxidizable substrates [53][54][55][56].

## 2.8. Urea Cycle Rewiring

In HCC, most urea cycle enzymes are downregulated, along with low concentrations of urea cycle metabolites such as arginine, ornithine, and citrulline, which are common in advanced HCC stages. Two key enzymes in the urea cycle, argininosuccinate synthase 1 (ASS1) and carbamoyl phosphate synthase (CPS), show a hypermethylated state in HCC, which explains their reduced expression.

## 2.9. HCC Tumor Microenvironment Rewiring

Hepatocytes are the main cell type in the liver parenchyma, while the rest is represented by stromal cells, including endothelial cells, immune cells, Kupffer cells, and hepatic stellate cells. Stromal cells are also recruited by HCC cells in the tumor microenvironment (TME). Two important effector cells are tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSC), which are the main cell types found in the tumor infiltrate [57]. The crosstalk between cancer cell-released metabolites and immune cells sustains the tumor ecosystem. Indeed, upon an inflammatory challenge, macrophages acquire an inflammatory cytokine-producing M1-like phenotype, whereas anti-inflammatory stimuli lead to an immunosuppressive M2-like phenotype [58]. Hence, tumor onset is favored by macrophage-induced chronic inflammation. The immunosuppressive M2-like phenotype may then prevail, supporting growth, angiogenesis, and the metastatic process [59]. These observations are in line with the consolidated view that an inflammatory status promotes tumor onset. For example, tumor-secreted cytokines attract monocyte-derived TAM, which switches to a glycolytic fermentative metabolism and survives in the hypoxic TME [59][60].

# 3. Metabolism-Based Pharmacology Approaches in HCC

## 3.1. Inhibition of the Glycolytic Pathway and Pentose Phosphate Pathway

Several glycolysis inhibitors are currently being investigated as possible therapeutic molecules. The glucose analog 2-deoxy-D-glucose (2-DG) is converted to 2-DG-6-phosphate, which inhibits HK2 activity in a non-competitive manner. This inhibition is associated with reduced HCC growth and invasiveness. Moreover, 2-DG synergizes with the effect of sorafenib in HCC cell lines [61][62]. 3-Bromopyruvic acid (3-BrPA) inhibits several enzymes, including HK2, PGK1, succinate dehydrogenase, GAPDH, and LDH. Also, some studies have identified GAPDH as the principal target of 3-BrPA in HCC cell lines [63][64][65][66]. The plant-derived molecule koniginic acid also inhibits GAPDH by binding to the enzyme's active site. However, the potential applicability of koniginic acid in HCC has not been well demonstrated [67]. Several metabolites from plants are considered potential inhibitors of glycolysis in HCC. Among them, chrysin, a flavonoid found in Chinese medicinal plants, can reduce the expression of HK2, reduce glucose uptake and lactate production, and ultimately trigger the mitochondrial apoptotic pathway. The anti-HCC effects of chrysin have also been described in a mouse model [68]. Likewise, the plant-stress metabolite methyl jasmonate reduces mitochondrial transmembrane potential by sequestering HK2 and thereby elicits apoptosis in HCC cell lines and mice [69].

## 3.2. Lipid Metabolism Rewiring Inhibition

To date, little evidence exists to suggest the possibility of lipid metabolism as an effective therapeutic target in HCC. For example, the inhibition of the central lipogenic enzyme acetyl-CoA carboxylase (ACC) has been considered for pharmacological purposes. Indeed, some chemical inhibitors of ACC are now under investigation, such as ND-654, an allosteric inhibitor of ACC1 and ACC2, which also enhances the pharmacological effects of sorafenib [70]. Several fatty acid synthase (FASN) inhibitors, including orlistat, C93, C75, GSK2194069, and GSK837149A, have been evaluated in preclinical testing [71][72]. These compounds, however, show several side effects and significant toxicity. One stearoyl-CoA desaturase 1 (SCD1) inhibitor, A939572, was shown to be

efficacious in HCC cell lines and to increase sorafenib sensitivity by modulating ER stress-induced differentiation [73]. The transcription factor Liver X receptor alpha (LXRα) is involved in lipid homeostasis and is under evaluation as an anti-HCC target. TO901317 has been reported to increase LXRα expression and decrease glucose uptake by inhibiting GLUT1 expression [74].

### 3.3. Other Potential Metabolic Targets for HCC

So far, chemicals targeting other metabolic pathways have not provided conclusive indications. For example, it has been proposed to target epigenetic regulation and ubiquitin-dependent degradation to increase fructose-1,6-bisphosphatase 1 (FBP1) expression, which is decreased in HCC [75]. Several molecules are under testing in this respect, such as histone deacetylase inhibitors or lysine-specific histone demethylase 1 inhibitors [76][77][78]. Still, these chemicals show low specificity and a pleiotropic action. Proteasome inhibitors and dexamethasone have also been suggested to regain FBP1 levels, but likewise, the scarce selectivity results in many side effects [79]. Glutaminase 1 (GLS1), which converts glutamine into glutamate, is a key enzyme in tumor cell metabolism because it can promote lipid and nucleotide biosynthesis and reduce ROS generation, thereby protecting tumor cells from apoptosis. Some GLS1 inhibitors have been tested, but they showed toxicity and low selectivity [80][81].

## 4. Multitarget Metabolic Systems

### 4.1. Plant-Based Multi-Pathway Approach

As mentioned above, metabolism-based single-target approaches still show several critical points in terms of efficacy and side effects. To this end, a synergistic approach targeting multiple metabolic pathways or processes may offer some advantages to achieve higher efficacy, a lower dose, and lower toxicity. For example, plant extracts, because their composition is a blend of several metabolites, can provide an interesting option to target several metabolic pathways at the same time. Plant extracts have not been explored much in this direction. However, several studies have reported interesting effects of plant extracts on several types of tumors, most likely due to synergistic effects targeting multiple pathways [82][83][84][85][86]. The plant-derived polyphenol molecule resveratrol acts on multiple cellular targets and exhibits a wide range of biological activities, including positive effects on various metabolic pathways [87].

### 4.2. Targets Exploiting Synergistic Effects on Metabolic Pathways

In addition, and in support of a plant-based multi-pathway approach, selective inhibition of receptors controlling metabolism may offer an additional translational opportunity for HCC pharmacological management. In this respect, it has recently demonstrated that LPA receptor 6 (LPAR6) is directly involved in the control of HCC cell metabolism [88] and that ectopic expression of LPAR6 in HCC cells drives sorafenib resistance by triggering a “metabolic switch”, which increases lactic acid fermentation at the expense of OXPHOS. This sorafenib resistance can be overcome by reducing lactic acid fermentation through inhibition of LPAR6 using a recently developed novel LPAR6 antagonists [89][90][91]. LPAR6 has a pro-tumorigenic role in HCC [92] by controlling the trans-differentiation of

peritumoral tissue fibroblasts (PTFs) into carcinoma-associated fibroblasts (CAFs) [93] and its overexpression leads to a worse clinical outcome in HCC patients [92].

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