Glucometabolic Reprogramming in the Hepatocellular Carcinoma Microenvironment: Cause and Effect

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Abstract: Hepatocellular carcinoma (HCC) is a tumor that exhibits glucometabolic reprogramming, with a high incidence and poor prognosis. Usually, HCC is not discovered until an advanced stage. Sorafenib is almost the only drug that is effective at treating advanced HCC, and promising metabolism-related therapeutic targets of HCC are urgently needed. The “Warburg effect” illustrates that tumor cells tend to choose aerobic glycolysis over oxidative phosphorylation (OXPHOS), which is closely related to the features of the tumor microenvironment (TME). The HCC microenvironment consists of hypoxia, acidosis and immune suppression, and contributes to tumor glycolysis. In turn, the glycolysis of the tumor aggravates hypoxia, acidosis and immune suppression, and leads to tumor proliferation, angiogenesis, epithelial–mesenchymal transition (EMT), invasion and metastasis. In 2017, a mechanism underlying the effects of gluconeogenesis on inhibiting glycolysis and blocking HCC progression was proposed. Treating HCC by increasing gluconeogenesis has attracted increasing attention from scientists, but few articles have summarized it. In this review, we discuss the mechanisms associated with the TME, glycolysis and gluconeogenesis and the current treatments for HCC. We believe that a treatment combination of sorafenib with TME improvement and/or anti-Warburg therapies will set the trend of advanced HCC therapy in the future.

Keywords: hepatocellular carcinoma, tumor microenvironment, glycolysis, gluconeogenesis, Warburg effect

Introduction

Liver cancer is the second leading cause of cancer mortality worldwide and the 7th most frequently diagnosed cancer worldwide, with approximately 782,000 deaths and 841,000 new cases diagnosed annually.¹ Hepatocellular carcinoma (HCC) is the major type of primary liver cancer (PLC) and accounts for 75–85% of cases.² The main risk factors for HCC are hepatitis B virus (HBV), hepatitis C virus (HCV), cirrhosis, aflatoxin-contaminated foodstuffs, alcohol abuse, obesity, and type 2 diabetes.³⁻⁵ Decades ago, Otto Warburg observed that cancer cells rely on glycolysis for the generation of energy even in a normoxic environment, which was known as the “Warburg effect” or “aerobic glycolysis”.⁶⁻⁷ Aerobic glycolysis not only provides energy but also provides intermediates (nucleotides, amino acids, lipids and NADPH) for biosynthesis,⁸⁻⁹ which explains why aerobic glycolysis occurs prior to oxidative phosphorylation (OXPHOS) in proliferation cells such as tumor cells. The distinct proliferation characteristics and glucometabolic...
reprogramming of tumor create a unique TME different from the overall human environment. The HCC microenvironment consists of various cell types, growth factors, proteolytic enzymes, extracellular matrix (ECM) proteins and cytokines, which are widely known to contribute to hypoxia, acidosis and immune suppression.\textsuperscript{10} The “suitable” environment provided by the tumor microenvironment (TME) contributes to tumor proliferation, angiogenesis, invasion and metastasis. Aerobic glycolysis and TME can interact with each other and create a vicious spiral.

However, as the major metabolic organ in the body, liver plays an important role in glucose homeostasis by regulating synthesis and decomposition of glycogen. During fasting, approximately 80\% of endogenous glucose is produced by liver through gluconeogenesis.\textsuperscript{11,12} Gluconeogenesis is actually a reverse pathway of glycolysis and can inhibit glycolysis through downstream gluconeogenesis enzymes, such as phosphoenolpyruvate carboxykinase1 (PCK1) and fructose-1,6-bisphosphatase 1 (FBP1).\textsuperscript{13,14} In addition, gluconeogenesis uses lactate as one of the substrates to consume harmful byproducts of glycolysis. This glucose-metabolizing feature offers a unique opportunity to treat HCC. Nevertheless, the decrease of PCK1 and FBP1 expression in HCC compared to normal liver tissue lead to the suppression of gluconeogenesis and elevation of glycolysis.\textsuperscript{15,16} As an emerging hallmark of tumors, studies regarding glucose metabolism reprogramming used to focus on glycolysis. However, the correlation between gluconeogenesis and tumors is rarely reported but may provide insight for the treatment of HCC. In this review, we summarized the interaction between glucometabolic reprogramming and the HCC microenvironment. Furthermore, we discussed HCC treatment targeting the improvement of the TME, suppression of glycolysis and elevation of gluconeogenesis aiming to find promising metabolism-related therapeutic targets of HCC.

**Hypoxic Microenvironment**

Hypoxia is a typical microenvironment feature in nearly all solid tumors, and it contributes to their rapid and uncontrolled proliferation.\textsuperscript{17} Hypoxia-inducible factors (HIFs) are key transcription factors produced by tumor cells under hypoxia to cope with the hypoxic microenvironment. Furthermore, HIFs contribute to invasive growth, survival, metastasis, treatment resistance and poor prognosis of HCC.\textsuperscript{18} The HIF family includes three subtypes: HIF-1, HIF-2, and HIF-3. Among them, HIF-1 and HIF-2 are considered to be the most important factors for cells to respond to hypoxia. HIF-1 and HIF-2 consist of an oxygen-sensitive subunit HIF-\(\alpha\) and a constitutively expressed HIF-\(\beta\) subunit.\textsuperscript{19,20} Both HIF-\(\alpha\)1 and HIF-\(\alpha\)2 are reported correlating with tumors. Studies have shown that HIF-1\(\alpha\) regulates vascular endothelial growth factor (VEGF) during the acute phase of hypoxia, while VEGF is mainly regulated by HIF-2\(\alpha\) during long-term hypoxia.\textsuperscript{21} HIF-2\(\alpha\) is overexpressed in primary and metastatic tumors\textsuperscript{22} and is positively correlated with tumor angiogenesis.\textsuperscript{23} However, studies on HIF-2\(\alpha\) and liver cancer are rare, and HIF-1\(\alpha\) is the primary factor in liver cancer hypoxia. In the presence of oxygen, HIF-1\(\alpha\) is hydroxylated by prolyl hydroxylases (PHDs), leading to its rapid proteasomal degradation. Under hypoxic conditions, PHDs are no longer active to hydroxylate HIF-1\(\alpha\). HIF-1\(\alpha\) will be stabilized and translocated to the nucleus.\textsuperscript{24}

Accumulation of HIF-1\(\alpha\) can influence tumor survival and proliferation by regulating tumor glycometabolism in the following four ways. First, HIF-1\(\alpha\) can increase the uptake of glucose by upregulating the expression of glucose transporters (GLUT) such as GLUT1. Second, HIF-1\(\alpha\) promotes the expression of glycolytic enzymes and accelerates the conversion of glucose to pyruvate. Third, HIF-1\(\alpha\) can phosphorylate pyruvate dehydrogenase (PDH) by inducing the expression of pyruvate dehydrogenase kinase (PDK) and inactivate the PDH to prevent the conversion of pyruvate to acetyl CoA. Fourth, HIF-1\(\alpha\) upregulates the expression of lactate dehydrogenase A (LDHA) to stimulate the production of lactic acid.\textsuperscript{25}

In addition to the effect the of the glycometabolism of HCC, HIF-1\(\alpha\) can also influence HCC survival by regulating the oxidative stress level.\textsuperscript{26} Oxidative stress can mediate mitochondrial apoptosis and the immune response in liver cancer.\textsuperscript{27} Reactive oxygen species (ROS), byproducts of oxygen metabolism, are the main causes of oxidative stress, and their concentration changes play dual functions in the regulation of HCC process.\textsuperscript{28,29} Low levels of ROS may induce DNA mutation by oxidative DNA damage, which eventually increases the likelihood of HCC development.\textsuperscript{30,31} Overexpression of ROS can inhibit HCC by inducing apoptosis of hepatoma cells and inhibiting metastasis through ROS/Akt/NF-kB pathway, and suppressing liver cancer stem cell via ROS/\(\beta\)-Catenin/FOXO3a Signaling.\textsuperscript{32-34} In a hypoxic environment, the low oxygen content and the lack of oxygen as electron recipient lead to the imbalance of electron flow through
the mitochondrial electron chain, which contributes to the accumulation of ROS and causes irreversible cellular damages in tumors.\textsuperscript{35,36} However, HIF-1\(\alpha\) can promote HCC progression by preventing ROS accumulation through the following pathways. First, HIF-1\(\alpha\) prevents pyruvate from entering TCA cycle by inactivating PDH through PDKs and the conversion of pyruvate to lactate by upregulating LDHA expression.\textsuperscript{21,37,38} HIF-1\(\alpha\) therapy ensures that circulating tricarboxylic acid cycle (TCA) substrates cannot enter mitochondrial oxidation.\textsuperscript{36,39} Second, HIF-1\(\alpha\) can reduces ROS accumulation by inhibiting ROS production sites in the electron transport chain (ETC), such as complexes 1 and 4.\textsuperscript{40,41} Third, HIF-1\(\alpha\) decreases the number of mitochondrial cristae and the mitochondrial mass through HEY1/PINK1 pathway, and degrading mitochondria by inducing BNIP3 to restrict ROS production and promote ROS elimination.\textsuperscript{42} Glutamine is a key source of carbon, secondary only to glucose.\textsuperscript{43} Decomposition of glutamine will replenish the TCA cycle and provide abundant carbon and nitrogen for hepatocyte growth and proliferation.\textsuperscript{44,45} Some of the carbon can be used to produce NADPH to achieve redox equilibrium.\textsuperscript{46} At the same time, glutamic acid produced by glutamine decomposition will directly synthesize the antioxidant glutathione and neutralize ROS.\textsuperscript{47} Through various pathways, the tumor cells will eventually maintain ROS at an appropriate level that is conducive to their own growth and proliferation. At present, most chemotherapy drugs and radiotherapy kill tumor cells by inducing ROS production.\textsuperscript{48} Hence, interfering with or reversing of hypoxia and its effects or identifying a suitable way to increase ROS level in tumor cells can reduce the drug resistance of tumors and improve the therapeutic effect.\textsuperscript{32,49,50} In turn, tumor cell aerobic glycolysis can influence HIF-1\(\alpha\) by upregulating glutamine. The TCA cycle is the hinge of metabolism of glucose, fat and amino acid. Glutamine is the most abundant nonessential amino acid in blood serum. The “Warburg effect” of tumors leads to the conversion of pyruvate into lactic acid and the lack of carbon source for TCA cycle. Glutamine is not only a nitrogen source for amino acids and nucleotide synthesis but also the main carbon source for TCA cycle and macromolecule biosynthesis.\textsuperscript{51} Many tumor cells require much more glutamine than normal cells. Tumor cells take up a large amount of glutamine and then convert it into other metabolic intermediates, meeting the energy requirements for rapid proliferation.\textsuperscript{52} Glutamine can regulate the stability of HIF-1\(\alpha\) in response to hypoxia and support the survival of HCC cells by upregulating proline and hydroxyproline levels.\textsuperscript{53} The increase in the level of glutamine further exacerbates tumor hypoxia. Thus, targeting glutamine could be a new strategy in oncotherapy.

