REVIEW

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 blockading 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.[1,3–5] 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.[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.[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).[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.[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.[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.[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-α and a constitutively expressed HIF-β subunit.[19,20] Both HIF-1α and HIF-2α are reported correlating with tumors. Studies have shown that HIF-1α regulates vascular endothelial growth factor (VEGF) during the acute phase of hypoxia, while VEGF is mainly regulated by HIF-2α during long-term hypoxia.[21] HIF-2α is overexpressed in primary and metastatic tumors[22] and is positively correlated with tumor angiogenesis.[23] However, studies on HIF-2α and liver cancer are rare, and HIF-1α is the primary factor in liver cancer hypoxia. In the presence of oxygen, HIF-1α is hydroxylated by prolyl hydroxylases (PHDs), leading to its rapid proteasomal degradation. Under hypoxic conditions, PHDs are no longer active to hydroxylate HIF-1α. HIF-1α will be stabilized and translocated to the nucleus.[24]

Accumulation of HIF-1α can influence tumor survival and proliferation by regulating tumor glycometabolism in the following four ways. First, HIF-1α can increase the uptake of glucose by upregulating the expression of glucose transporters (GLUT) such as GLUT1. Second, HIF-1α promotes the expression of glycolytic enzymes and accelerates the conversion of glucose to pyruvate. Third, HIF-1a 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-1a upregulates the expression of lactate dehydrogenase A (LDHA) to stimulate the production of lactic acid.[25]

In addition to the effect the of the glycometabolism of HCC, HIF-1α can also influence HCC survival by regulating the oxidative stress level.[26] Oxidative stress can mediate mitochondrial apoptosis and the immune response in liver cancer.[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.[28,29] Low levels of ROS may induce DNA mutation by oxidative DNA damage, which eventually increases the likelihood of HCC development.[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/β-Catenin /FOXO3a Signaling.[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.[35,36] However, HIF-1α can promote HCC progression by preventing ROS accumulation through the following pathways. First, HIF-1α prevents pyruvate from entering TCA cycle by inactivating PDH through PDKs and the conversion of pyruvate to lactate by upregulating LDHA expression.[21,37,38] HIF-1α therapy ensures that circulating tricarboxylic acid cycle (TCA) substrates cannot enter mitochondrial oxidation.[36,39] Second, HIF-1α can reduces ROS accumulation by inhibiting ROS production sites in the electron transport chain (ETC), such as complexes 1 and 4.[40,41] Third, HIF-1α 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.[42] Glutamine is a key source of carbon, secondary only to glucose.[43] Decomposition of glutamine will replenish the TCA cycle and provide abundant carbon and nitrogen for hepatocyte growth and proliferation.[44,45] Some of the carbon can be used to produce NADPH to achieve redox equilibrium.[46] At the same time, glutamic acid produced by glutamine decomposition will directly synthesize the antioxidant glutathione and neutralize ROS.[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.[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.[32,49,50] In turn, tumor cell aerobic glycolysis can influence HIF-1α 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.[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.[52] Glutamine can regulate the stability of HIF-1a in response

to hypoxia and support the survival of HCC cells by upregulating proline and hydroxyproline levels.[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) ≥7.4 rather than a higher pH_e of ~7.4 and a lower pH_i of ~7.2 in normal cells.[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.[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.[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.[59] LDH enzymes with high M-subunits (encoded by LDHA) promote the conversion from pyruvate to lactate.[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.[59] Moreover, pyruvate dehydrogenase kinase (PDK) can prevent pyruvate from entering mitochondria for OXPHOS.[60] Upregulation of LDHA and PDK synergistically promotes the production of lactate in HCC.[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.[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.[62] MCT4 is a low-affinity lactate transporter that promotes lactate release from glycolytic tumor cells.[63] Although lactate could be incepted as a fuel, lactate
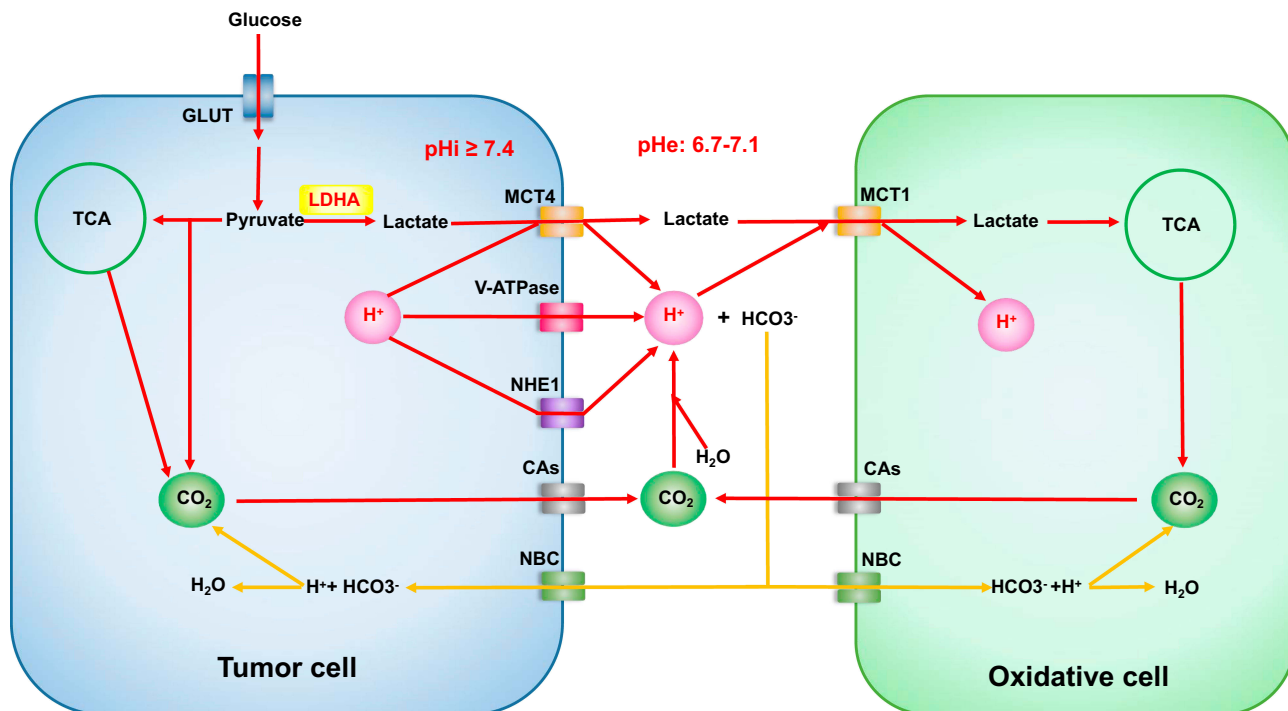
Figure 1 Overview of tumor acid-base microenvironment. OXPHOS of the oxidative cells is a compensatory mechanism of tumor acidosis, but the acidosis cannot be reversed.
Abbreviations: GLUT, glucose transporters; TCA, tricarboxylic acid cycle; LDH-A, lactate dehydrogenase A; MCT4, monocarboxylate transporter4; MCT1, mono-carboxylate transporter1; V-ATPase, vacuolar ATPase; NHE1, sodium-hydrogen exchanger1; CAs, carbonic anhydrases; NBC, sodium bicarbonate cotransporters.