**Acid-Base Microenvironment**

Acid-base characteristic of the TME is widely recognized as the acidification of extracellular pH (pH\(_e\)), which is so-called tumor acidosis (Figure 1). Tumor cells have a lower pH\(_e\) of \(-6.7\)–\(-7.1\) and a higher intracellular pH (pH\(_i\)) \(\geq 7.4\) rather than a higher pH\(_e\) of \(-7.4\) and a lower pH\(_i\) of \(-7.2\) in normal cells.\textsuperscript{54} Recently scientists have proposed using high-resolution pH mapping to monitor pH\(_e\) in HCC, which could be a biomarker for metabolic changes and monitoring tumor aggressiveness and therapeutic outcome.\textsuperscript{55–57} Tumor acidosis is the consequence of lactate and H\(^+\) ions accumulation, which are produced by glycolysis and oxidative metabolism. Most tumor cells, also called as glycolytic tumor cells, prefer glycolysis rather than OXPHOS, leading to increases in lactate and H\(^+\) production. However, some tumor cells still use oxidative metabolism and are called oxidative tumor cells.\textsuperscript{58} The accumulation of lactate in HCC microenvironment is mainly due to the increases of intracellular lactate production and extracellular transport. The interconversion of pyruvate and lactate plays a critical role in intracellular lactate production, which is primarily catalyzed by the lactate dehydrogenase (LDH) family.\textsuperscript{59} LDH enzymes with high M-subunits (encoded by LDHA) promote the conversion from pyruvate to lactate.\textsuperscript{59} In contrast to LDHA, LDH enzymes with high H-subunits are encoded by lactate dehydrogenase B (LDHB) and promote the conversion from lactate to pyruvate.\textsuperscript{59} Moreover, pyruvate dehydrogenase kinase (PDK) can prevent pyruvate from entering mitochondria for OXPHOS.\textsuperscript{60} Upregulation of LDHA and PDK synergistically promotes the production of lactate in HCC.\textsuperscript{60,61} Monocarboxylate transporter (MCT) expression on tumor cell membranes is associated with lactate passive transport and prevents glycolytic tumor cells from intracellular lactate accumulation.\textsuperscript{59,62} MCT1 and MCT4 are major proteins expressed in tumors. MCT1 is a high-affinity lactate transporter that participates in exogenous lactate uptake by endothelial cells and oxidative tumor cells.\textsuperscript{62} MCT4 is a low-affinity lactate transporter that promotes lactate release from glycolytic tumor cells.\textsuperscript{63} Although lactate could be incepted as a fuel, lactate...
accumulation in the TME still exists due to a large amount of lactate release. As a production of oxidative metabolism, CO$_2$ can be hydrated to H$_2$CO$_3$ and then dissociates to HCO$_3^-$ + H$^+$. Furthermore, the production of H$^+$ can also be associated with the metabolism of amino acids and the hydrolysis of ATP. In addition to lactate/H$^+$ symporter MCTs, H$^+$ can also be actively transported by H$^+-$ATPases and Na$^+$/H$^+$ exchangers (NHEs). However, carbonic anhydrases (CAs) colocalize with Na$^+$/HCO$_3^-$ cotransporters (NBCs) transporting Na$^+$ and HCO$_3^-$ into tumor cells and maintaining a mildly alkaline level of pH$_i$ and a dynamic balance of Na$^+$. V-ATPase, CAIX and CAXII are selectively overexpressed in HCC.

TME acidosis could influence HIFs reprogramming by increased O$_2$ consumption. An upregulation of HIF-1$\alpha$ under acidosis has been reported in glioma and HEK293 cell. Additionally, HIF-2$\alpha$ has been reported to play a critical role in regulating metabolic adaptation to acidosis in liver cancer and glioma. Mild extracellular acidosis could restructure mitochondria and promote mitochondria fusion. Excess H$^+$ and lactate decrease immunological cell function by inhibiting glycolysis and IFN-$\gamma$ production. Moreover, TME acidosis has been reported to contribute to angiogenesis, invasion and metastasis.

Immune Microenvironment
Tumor immune microenvironment of liver cancer is mainly associated with T cells, NK cells and tumor-associated macrophages (TAMs). In HCC, dysfunction of the above immune cells leads to reductions in inflammation and the immune response, which contributes to tumor progression. Metabolism reprogramming in these cells is closely associated with their functional change in the TME.

T cells play a critical role in antitumor immunity. Different subsets of T cells incline to different types of metabolism pathways. Naïve T cells have two types of subsets: CD4$^+$ T cells and CD8$^+$ T cells. Both of them express a resting mode for OXPHOS, which is accompanied by low lactate levels and low nutrient uptake. After activation, both CD4$^+$ and CD8$^+$ naïve T cells differentiate into long-lived memory T cells (T$_M$ cells) and short-lived effector T cells (T$_E$ cells). CD4$^+$ T cells can differentiate into two affected subsets: helper T cells (Th cells) and regulatory T cells (T$_{reg}$ cells). CD8$^+$ T cells can also differentiate into cytotoxic T cells (CTL).
and T_{reg} cells upon activation. Metabolic reprogramming occurs during the process of activation. Long-lived T_{M} cells and T_{reg} cells tend to go through fatty acid oxidation (FAO). FAO in T_{M} cells can fuel OXPHOS and enhance mitochondrial capacity, which could be a sign of rapid response to infection or cancer recurrence. T_{E} cell expansion can be accomplished in just a few days for the immune response and most T_{E} cells die after antigen clearance. However, short-lived T_{E} cells require a fast energy supply. Both elevated aerobic glycolysis and OXPHOS have been observed in T_{E} cells (except T_{reg} cells) activation.

Immune checkpoints can be negative regulators of the immune response by inhibiting effector lymphocytes (Figure 2). When T_{E} cells are activated, generation of IFN-γ could enhance the antigen presentation and promote T cell maturity. Nevertheless, scientists found that this process could upregulate expression of immune checkpoints, such as programmed death-1 (PD-1). This upregulation could provide negative feedback for the immune response in the normal microenvironment, preventing damage from a hyperimmune response and maintaining peripheral tolerance. However, tumors can take advantage of this mechanism of immune evasion. PD-1 and cytolytic T lymphocyte-associated antigen-4 (CTLA-4), the most focused checkpoints for T cells, are correlated with glucose metabolism. PD-1, programmed death-1, is also known as CD279, PDCD1, SLEB2, hPD-1, hPD-I, hSLE1 [NCBI Gene ID: 5133]. A high level of PD-1 expression can be a characteristic of exhausted T cells. After being activated by its ligands, such as programmed death-ligand (PD-L1) (CD274, B7-H, B7H1, hPD-L1, PDCD1L1, PDCD1LG1 [NCBI Gene ID: 29,126]), PD-1 can send...
inhibitory signals to T cells. Expression of myocyte-specific enhancer factor 2D (MEF2D) by HCC cells can upregulate their PD-L1 expression and enhance their combination with PD-1. PD-1 can inhibit aerobic glycolysis in T cells in 3 ways: i, PD-1 inhibits expression of GLUT1 leading to reductions in glucose uptake and transmission; ii, PD-1 inhibits a rate-limiting enzyme of aerobic glycolysis, hexokinase2 (HK2); and iii, PD-1 reduces mitochondrial number and induces mitochondrial dysfunction by reducing the number and length of mitochondrial cristae. In addition, PD-1 induces FAO by upregulating the rate-limiting enzyme of FAO, carnitine palmityltransferase (CPT1A). Metabolic reprogramming from aerobic glycolysis to FAO makes the dynamics lean toward long-lived T M cells. PD-L1 expression in tumor cells is associated with vascular formation in HCC patients. PD-1/PD-L1 can be a target for the treatment of HCC. Blocking PD-1 can reinvigorate exhausted CD8⁺ T cells and program them into durable memory CD8⁺ T cells. However, this reinvigoration CD8⁺ T cells can be re-exhausted in a high antigen concentration environment. Conversely, CTLA-4 inhibits aerobic glycolysis rather than enhancing FAO. Blockade of PD-1 and CTLA-4 can reverse the inhibition of aerobic glycolysis and effector function in T cells. Different from off-targets of the classical immune checkpoint blockers, Treg cells are sensitive to anti-CTLA-4 antibodies and can induce antibody-dependent cell-mediated cytotoxicity. T cell immunoglobulin and ITIM domain (TIGIT) is also involved in the regulation of CD8⁺ T cell metabolism by downregulating GLUT1 and HK1/HK2.

Similar to T cells, activated NK cells preferentially go through aerobic glycolysis by maintaining proliferation and effector function and memory NK cells more likely to use FAO to fuel OXPHOS. NK cells can be divided into two different phenotypic and functional subsets depending on the expression levels of CD56 receptor: CD56dim cells and CD56bright cells. CD56dim cells are generally deemed to be cytotoxic cells with less GLUT1 expression, whereas the CD56bright cells are considered to be IFN-γ producers with higher GLUT1 expression. Either IL-2 or IL-12/15 cytokine combinations can activate NK cells and increase OXPHOS levels for energy supply. Sine ‘ad E. Keating and his colleagues found an interesting phenomenon in which CD56bright cells showed higher GLUT1 expression and levels of aerobic glycolysis than CD56dim cells, leading to a higher level of activation. Downregulation of aerobic glycolysis in CD56bright cells restricts IFN-γ production. Lactate accumulation and acidification can impair activation and IFN-γ production of NK cells by diminishing nuclear factor of activated

**Figure 2** Interaction between tumor cells and immune cells by immune checkpoints. T cells and NK cells express various immune checkpoints, which can bind to ligands on tumor cells, Treg cells and Kupffer cells and be inhibited (CD96 binds to CD111 and CD115; TIGHT binds to CD115 and CD112; NKG2A binds to HLA-E; PD-L1 binds to CD80 and CD86).

**Abbreviations:** PD-1, programmed death-1; CTLA-4, cytolytic T lymphocyte-associated antigen-4; NKG2A, natural killer cell group 2A; TIGIT, T-cell immunoglobulin and ITIM domain; HLA-E, human leukocyte antigen-E.
Liver-resident natural killer (LrNK) cells (NFAT). Moreover, liver-resident natural killer (LrNK) in the TME displayed a downregulation of NKG2D and impaired cytotoxicity and cytokine production, which could be recovered by IL-15. Zhou et al. found that LrNK contributes to the tolerogenic microenvironment of the liver by inhibiting T\textsubscript{E} cells. LrNK inhibits T\textsubscript{E} cells proliferation and production of INF-\gamma and TNF-\alpha through a PD-1-PD-L1 axis.

Immune checkpoints: natural killer cell group 2A (NKG2A)/CD94, TIGIT and CD96 were found to lead to NK cell exhaustion and to predict poor prognosis in HCC. Scientists found that CD49a\textsuperscript{+} NK cells, which expressed higher levels of immune checkpoints molecules PD-1, TIGIT and CD96, were correlated with a poor prognosis in HCC. However, few studies have assessed the correlation between immune checkpoints and metabolic reprogramming in NK cells. NK cell education is the process of NK-cell subsets to obtain functional competence. Caroline Pfeifer found that educated NK cells presented with distinct self-inhibitory receptors and went through distinct glycolytic profile and functions. Educated NK cells presented with NKG2A (NKG2A-educated NK cells) showed no obvious upregulation in GLUT1 expression, glycolysis or functionality compared with educated NK cells presented with killer cell immunoglobulin (KIR) (KIR-educated cells). Furthermore, compared with KIR-educated NK cells NKG2A-educated NK cells could better survive glycolysis blockade, allowing NKG2A-educated NK cells to adapt to the hypoxic and low-glucose environment of the tumor. Upregulation of the NKG2A ligand on tumor cells further promotes immune evasion from NK cells in the TME. In addition, other experiments have proven that anti-NKG2A mAb could block immune evasion by unleashing not only NK cells but also T cells.

TAM infiltration takes part in tumor invasion and metastasis. Macrophages exhibit two diverse phenotypes: M1-classic activation and M2-alternative activation. The M1 type is characterized by pro-inflammatory (IL-1, TNF-\alpha) cytokines and IFN-\gamma production and can phagocytize tumor cells and induce tumor cell apoptosis. The M2 type is characterized by the production of anti-inflammatory cytokines (IL-6, IL-10, TGF-\beta) and can induce angiogenesis and tumor cell generation. TAMs were attracted and activated by tumor secretory factors (VEGF, PDGF, TGF-\beta, CCL2, and M-CSF). TAMs mostly exist in the form of M2 in the TME, which could be closely associated with the byproduct of glycolysis in HCC. Lactate secreted by hepatoma cells induces VEGF and arginase1 (Arg1) via HIF-1\alpha to promote M2-like polarization of TAMs. As liver-specific macrophages, Kupffer cells survive anoxia by glycolysis and produce PD-L1 ligand to suppress T\textsubscript{E} cells. The dominant TAMs in orthotopic HCC exhibit Kupffer cell (KC) properties and are known as KC-like TAMs (kclTAM). TAMs could be "helpers" and target tumorigenesis and development.

**Signaling Pathways Involved in HCC Glucometabolic Reprogramming**

Tumor cells prefer to choose glycolysis over OXPHOS in hypoxic or even normoxic environment, relying on HIF-1\alpha and c-MYC synergies. c-MYC also plays as an important role in glycolysis in HCC as HIF-1\alpha. The importance of collaboration between c-MYC and HIF-1\alpha was demonstrated to activate the Warburg effect by inhibiting IDH1-AS1 in multiple tumors under normal oxygen. c-MYC was reported to participate overexpression of MTR4 in HCC, which drives the expression of glycolytic genes such as GLUT1 and PKM2. PFK2 in turn was found to up-regulate c-MYC expression in glioma. A positive feedback loop between MYC and PFK2 was demonstrated to sustain tumor cell aerobic glycolysis in a Drosophila tumor model. c-MYC could be a promising target for HCC treatment, especially in advanced stages. Moreover, some cytokines and signal pathways can also directly or indirectly affect the glycolysis of tumor cells by increasing the stability and transcription activity of HIF-1\alpha. The major regulatory mechanisms of HIF-1\alpha involved in HCC glucometabolic reprogramming are described in detail below and shown in Figure 3.

Phosphatidylinositol-3-kinase (PI3K)/AKT signaling promotes the proliferation of hepatoma cell and EMT in HCC, which contributes to HCC growth, migration and invasion. They transmit cell surface receptor signals and affect a variety of tissue-dependent cellular functions. The PI3K/AKT/mTOR signaling pathway not only directly mediates aerobic glycolysis but also regulates HIF-1\alpha. Impairing insulin signaling by inhibiting PI3K/AKT pathway could promote gluconeogenesis in the liver. Molecules can treat HCC by inhibiting PI3K/AKT activation, such as MiR-612.

The Wnt/\beta-catenin pathway has been reported to occur in both early and late stages of HCC and suppress mitochondrial respiration and promotes glycolysis. HIF-1\alpha can stimulate Wnt/\beta-catenin signaling via the
Wnt signaling further drives PROX1 was also found to participate in the TGF-α
Mechanisms of glycolysis regulation by HIF-1 can induce In can directly regulate glycolysis-related enzymes to affect tumor cell
VEGFs and their coactivator BCL9 in HCC. Wnt signaling further drives HCC proliferation through MYC, frizzled (FZD), Glypican-3 (GPC3), EGFR and CTNNBIP1. Moreover, activation of the Wnt/β-catenin pathway increases the EMT-associated activity of HIF-1α and enhances the proliferation, EMT, invasion and metastasis of HCC. Molecules that activate the Wnt/β-catenin pathway can provide therapeutic targets and predictors for molecular precision therapy of HCC, such as LINC00346 and Linc00210 (long noncoding RNAs), and PBOV1 and PROX1 (oncogene). PROX1 was also found to be a target for treating HCC sorafenib tolerance.

Transforming growth factor-β1 (TGF-β1) is a common cytokine that regulates a variety of cellular processes. In advanced tumor, TGF-β1 acts as an oncogenic factor and induces tumor proliferation, EMT invasion and metastasis. TGF-β1 contributes to the metabolic reprogramming of tumor cells by upregulating the expression of key enzymes of the glycolytic pathway via the Smad, p38 MAPK and PI3K/AKT signaling pathways. TGF-β1 promotes tumor progression by reducing mitochondrial respiration and enhancing glutamine anaplerosis and the pentose phosphate pathway (PPP) cycle. TGF-β1 and its mediated signaling pathway can still induce HIF-α to participate in the process of metabolic reprogramming under normoxic conditions.