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_2CO_3 and then dissociates to $HCO_3^- + H^+$.[64] 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).[65] However, carbonic anhydrases (CAs) colocalize with Na^+/HCO_3^- cotransporters (NBCs)[66] transporting Na^+ and HCO_3^- into tumor cells and maintaining a mildly alkaline level of pH_i and a dynamic balance of Na^+.[65] V-ATPase, CAIX and CAXII are selectively overexpressed in HCC.[67]

TME acidosis could influence HIFs reprogramming by increased O_2 consumption. An upregulation of HIF-1α under acidosis has been reported in glioma and HEK293 cell.[68,69] Additionally, HIF-2α has been reported to play a critical role in regulating metabolic adaptation to acidosis in liver cancer and glioma.[68,69] Mild extracellular acidosis could restructure mitochondria and promote mitochondria fusion. Excess H^+ and lactate decrease immunological cell function by inhibiting glycolysis and IFN-γ production. Moreover, TME

acidosis has been reported to contribute to angiogenesis, invasion and metastasis.[65]

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.[70] 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.[71,72] 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).[73–75] CD4$^+$ T cells can differentiate into two affected subsets: helper T cells (Th cells) and regulatory T cells (T_{reg} cells).[75–77] CD8$^+$ T cells can also differentiate into cytotoxic T cells (CTL)

and T_{reg} cells upon activation.[78] 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).[79–83] 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.[84] 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.[85,86] Both elevated aerobic glycolysis and OXPHOS have been observed in T_E cells (except T_{reg} cells) activation.[85] Many scientists pointed out that aerobic glycolysis/OXPHOS levels are upregulated in these activated T_E cells compared to resting T cells, which indicates a "switch" from OXPHOS to aerobic glycolysis.[82,85,87-90] Metabolism reprogramming is associated with T cells fate, in which mitochondria play a critical role.[91] The function of mitochondria is correlated with the number and polarization of mitochondria, the number and length of mitochondrial cristae, and mitochondrial dynamics.[85] T_E cells with a dominant metabolic pathway of aerobic glycolysis have punctate mitochondria, which accelerate cell proliferation.[79,92] T_M cells with a dominant metabolic pathway of OXPHOS maintain fused networks,[79,92] which maximize OXPHOS activity. Control of T_{reg} cells suppress function by mitochondria is closely related to mitochondrial complex III.[81] Crosstalk among these cells keeps the dynamic balance of immune response in vivo. T_E cells play a role as immune guards, and T_M cells can be supplements for T_E cells to react rapidly after restimulation. T_{regs} can inhibit overreaction of T_E cells and are essential for protection from autoimmunity and excessive inflammation.[93] However, the disruption of this balance leads to the immune escape during tumorigenesis.

Tumorigenesis starts from mutations of tumor-related genes and an uncontrolled cell cycle. In addition to uncontrolled proliferation, tumor progression and migration require the suppression of self-programmed death and evasion of immunosurveillance.[94] Immune tolerance and immune evasion play a critical role in poor HCC prognosis and are always concomitant with energy, exhaustion and senescence of T cells, especially T_E cells.[95] In the TME, T cells' metabolic pathways can be influenced by glucose competition, extracellular lactate accumulation and interaction between tumor cells and T cells. As a result of glucose competition, T cells cannot obtain enough glucose, which inhibits T cell proliferation and function.[87,95] The glucose transporter GLUT1 of T cells is downregulated in

tumors, which is essential for glucose uptake and aerobic glycolysis. Macintyre et al suggested that GLUT1 deficiency prevented CD4$^+$ T cells activation and effector functions, and that T_E cell expansion and IFN-γ production decreased.[96] Stromal cells (tumor cells and cancer-associated fibroblasts) contribute to lactate levels, which lead to cell invasiveness and metastasis.[92] It has been demonstrated that tumor cells can inhibit T cell proliferation by expressing indoleamine 2.3-dioxygenase and inhibit T cell function by deriving lactate and blocking lactate export in T cells.[97,98] As a byproduct of the "Warburg effect", lactate production and acidification can lead to immune evasion by diminishing the IFN-γ production of T cells through the NF-kB pathway.[99] In contrast, T_{reg} cell proportions can be found increased in the HCC microenvironment.[100] It has been reported that tumor-infiltrating T_{reg} cells express higher levels of GLUT1 and glycolysis-related genes than T_E cells on the cell surface, which leads to a higher uptake of glucose and an increased level of aerobic glycolysis.[101] T_{reg} cells play a synergistic action with tumor cells for glucose competition, which induces T cell senescence and exhaustion by starving effector T cells.[102] In the TME, immune tolerance and evasion are only displayed in local tumor rather than the whole body, which is tightly associated with the interaction between tumors and T cells by "immune checkpoints".[103]