The EGFR/MEK/ERK/HIF-1α/VEGFA cycle regulates glucose metabolism and promotes HCC proliferation, angiogenesis and metastasis. VEGFs and their cognate receptors (VEGFRs) are critical in the regulation of vessel formation in angiogenesis. HIF-1α can induce angiogenesis by binding to the VEGF gene promoter and upregulating VEGF expression. In addition, TGF-β1 can induce VEGF expression via Smad and HIF-2α.

The Notch signaling pathway plays a critical role in crosstalk between glucometabolic reprogramming and
HCC microenvironment. High expression of Notch1 indicates a poor prognosis in HCC.\textsuperscript{157} Notch/Hes1 signaling could induce glycolysis by inactivation of p53 and activation of the NF-kB pathway.\textsuperscript{126,158} As a target gene of NF-kB, the transcriptional activity of HIF-1α was significantly increased by activated Notch1.\textsuperscript{158} In turn, HIF-1α was reported to upregulate the expression and function of Notch in HCC.\textsuperscript{159,160} Notch can promote the proliferation of hepatoma cells through the PI3K-Akt, mTOR and Ras pathways.\textsuperscript{126} Moreover, Notch inhibits hepatoma cells apoptosis by downregulation of ROS production via the NICD1/Hey1/PINK1 pathway and inactivation of p53.\textsuperscript{36,126} Notch promotes EMT, invasion and metastasis in HCC through NICD/snail and Wnt3a pathway.\textsuperscript{157,161-163} However, there is a dispute that blocking Notch promotes HCC progression and metastasis by accelerating proliferation of keiTAMs via Wnt signaling and IL-10 production through c-MYC.\textsuperscript{164} More evidence is needed in the future.

Unlike the above pathways, AMP-activated protein kinase (AMPK) is the main activation pathway of the anti-Warburg effect in HCC. Activation of AMPK inhibits glycolysis and promotes OXPHOS, which restricts the proliferation of hepatoma cells.\textsuperscript{165-167} Activation of AMPK/mTOR by glycochenodeoxycholate can also promote HCC invasion and migration by activating autophagy.\textsuperscript{168} Moreover, upregulation of AMPK reduces the expression of hepatocellular cancer stem cell markers in long-term sorafenib therapy, which provides a new target for overcoming the chemotherapy resistance of HCC.\textsuperscript{169} AMPK treatment options such as upregulation of HSF1, NOD2 and PEDF or inhibition of 6PGD and GSK-3β could have potential in HCC treatment.\textsuperscript{165-167,170,171} Among them HSF1 also participates in the promotion of gluconeogenesis.\textsuperscript{172} Treatments that both inhibit glycolysis and promote gluconeogenesis at the same time are expected to be promising HCC treatment solutions.

**Discussion**

Along with the improvement of tumor cognition, including disorder of cell cycle, gene mutations and immune evasion, the development of oncotherapy has gone through 3 stages: chemotherapy, targeted therapy and immunotherapy. HCC shows hidden clinical symptoms in the early stage of the disease; thus, the diagnosis often occurs in the advanced stage or metastasis, which is prone to recurrence. Sorafenib is almost the only systemic treatment options for patients with advanced HCC.\textsuperscript{173} However, sorafenib treatment of advanced HCC is prone to drug resistance and cannot achieve the desired therapeutic effect, which is closely related to the TME.\textsuperscript{173} Recently, an increasing number of scientists have focused on the effect of TME in tumorigenesis and development, which could be the fourth stage of tumor cognition and therapy. The Warburg effect is the foundation of tumorigenesis, proliferation, migration and metastasis, and it contributes to a unique TME. In this review, we focused on metabolism reprogramming in three aspects of TME: hypoxia, acid-base status and immune microenvironment. Lately, anti-Warburg therapies which not only focus on the characteristics of the TME or directly inhibit glycolysis but also inhibit glycolysis by increasing gluconeogenesis, have become a popular area of research.

**Treatments for the Hypoxia Microenvironment**

Hypoxia is considered to be a major obstacle to tumor treatment.\textsuperscript{174} At present, the main idea of hypoxia treatment for HCC is to directly provide/generate oxygen at the tumor site to increase the partial oxygen pressure or indirectly reduce the level of HIF-1α and interfere with a HIF-1α-related signaling pathway to decrease hypoxia effects.\textsuperscript{17,18} Increasing local oxygen pressure and reversing hypoxia can use nanotechnology to introduce O\textsubscript{2} into the tumor or generate oxygen at the tumor site by increasing the decomposition of endogenous hydrogen peroxide and light-triggered water splitting.\textsuperscript{18,175} However, most of these approaches are in the early stage of development and need more time to evaluate their availability in HCC therapy. Many anticancer drugs aimed at HIF-1α have been reported. Heat shock protein 70 (Hsp70), benzopyranyl 1,2,3-triazole and BIX01294 reduce HIF-1α levels by promoting ubiquitination and proteasome degradation of HIF-1α.\textsuperscript{176-178} Drugs that inhibit the expression and accumulation of HIF-1α transcriptional activity and protein accumulation include cardenolides and ezn-2208.\textsuperscript{177,179} Moreover, some inhibitors act on HIF-1α-related signaling pathways, such as glyceollins and apigenin, which inhibit the PI3K/AKT pathway to downregulate HIF-1α.\textsuperscript{180,181} Semaxanib causes low HIF-1 DNA-binding activity by inhibiting PI3K activity and AKT phosphorylation.\textsuperscript{182} HIF-1α inhibitors can not only improve the effect of the hypoxia microenvironment on HCC progression but also increase the sensitivity of hepatocytes to targeted therapy. Simvastatin can inhibit HIF-1α/PPAR-γ/PKM2-mediated glycolysis in hepatocytes and resensitize it to
In addition to treatment, HIF-1-related genes have also been used in the establishment of a novel integrated scoring system, which could contribute to the precise treatment of HCC patients. Due to complicated regulations and overlap mechanisms, the clinical trials of HIF-1α inhibitors targeting tumor hypoxia have failed to achieve significantly satisfactory results.

Treatments for the Acid-Base Microenvironment

There are two distinct approaches in tumor acidosis: I. modulating pH to restore chemosensitivity and correct defective immune mechanisms and II. Utilizing an acidic TME to enhance the effect of drugs. The pH can be adjusted in 3 ways: reducing acid production, increasing acid consumption and providing an outside pH buffer. Elevating gluconeogenesis can both reduce acid production and increase acid consumption by inhibiting glycolysis and using lactate as a substrate. As a promising treatment, the mechanisms and options for increasing gluconeogenesis to treat HCC will be explored in detail in “Treatments for aerobic glycolysis”. Targeted therapy of proton pump/transporters could also reduce acid production. Omeprazole, a proton pump inhibitor (PPI), has been used to analyze the role of V-ATPase in HCC and proved to have a wide range of antitumor effects at the preclinical and clinical levels. Since HCC showed partial drug resistance to a CAXII inhibitor compound in an anoxic TME, modifications of compound 25 need to be studied to improve its antitumor effect. As mentioned above, outside provision of a pH buffer could be anti-acidifying strategies in HCC. Oral or transarterial chemoembolization (TACE) pH buffer can restrict local invasive growth and metastasis by reducing intratumoral and peritumoral acidosis rather than altering the pH of healthy tissues or blood. Patients with large HCC showed a marked enhancement of the anticancer activity after TACE with bicarbonate local infusion into tumor. Sodium bicarbonate, with a pKa of 6.1, is sufficient to meet the above requirements. However, published clinical trials have indicated that pH buffer with a pKa of approximately 7 is a more ideal treatment. Alternatively, some drugs have shown an enhanced effect in tumor acidic microenvironment. As protonable weak bases, PPIs can be selectively aggregated and activated in acidic region. An acidic TME can not only be the target of PPI but also promote PPI activation. Drugs with the same characteristics as PPIs can be considered for cancer combination therapy. More fundamental studies and clinical trials need to be performed.

Treatments for the Immune Microenvironment

Two treatment strategies aiming at liver cancer immunotherapy include the enhancement of normal immune mechanisms and the correction of defective immune mechanisms.

For enhancement immunotherapy, cytokines (IL-2, IFNs), cancer vaccines and cell therapy (CAR-T) have been approved by the FDA. Although some of the above methods have achieved a certain effect in liver cancer therapy, high-frequency negative trials with high toxicity pushed scientists to find other ways. Immune checkpoints inhibitors targeting the TME but with lower toxicity have begun to emerge. Immune checkpoint therapy could satisfy the following three principles at the same time: normalizing tumor immunity, targeting the TME and reset of immune response in TME. Treatment with the CTLA-4 inhibitor, tremelimumab, led to a transient complete viral response in 25% of HCC patients with HCV infection (ClinicalTrials.gov Identifier: NCT01008358). PD-1 and PD-L1 inhibitors showed lower levels of immune-related adverse events (irAEs) than CTLA-4 inhibitors, with an incidence of 27% versus 72% for all grades and 6% versus 24% for grade 3 or higher in HCC. The irAEs of PD-1 or PD-L1 inhibitors are not related to dose; however, the effect is dose-dependent for CTLA-4 inhibitors. The PD-1 inhibitor MEDI4736 resulted in lower hepatotoxicity than CTLA-4 antibody in HCC patients. Although antitumor activity of PD-1 antibody is promising, less than 20% of HCC patients respond to it. Clinical trials targeting at the effect of PD-1 blockade combined with other treatments have been launched. A trial examining the combination of PD-1 blockade and incomplete thermal ablation in patients with advanced HCC has just been completed, the results of which will provide us with a more in-depth understanding of the efficacy once they are published (ClinicalTrials.gov Identifier: NCT03939975). A clinical trial investigating CTLA-4 and PD-L1 combination blockade after transarterial chemoembolization (DEB-TACE) in intermediate-stage HCC patients is underway (ClinicalTrials.gov Identifier: NCT03638141). Given the complexity of liver cancer and the irAEs of immunotherapy alone, more than 16 clinical trials are ongoing in an attempt to explore the therapeutic effects and side effects of combined...
locoregional immunotherapy. Lately, it has been revealed that exhaustion of CD8+ T cells is not the major cause for tumor immune evasion, but rather, it is a lack of stem-like CD8 T cells, providing a fresh perspective to this field of research. Stem-like CD8 T cells can differentiate into CTLs and maintain tumor immune response within the comfortable environment provided by antigen-presenting cells. This new discovery may be able to explain why the immune checkpoint treatment is only 20–30% efficient. However, tumor immune evasion has a large and complicated network, which cannot be explained by one single mechanism. The role of stem-like CD8 T cells in tumor immune evasion requires further exploration in the future.

Currently, scientists believe that NK cells are equally as important as T cells and can be used in conjunction with T cells for tumor immunotherapy. The antiviral activity of hepatic T cells has been found to be controlled by LrNK via the PD-1-PD-L1 axis. Moreover, anti-NKG2A mAb was revealed play an important role in unleashing both T and NK cells. The results of the above experiments suggest that PD-1-PD-L1 and NKG2A blockade are important targets for tumor treatment. However, no clinical trials investigating NKG2A blockade in HCC patients have yet been conducted.

**Treatments for Aerobic Glycolysis**

Strategies targeting for the “anti-Warburg effect” have been primarily considered for key transporter and enzymes involved in glycolysis. However, increasing gluconeogenesis could suppress glycolysis at the same time, which became the new target for the “anti-Warburg effect”.

The selective inhibitors of GLUTs include benzamides and rapafucins, which can inhibit GLUTs and inhibit glucose uptake to prevent or reduce the proliferation of tumor cells. Benzamides can directly bind to GLUT1 and inhibit GLUT1 function without affecting GLUT1 protein levels. Benzamides have no obvious toxicity to normal tissues. New GLUT inhibitors such as rapafucins are being explored. As the healthy tissues also need glucose, it is necessary to select tumor-specific GLUT inhibitors and make appropriate assessments to reduce the toxicity to normal cells.

HK2 is the first rate-limiting enzyme for glucose metabolism. Studies have shown that blockade of HK2 in human hepatoma cells can inhibit the occurrence of tumor and increase cell death. 2-DG is a well-known HK2 inhibitor, that has been reported to inhibit hexokinase by competing with glucose. Lonidamin is a mitochondrial HK inhibitor that suppresses the activity of HK1 and HK2. Others such as 3-bromopyruvic acid (3-BrPA), ketoconazole and posaconazole can also affect tumor metabolism and growth by blocking HK.

Phosphofructokinase 1 (PFK1) is the second rate-limiting enzyme of glycolysis, and tumor formation can be impaired by the O-GlcNAcylation of PFK1 at serine 529. Metformin can target the HIF-1α/PEKFB3/PFK1 pathway in hepatoma cells and reduce hepatoma cell proliferation by inhibiting glycolysis. Pyruvate kinase (PK) is the third rate-limiting enzyme in glycolysis. It has multiple subtypes, among which PKM2 is upregulated in a variety of cancers. Shikonin is an inhibitor of PKM2 that can reduce the glycolytic rate of tumors. However, its toxicity and poor solubility limit its application. Recent studies have found that metformin can also induce tumor cell death and increase sensitivity to chemotherapy drugs by inhibiting PKM2 in osteosarcoma.

LDHA is located at the bifurcation point of glycolysis and oxidative phosphorylation. Inhibition of LDHA may be a promising antitumor strategy. The piperidinedione derivatives miR-30a-5p, miR-41 and GNE-140 have been indicated to inhibit LDHA in breast and pancreatic cancer. However, glycolysis inhibitors cannot induce cell death to achieve long-term tumor remission and its safety needs further verification. At present, the effect of glycolysis inhibitors alone is not very significant. In the future, it will be necessary to further study the mechanism or a combination of multiple methods for treatment to achieve the purpose of controlling tumors.

The main substrates of hepatic gluconeogenesis are lactate, pyruvate, glycerol and glycosylated amino acids (such as glutamate). Hepatic glycosylation relies on the initial gluconeogenesis enzymes phosphoenolpyruvate carboxykinase (PEPCK), downstream fructose-1, 6-bisphosphatase (FBP) and the final-step glucose-6-phosphatase (G6PC). PEPCK has two isoforms: a cytosolic isoform, PCK1, and a mitochondrial isoform, PCK2. Unlike the well-known PCK1, it was recently demonstrated that PCK2 contributed to gluconeogenesis with less efficiency than PCK1. FBP also has two isoforms: liver isoform, FBP1 and muscle isoform, FBP2. Studies of increasing gluconeogenesis are mainly focused on PCK1 and FBP1.

PCK1 and PCK2 are downregulated in HCC and suggest a poor prognosis. Bian found that Nur77 could stabilize PCK1 by attenuating its sumoylation and ubiquitination and then suppress HCC. PCK1 was also found to inhibit hepatoma cell proliferation by downregulating cell cycle progression through the AMPK pathway.

FBP1 appears to be a tumor suppressor and poor prognostic marker in HCC. Gene set enrichment analysis with 594
cases of HCC demonstrated that lower FBP1 expression was correlated with advanced tumor stage, poor overall survival and higher tumor recurrence rates. Two double-negative feedback loops have been indicated for FBP1 expression in HCC. The first loop is FBP1 and enhancer of zeste homolog 2 (EZH2): EZH2 can inhibit FBP1 and FBP1 physically competed for EZH2 binding in turn. The second loop is FBP1 and polycomb repressive complex 2 (PRC2). PRC2 can downregulate FBP1, and conversely, FBP1 can interfere with PRC2 functions. Histone deacetylases and FXI1 inhibitor stabilize FBP1 in HCC to inhibit of tumor growth with PCR2 functions. downregulate FBP1, and conversely, FBP1 can interfere with PCR2 functions. Histone deacetylases and FXI1 inhibitor stabilize FBP1 in HCC to inhibit of tumor growth and invasion. However, more clinical trials are needed.

Conclusion

Tumor aerobic glycolysis is closely associated with TME. They promote each other to provide a suitable growth environment for tumor. Combination treatment of sorafenib with TME improvement and/or anti-Warburg therapies represents the future of advanced HCC therapy. Treatment options that elicit responses with “anti-Warburg effects” are more promising for HCC therapy, such as those that promote the elevation of gluconeogenesis. However, these treatments still need clinical trials for verification.