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.[104] PD-1, programmed death-1, is also known as CD279, PDCD1, SLEB2, hPD-1, hPD-l, hSLE1 [NCBI Gene ID: 5133]. A high level of PD-1 expression can be a characteristic of exhausted T cells.[105] 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
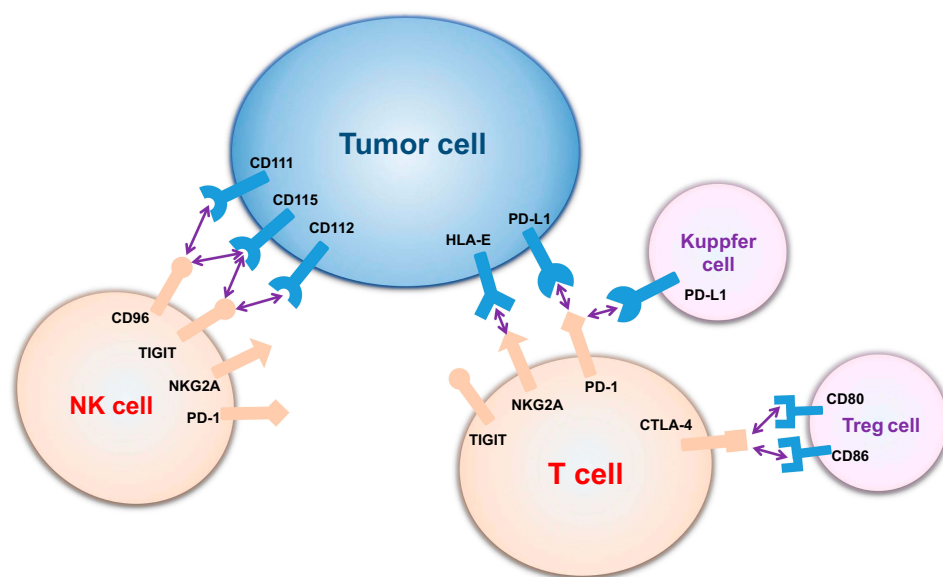
Figure 2 Interaction between tumor cells and immune cells by immune checkpoints. T cells and NK cells express various immune checkpoints, which can bind to ligands on tumor cells, T_{reg} cells and Kupffer cells and be inhibited (CD96 binds to CD111 and CD115; TIGHT binds to CD115 and CD112; NKG2A binds to HLA-E; PD-1 binds to PD-L1; and CTLA-4 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.

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.[106] 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.[85,89] In addition, PD-1 induces FAO by upregulating the rate-limiting enzyme of FAO, carnitine palmitoyl-transferase (CPT1A).[89] Metabolic reprogramming from aerobic glycolysis to FAO makes the dynamics lean toward long-lived T_M cells.[89] PD-L1 expression in tumor cells is associated with vascular formation in HCC patients.[107] 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.[108] However, this reinvigoration CD8+ T cells can be re-exhausted in a high antigen concentration environment.[108] Conversely, CTLA-4 inhibits aerobic glycolysis rather than enhancing FAO.[89] Blockade of PD-1 and CTLA-4 can reverse the inhibition of aerobic glycolysis and effector function in T cells.[85,89,92,95,109] Different from off-targets of the classical immune checkpoint

blockers, T_{reg} cells are sensitive to anti-CTLA-4 antibodies and can induce antibody-dependent cell-mediated cytotoxicity.[110,111] T cell immunoglobulin and ITM domain (TIGIT) is also involved in the regulation of CD8+ T cell metabolism by downregulating GLUT1 and HK1/HK2.[112]