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Disclosure

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. doi:10.3322/caac.21492
2. Wang P, Song X, Cao D, et al. Oncogene-dependent function of BRG1 in hepatocarcinogenesis. Cell Death Dis. 2020;11(2):91. doi:10.1038/s41419-020-2289-3
3. Zhang G, Tang X, Liang L, et al. DNA and RNA sequencing identified a novel oncogene VPS35 in liver hepatocellular carcinoma. Oncogene. 2020;39(16):3229–3244. doi:10.1038/s41388-020-1215-6
4. Vitale A, Trevisani F, Farinati F, Cillo U. Treatment of hepatocellular carcinoma in the Precision Medicine era: from treatment stage migration to therapeutic hierarchy. Hepatology. 2020. doi:10.1002/hep.31187
5. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69(1):7–34. doi:10.3322/caac.21551
6. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol. 1927;8(6):519–530. doi:10.1085/jgp.8.6.519
7. Sanderson SM, Locasale JW. Revisiting the Warburg effect: some tumors hold their breath. Cell Metab. 2018;28(5):669–670. doi:10.1016/j.cmet.2018.10.011
8. Wang T, Marquardt C, Foker J. Aerobic glycolysis during lymphocyte proliferation. Nature. 1976;261:4.
9. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. Cell Metab. 2015;23(1):27–47. doi:10.1016/j.cmet.2015.12.006
10. Novikova MV, Khromova NV, Kopnin PB. Components of the hepatocellular carcinoma microenvironment and their role in tumor progression. Biochemistry (Moscow). 2017;82(8):861–873. doi:10.1134/S0006297917080016
11. Sharabi K, Tavares CD, Rines AK, Puigserver P. Molecular pathophysiology of hepatic glucose production. Mol Aspects Med. 2015;46:21–33. doi:10.1016/j.mam.2015.09.003
12. Goldstein I, Hager GL. Transcriptional and chromatin regulation during fasting - the genomic era. Trends Endocrinol Metab. 2015;26(12):699–710. doi:10.1016/j.tem.2015.09.005
13. Hirata H, Sugimachi K, Komatsu H, et al. Decreased expression of fructose-1,6-bisphosphatase associates with glucose metabolism and tumor progression in hepatocellular carcinoma. Cancer Res. 2016;76(11):3265–3276. doi:10.1158/0008-5472.CAN-15-2601
14. Bian XL, Chen HZ, Yang PB, et al. Nur77 suppresses hepatocellular carcinoma via switching glucose metabolism toward gluconeogenesis through attenuating phosphoenolpyruvate carboxykinase sumoylation. Nat Commun. 2017;8:14420. doi:10.1038/ncomms14420
15. Liao K, Deng S, Xu L, et al. A feedback circuitry between polycomb signaling and fructose-1,6-bisphosphatase enables hepatic and renal tumorigenesis. Cancer Res. 2020;80(4):675–688. doi:10.1158/0008-5472.CAN-19-2060
16. Liu MX, Jin L, Sun SJ, et al. Metabolic reprogramming by PCK1 promotes TCA cataplerosis, oxidative stress and apoptosis in liver cancer cells and suppresses hepatocellular carcinoma. Oncogene. 2018;37(12):1637–1653. doi:10.1038/s41388-017-0070-6
17. Jing X, Yang F, Shao C, et al. Role of hypoxia in cancer therapy by regulating the tumor microenvironment. Mol Cancer. 2019;18(1):157. doi:10.1186/s12943-019-1089-9
18. Sahu A, Kwon I, Tae G. Improving cancer therapy through the nanomaterials-assisted alleviation of hypoxia. Biomaterials. 2020;228:119578. doi:10.1016/j.biomaterials.2019.119578
19. Briggs KJ, Koivunen P, Cao S, et al. Paracrine induction of HIF by glutamate in breast cancer: Egln1 senses cysteine. Cell. 2016;166(1):126–139. doi:10.1016/j.cell.2016.05.042
20. Shang RZ, Qu SB, Wang DS. Reprogramming of glucose metabolism in hepatocellular carcinoma: progress and prospects. World J Gastroenterol. 2016;22(45):9933–9943. doi:10.3748/wjg.v22.i45.9933
21. Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab. 2006;3(3):187–197. doi:10.1016/j.cmet.2006.01.012
22. Rohwer N, Cramer T. Hypoxia-mediated drug resistance: novel insights on the functional interaction of HIFs and cell death pathways. Drug Resist Updat. 2011;14(3):191–201. doi:10.1016/j.drup.2011.03.001
23. Qing G, Simon MC. Hypoxia inducible factor-2alpha: a critical mediator of aggressive tumor phenotypes. Curr Opin Genet Dev. 2009;19(1):60–66. doi:10.1016/j.gde.2008.12.001
24. Dabral S, Muecke C, Valasarajan C, et al. A RASSF1A-HIF1alpha loop drives Warburg effect in cancer and pulmonary hypertension. Nat Commun. 2019;10(1):2130. doi:10.1038/s41467-019-10044-z
25. Zhang X, Li Y, Ma Y, et al. Yes-associated protein (YAP) binds to HIF-1alpha and sustains HIF-1alpha protein stability to promote hepatocellular carcinoma cell glycolysis under hypoxic stress. J Exp Clin Cancer Res. 2018;37(1):216. doi:10.1186/s13046-018-0892-2

26. Shi DY, Xie FZ, Zhai C, Stern JS, Liu Y, Liu SL. The role of cellular oxidative stress in regulating glycolysis, energy metabolism in hepatoma cells. Mol Cancer. 2009;8:32. doi:10.1186/1476-4598-8-32

27. Zhang X, Yang L, Yi X, Yang A, Li Z, Wang D. The anti-carcinogenesis properties of eriavin in the modulation of oxidative stress-mediated apoptosis and immune response in liver cancer. Aging (Albany NY). 2019;11(22):10284–10300. doi:10.18632/aging.102456

28. Lee D, Xu IM, Chiu DK, et al. Induction of oxidative stress through inhibition of thioredoxin reductase 1 is an effective therapeutic approach for hepatocellular carcinoma. Hepatology. 2019;69(4):1768–1786. doi:10.1002/hep.30467

29. Huang Q, Zhan L, Cao H, et al. Increased mitochondrial fission promotes autophagy and hepatocellular carcinoma cell survival through the ROS-modulated coordinated regulation of the NFKB and TP53 pathways. Autophagy. 2016;12(6):999–1014. doi:10.1080/15548627.2016.1166318

30. Shen J, Chen M, Lee D, et al. Histone chaperone FACT complex mediates oxidative stress response to promote liver cancer progression. Gut. 2020;69(2):329–342. doi:10.1136/gutjnl-2019-318668

31. Lu J, Tan M, Cai Q. The Warburg effect in tumor progression: mitochondrial oxidative metabolism as an anti-metastasis mechanism. Cancer Lett. 2015;356(2 Pt A):156–164. doi:10.1016/j.canlet.2014.04.001

32. Chang H, Li J, Qu K, et al. CRF1 overexpression facilitates tumor growth and metastasis through inducing ROS/NF-kappaB pathway in hepatocellular carcinoma. Cell Death Dis. 2020;11(5):332. doi:10.1038/s41419-020-2258-7

33. Geng X, Geng Z, Li H, Zhang Y, Li J, Chang H. Over-expression of TF2BM facilitates cell growth and metastasis via activating ROS-Akt-NF-kappaB signalling in hepatocellular carcinoma. Liver Int. 2020;40(7):1756–1769. doi:10.1111/liv.14440

34. Zheng X, Li C, Yu K, et al. Aquaporin-9, mediated by IGF2, suppresses liver cancer stem cell properties via augmenting ROS/beta-catenin/FoxO3a signaling. Mol Cancer Res. 2020;18(7):992–1003. doi:10.1158/1541-7786.MCR-19-1180

35. Fuhrmann DC, Brun E. Mitochondrial composition and function under the control of hypoxia. Redox Biol. 2017;12:208–215. doi:10.1016/j.redox.2017.02.012

36. Kung-Chun Chiu D, Pui-Wah Tse A, Law CT, et al. Hypoxia regulates the mitochondrial activity of hepatocellular carcinoma cells through HIF/HEY1/PINK1 pathway. Cell Death Dis. 2019;10(12):934. doi:10.1038/s41419-019-2155-3

37. Lu CW, Lin SC, Chen KF, Lai YY, Tsai SJ. Induction of pyruvate dehydrogenase kinase-3 by hypoxia-inducible factor-1 promotes metabolic switch and drug resistance. J Biol Chem. 2008;283(42):28106–28114. doi:10.1074/jbc.M803508200

38. Dang CV. The interplay between MYC and HIF in the Warburg effect. Ernst Schering Found Symp Proc. 2007;4(2):35–53.

39. Brahim-Horn MC, Giuliano S, Saland E, et al. Knockout of Vdac1 activates hypoxia-inducible factor through reactive oxygen species generation and induces tumor growth by promoting metabolic reprogramming and inflammation. Cancer Metab. 2015;3:8. doi:10.1186/s40170-015-0133-5

40. Fukuda R, Zhang H, Kim JW, Shimoda L, Dang CV, Senemaa GL. HIF-1 regulates cytochrome oxide subunits to optimize efficiency of respiration in hypoxic cells. Cell. 2007;129(1):111–122. doi:10.1016/j.cell.2007.01.047

41. Tello D, Bals E, Acosta-Iborra B, et al. Induction of the mitochondrial NDUF4L2 protein by HIF-1alpha decreases oxygen consumption by inhibiting Complex I activity. Cell Metab. 2011;14(6):768–779. doi:10.1016/j.cmet.2011.10.008

42. Zhang H, Bosch-Marce M, Shimoda LA, et al. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem. 2008;283(16):10892–10903. doi:10.1074/jbc.M80102200

43. Dai W, Xu L, Yu X, et al. OGDHL silencing promotes hepatocellular carcinoma by reprogramming glutamine metabolism. J Hepatol. 2020;72(5):909–923. doi:10.1016/j.jhep.2019.12.015

44. DeBerardinis RJ, Mancuso A, Daikhin E, et al. Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc Natl Acad Sci U S A. 2007;104(19):19345–19350. doi:10.1073/pnas.0709747104

45. Levy PL, Duponchel S, Eischeid H, et al. Hepatitis C virus infection triggers a tumor-like glutamine metabolism. Hepatology. 2017;65(3):789–803. doi:10.1002/hep.29849

46. Shanware NP, Bray K, Eng CH, et al. Glutamine deprivation stimulates mTOR-JNK-dependent chemokine secretion. Nat Commun. 2014;5:4900. doi:10.1038/ncomms5900

47. Li B, Cao Y, Meng G, et al. Targeting glutaminase 1 attenuates stemness properties in hepatocellular carcinoma by increasing reactive oxygen species and suppressing Wnt/beta-catenin pathway. EBioMedicine. 2019;39:239–254. doi:10.1016/j.ebiom.2018.11.063

48. Fulda S, Galluzzi L, Kroemer G. Targeting mitochondria for cancer therapy. Nat Rev Drug Discov. 2010;9(6):447–464. doi:10.1038/nrd3137

49. Lippmann J, Petri K, Fulda S, Liese J. Redox modulation and induction of ferroptosis as a new therapeutic strategy in hepatocellular carcinoma. Transl Oncol. 2020;13(8):100785. doi:10.1016/j.tranon.2020.100785

50. Pibiri M, Sulas P, Camboni T, Leoni VP, Simbula G. Alpha-lipoic acid induces endoplasmic reticulum stress-mediated apoptosis in hepatoma cells. Sci Rep. 2020;10(1):7139. doi:10.1038/s41598-020-64004-5

51. Huang X, Gan G, Wang X, Xu T, Xie W. The HGF-MET axis coordinates liver cancer metabolism and autophagy for chemotherapeutic resistance. Autophagy. 2019;15(7):1258–1279. doi:10.1080/15548627.2019.1580105

52. Long Y, Tsai WB, Wang D, et al. Argininosuccinate synthetase 1 (ASS1) is a common metabolic marker of chemosensitivity for targeted arginine- and glutamine-starvation therapy. J Exp Clin Cancer Res. 2021;40(2):472. doi:10.1186/s13046-021-01977-x

53. Tang L, Zeng J, Geng P, et al. Global metabolic profiling identifies a pivotal role of proline and hydroxyproline metabolism in supporting hypoxic response in hepatocellular carcinoma. Clin Cancer Res. 2018;24(2):474–485. doi:10.1158/1078-0432.CCR-17-1707

54. Webb BA, Chimenti M, Jacobson MP, Barber DL. Dysregulated pH: a perfect storm for cancer progression. Nat Rev Cancer. 2011;11(9):671–677. doi:10.1038/nrc3110

55. Savic LJ, Schober JT, Peters D, et al. Molecular imaging of extracellular tumor pH to reveal effects of locoregional therapy on liver cancer microenvironment. Clin Cancer Res. 2020;26(2):428–438. doi:10.1158/1078-0432.CCR-19-1702

56. Coman D, Peters DC, Walsh JJ, et al. Extracellular pH mapping of tumor extracellular space/interstitial fluid and its implications for diagnostic and therapeutic resistance. Cancer Res. 2016;76(2):377–386. doi:10.1158/0008-5472.CAN-15-2295

57. Wang S, Shi Q, Yang Z, et al. Targeting a glutamine metabolic marker (ASS1) using 11C-acetate PET/CT in patients with hepatocellular carcinoma. J Nucl Med. 2018;59(12):2153–2159. doi:10.2967/jnumed.118.205946
129. Liang J, Cao R, Zhang Y, et al. PKM2 dephosphorylation by Cdc25A promotes the Warburg effect and tumorigenesis. Nat Commun. 2016;7:12431. doi:10.1038/ncomms12431

130. Wong KKL, Liao JZ, Verheyen EM. A positive feedback loop between Myc and aerobic glycolysis sustains tumor growth in a Drosophila tumor model. Elife. 2019;8. doi:10.7554/eLife.46315

131. Weng Q, Chen M, Yang W, et al. Integrated analyses identify miR-34c-3p/MAMG3 axis for the Warburg metabolism in hepatocellular carcinoma. FASEB J. 2020;34(4):5420–5434. doi:10.1096/fj.201902895R

132. Yin Y, Sun M, Zhan X, et al. EGF signaling confers resistance to BET inhibition in hepatocellular carcinoma through stabilizing oncogenic MYC. J Exp Clin Cancer Res. 2019;38(1):83. doi:10.1186/s13046-019-10826

133. Peng M, Wei-Guo T, Jin-Wu H, et al. HSP4 triggers epithelial-mesenchymal transition and promotes motility capacities of hepatocellular carcinoma cells via activating AKT. Liver Int. 2020;40(5):1211–1223.

134. Dou C, Zhou Z, Xu Q, et al. Hypoxia-induced TUF1 promotes the growth and metastasis of hepatocellular carcinoma by activating the Ca(2+)/PI3K/AKT pathway. Oncogene. 2019;38(8):1239–1255. doi:10.1038/s41388-018-0505-8

135. Cheng SC, Quintin J, Cramer RA, et al. mTOR- and HIF-1alpha-mediated aerobic glycolysis as metabolic basis for trained immunity. Science. 2014;345(6204):1250684. doi:10.1126/science.1250684

136. Liao YJ, Lee TS, Twu YC, et al. Glycine N-methyltransferase deficiency in female mice impairs insulin signaling and promotes gluconeogenesis by modulating the PI3K/Akt pathway in the liver. J Biomed Sci. 2016;23(1):69. doi:10.1186/s12929-016-0278-y

137. Liu Y, Lu L, Wen D, et al. MiR-612 regulates invadopodia of hepatocellular carcinoma by HADHA-mediated lipid reprogramming. J Hematol Oncol. 2020;13(12):10. doi:10.1186/s13045-019-0841-3

138. Kim E, Lisby A, Ma C, et al. Promotion of growth factor signaling as a critical function of beta-catenin during HCC progression. Nat Commun. 2019;10(1):1909. doi:10.1038/s41467-019-09780-z

139. de la Coste A, Romagnolo B, Billuart P, et al. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. Proc Natl Acad Sci U S A. 1998;95(15):8847–8851. doi:10.1073/pnas.95.15.8847

140. Lecarpentier Y, Schussler O, Hebert JL, Vallée A. Multiple targets of the canonical WNT/beta-catenin signaling in cancers. Front Oncol. 2019;9:1248. doi:10.3389/fonc.2019.01248

141. Xu W, Zhou W, Cheng M, et al. Hypoxia activates Wnt/beta-catenin signaling by regulating the expression of BCL9 in human hepatocellular carcinoma. Sci Rep. 2017;7:40446. doi:10.1038/srep40446

142. Zhang N, Chen X. A positive feedback loop involving the LINCO0346/beta-catenin/MYC axis promotes hepatocellular carcinoma development. Cell Oncol (Dordr). 2020;43(1):137–153. doi:10.1007/s13402-019-00478-4

143. Fu X, Zhu X, Qin F, et al. Linc00210 drives Wnt/beta-catenin signaling activation and liver tumor progression through CTNNBIP1-dependent manner. Mol Cancer. 2018;17(1):73. doi:10.1186/s12943-018-0783-3

144. Li N, Wei L, Liu X, et al. A frizzled-like cysteine-rich domain in glycipan-3 mediates wnt binding and regulates hepatocellular carcinoma tumor growth in mice. Hepatology. 2019;70(4):1231–1245. doi:10.1002/hep.30646

145. Huang JL, Fu YP, Gan W, et al. Hepatic stellate cells promote the progression of hepatocellular carcinoma through microRNA-1246-ROAlpha-Wnt/beta-Catenin axis. Cancer Lett. 2020;476:140–151. doi:10.1016/j.canlet.2020.02.012

146. Zhang Q, Bai X, Chen W, et al. Wnt/beta-catenin signaling enhances hypoxia-induced epithelial-mesenchymal transition in hepatocellular carcinoma via crosstalk with hif-1alpha signaling. Carcinogenesis. 2013;34(5):962–973. doi:10.1093/carcin/bgt027

147. Liu Y, Ye X, Zhang JB, et al. PROX1 promotes hepatocellular carcinoma proliferation and sorafenib resistance by enhancing beta-catenin expression and nuclear translocation. Oncogene. 2015;34(44):5524–5535. doi:10.1038/onc.2015.7

148. Guo Y, Wu Z, Shen S, et al. Nanomedicines reveal how PBOV1 promotes hepatocellular carcinoma for effective gene therapy. Nat Commun. 2018;9(1):3430. doi:10.1038/s41467-018-05764-7

149. Batlle E, Massague J. Transforming growth factor-beta signaling in immunity and cancer. Immunity. 2019;50(4):924–940. doi:10.1016/j.immuni.2019.03.024

150. Liu Z, Wang Y, Dou C, et al. Hypoxia-induced up-regulation of VASP promotes invasiveness and metastasis of hepatocellular carcinoma. Theranostics. 2018;8(17):4649–4663. doi:10.7515/thno.26789

151. Soukupova J, Malfettone A, Hyrošlová V, et al. Role of the transforming growth factor-beta in regulating hepatocellular carcinoma oxidative metabolism. Sci Rep. 2017;7(1). doi:10.1038/s41598-017-12837-y

152. Chae KS, Kang MJ, Lee JH, et al. Opposite functions of HIF-alpha isoforms in VEGF induction by TGF-betal under non-hypoxic conditions. Oncogene. 2011;30(10):1213–1228. doi:10.1038/onc.2010.498

153. Lin J, Cao S, Wang Y, et al. Long non-coding RNA UBE2CP3 enhances HCC cell secretion of VEGFA and promotes angiogenesis by activating ERK1/2/HIF-1alpha/VEGFA signaling in hepatocellular carcinoma. J Exp Clin Cancer Res. 2018;37(1):113. doi:10.1186/s13046-018-0727-1

154. Xuan Z, Zhao L, Li Z, et al. EPS8L3 promotes hepatocellular carcinoma proliferation and metastasis by modulating EGFR dimerization and internalization. Am J Cancer Res. 2020;10(1):60–77.

155. Zhou HJ, Xu Z, Wang Z, et al. SUMOylation of VEGFR2 regulates its intracellular trafficking and pathological angiogenesis. Nat Commun. 2018;9(1):3303. doi:10.1038/s41467-018-05812-2

156. Krzywinska E, Kantari-Mimoun C, Kerdrès Y, et al. Loss of HIF-1alpha in natural killer cells inhibits tumour growth by stimulating non-productive angiogenesis. Nat Commun. 2017;8(1):1597. doi:10.1038/s41467-017-01599-w

157. Zhang L, Chen J, Yong J, Qiao L, Xu L, Liu C. An essential role of RNF187 in Notch1 mediated metastasis of hepatocellular carcinoma. J Exp Clin Cancer Res. 2019;38(1):384. doi:10.1186/s13046-019-1382-x

158. Moriyama H, Moriyama M, Ozawa T, et al. Notch signaling regulates its intracellular trafficking and pathological angiogenesis. Nat Commun. 2018;9(1):3303. doi:10.1038/s41467-018-05812-2

159. Landor SK, Lendahl U. The interplay between the cellular calcium dependent ROS/Nrf2/Notch pathway in hepatocellular carcinoma. Cell Prolif. 2019;52(3):e12581. doi:10.1111/cpr.12581
163. Zhou J, Zheng X, Feng M, et al. Uregulated MMP28 in hepatocellular carcinoma promotes metastasis via Notch3 signaling and predicts unfavorable prognosis. *Int J Biol Sci*. 2019;15(4):812–825. doi:10.7150/ijbs.31335

164. Ye YC, Zhao JL, Lu YT, et al. NOTCH signaling via WNT regulates the proliferation of alternative, CCR2-independent tumor-associated macrophages in hepatocellular carcinoma. *Cancer Res*. 2019;79(16):4160–4172. doi:10.1158/0008-5472.CAN-18-1691

165. Chen H, Wu D, Bao L, et al. 6PGD inhibition sensitizes hepatocellular carcinoma to chemotherapy via AMPK activation and metabolic reprogramming. *Biomed Pharmacotherapy*. 2019;111:1351–1358. doi:10.1016/j.biopha.2019.01.028

166. Jin X, Moskophidis D, Mivechi NF. Heat shock transcription factor 1 is a key determinant of HCC development by regulating hepatic steatosis and metabolic syndrome. *Cell Metab*. 2011;14(1):91–103. doi:10.1016/j.cmet.2011.03.025

167. Ma X, Qiu Y, Sun Y, et al. NOD2 inhibits tumorigenesis and increases chemosensitivity of hepatocellular carcinoma by targeting AMPK pathway. *Cell Death Dis*. 2020;11(3):174. doi:10.1038/s41419-020-2368-5

168. Gao L, Lv G, Li R, et al. Glyceroenolpyruvate holophosphate hepatocellular carcinoma invasion and migration by AMPK/mTOR dependent autophagy activation. *Cancer Lett*. 2019;454:215–223. doi:10.1016/j.canlet.2019.04.009

169. Bort A, Sanchez BG, Mateos-Gomez PA, Vara-Ciruelos D, Rodríguez-Henche N, Diaz-Laviada I. Targeting AMP-activated kinase impacts hepatocellular cancer stem cells induced by long-term treatment with sorafenib. *Mol Oncol*. 2019;13(5):1311–1331. doi:10.1002/1878-0261.12488

170. Li C, Huang Z, Zhu L, et al. The contrary intracellular and extracellular functions of PEDF in HCC development. *Cell Death Dis*. 2019;10(10):742. doi:10.1038/s41419-019-1976-4

171. Fang G, Zhang P, Liu J, et al. Inhibition of GSK-3beta activity suppresses HCC malignant phenotype by inhibiting glycolysis via activating AMPK/mTOR signaling. *Cancer Lett*. 2019;463:11–26. doi:10.1016/j.canlet.2019.08.003

172. Qiao A, Jin X, Pang J, Moskophidis D, Mivechi NF. The transcriptional regulator of the chaperone response HSF1 controls hepatic bioenergetics and protein homeostasis. *J Cell Biol*. 2017;216(3):723–741. doi:10.1083/jcb.201607091

173. Garten A, Grohmann T, Kluckova K, Lavery GG, Kiess W, Penke M. Sorafenib-induced apoptosis in hepatocellular carcinoma. *Expert Opin Drug Metab Toxicol*. 2015;11(5):925–937. doi:10.1517/17420287.2015.888231

174. Ye YC, Zhao JL, Lu YT, et al. NOTCH signaling via WNT suppresses glucose metabolism and hepatocellular carcinoma growth by restoring FBP1 expression. *Sci Rep*. 2017;7:43864. doi:10.1038/srep43864

175. Chen H, Wu D, Bao L, et al. 6PGD inhibition sensitizes hepatocellular carcinoma to chemotherapy via AMPK activation and metabolic reprogramming. *Biomed Pharmacother*. 2019;111:1351–1358. doi:10.1016/j.biopha.2019.01.028

176. Luo W, Zhong J, Chang R, Hu H, Pandey A, Semenza GL. Hsp70 promotes tumor hypoxia via nanomaterials chemistry for efficient treatment of solid tumors. *Acc Chem Res*. 2018;51(10):2502–2511. doi:10.1021/acs.accounts.8b00214

177. Lu D, Li N, Zhang YF, et al. Persistent polyfunctional chimeric antigen receptor T cells that target glypecan 3 eliminate orthotopic hepatocellular carcinomas in mice. *Cell. Mol. Life Sci.* 2016;73(19):3915–3930. doi:10.1007/s00018-016-2308-1

178. Feng J, Dai W, Mao Y, et al. Simvastatin re-sensitizes hepatocellular carcinoma cells to sorafenib by inhibiting HIF-1alpha/PPAR-gamma/PKM2-mediated glycolysis. *J Exp Clin Cancer Res*. 2020;39(1):24. doi:10.1186/s13046-020-1528-x

179. Yang J, Jin X, Yan Y, et al. Inhibiting histone deacetylases suppresses glucose metabolism and hepatocellular carcinoma growth by restoring FB1P expression. *Sci Rep*. 2017;7:43864. doi:10.1038/srep43864

180. Tang Y, Zhang Y, Wang C, et al. Overexpression of PCK1 gene antagonizes hepatocarcinogenesis through the activation of gluconeogenesis and suppression of glycolysis pathways. *Cell Physiol Biochem*. 2018;47(1):344–355. doi:10.1055/s-0044-20573

181. Taylor S, Spugnini EP, Assaraf YG, Azzarito T, Rauch C, Fais S. Microenvironment acidity as a major determinant of tumor chemoresistance: proton pump inhibitors (PIPs) as a novel therapeutic approach. *Drug Resist Updat*. 2015;23:41–69. doi:10.1016/j.drup.2015.08.004

182. Estrella V, Chen T, Lloyd M, et al. Acidity generated by the tumor microenvironment drives local invasion. *Cancer Res*. 2013;73(5):1524–1535. doi:10.1158/0008-5472.CAN-12-2796

183. Feng J, Dai W, Mao Y, et al. Simvastatin re-sensitizes hepatocellular carcinoma cells to sorafenib by inhibiting HIF-1alpha/PPAR-gamma/PKM2-mediated glycolysis. *J Exp Clin Cancer Res*. 2020;39(1):24. doi:10.1186/s13046-020-1528-x

184. Chao M, Wu H, Jin K, et al. A nonrandomized cohort and a randomized study of local control of large hepatocarcinoma by targeting intratumoral lactic acidosis. *Elife*. 2016;5(4):e12554. doi:10.7554/eLife.15691

185. Lu ZN, Tian B, Guo XL. Repositioning of proton pump inhibitors in cancer therapy. *Cancer Chemother Pharmacol*. 2017;80(5):925–937. doi:10.1007/s00280-017-4342-6

186. Liu D, Li N, Zhang YF, et al. Persistent polyfunctional chimeric antigen receptor T cells that target glypican 3 eliminate orthotopic hepatocellular carcinomas in mice. *Gastroenterology*. 2020.

187. Harding JJ, El Dika I, Abou-Alfa GK. Immunotherapy in hepatocellular carcinoma. *Expert Opin Biol Ther*. 2019;19(4):477–487. doi:10.1080/14796694.2018.1513370

188. Sangro B, Gomez-Martin C, de la Mata M, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma. *Gastroenterology*. 2019;156(5):2777–2784. doi:10.1053/j.gastro.2018.11.022

189. Song H, Brooks AL, et al. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma. *Gastroenterology*. 2019;156(5):2777–2784. doi:10.1053/j.gastro.2018.11.022

190. Wang FF, Chen Y, Song SY, et al. Immune-related adverse events associated with anti-PD-1/PD-L1 treatment for malignancies: a meta-analysis. *Front Pharmacol*. 2017;8:730. doi:10.3389/fphar.2017.00730