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: CD56^{dim} cells and CD56^{bright} cells.[113] CD56^{dim} cells are generally deemed to be cytotoxic cells with less GLUT1 expression, whereas the CD56^{bright} cells are considered to be IFN-γ producers with higher GLUT1 expression.[113] Either IL-2 or IL-12/15 cytokine combinations can activate NK cells and increase OXPHOS levels for energy supply.[114] Siné ´ad E. Keating and his colleagues found an interesting phenomenon in which CD56^{bright} cells showed higher GLUT1 expression and levels of aerobic glycolysis than CD56^{dim} cells, leading to a higher level of activation. Downregulation of aerobic glycolysis in CD56^{bright} cells restricts IFN-γ production.[113] Lactate accumulation and acidification can impair activation and IFN-γ production of NK cells by diminishing nuclear factor of activated

T cells (NFAT).[99] 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.[115] Zhou et al found that LrNK contributes to the tolerogenic microenvironment of the liver by inhibiting T_E cells. LrNK inhibits T_E cells proliferation and production of INF-γ and TNF-α through a PD-1-PD-L1 axis.[116]

Immune checkpoints: natural killer cell group 2A (NKG2A)/CD94, TIGIT and CD96 were found to lead to NK cells exhaustion and to predict poor prognosis in HCC.[117–119] Scientists found that CD49a+ NK cells, which expressed higher levels of immune checkpoints molecules PD-1, TIGIT and CD96, were correlated with a poor prognosis in HCC patients.[120] 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.[121] Caroline Pfeifer found that educated NK cells presented with distinct self-inhibitory receptors and went through distinct glycolytic profile and functions.[121] 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).[121] 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.[121] Upregulation of the NKG2A ligand on tumor cells further promotes immune evasion from NK cells in the TME.[121] In addition, other experiments have proven that anti-NKG2A mAb could block immune evasion by unleashing not only NK cells but also T cells.[122]

TAM infiltration takes part in tumor invasion and metastasis.[123] Macrophages exhibit two diverse phenotypes: M1-classic activation and M2-alternative activation. The M1 type is characterized by pro-inflammatory (IL-1, TNF-α) cytokines and IFN-γ 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-β) and can induce angiogenesis and tumor cell generation. TAMs were attracted and activated by tumor secretory factors (VEGF, PDGF, TGF-β, CCL2, and M-CSF).[10] 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-1a to promote M2-like polarization of TAMs.[124] As liver-specific macrophages, Kupffer cells survive anoxia by glycolysis and produce PD-L1 ligand to suppress T_E cells.[125] The dominant TAMs in orthotopic HCC exhibit Kupffer cell (KC) properties and are known as KC-like TAMs (kclTAM).[126] 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-1α and c-MYC synergies.[127] c-MYC also plays as important a role in glycolysis in HCC as HIF-1α. The importance of collaboration between c-MYC and HIF-1α was demonstrated to activate the Warburg effect by inhibiting IDH1-AS1 in multiple tumors under normal oxygen.[127] c-MYC was reported to participate overexpression of MTR4 in HCC, which drives the expression of glycolytic genes such as GLUT1 and PKM2.[128] PFK2 in turn was found to up-regulate c-MYC expression in glioma.[129] A positive feedback loop between MYC and PFK2 was demonstrated to sustain tumor cell aerobic glycolysis in a Drosophila tumor model.[130] c-MYC could be a promising target for HCC treatment, especially in advanced stages.[131,132] 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-1α. The major regulatory mechanisms of HIF-1α 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.[3,133,134] 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-1α.[135] Impairing insulin signaling by inhibiting PI3K/AKT pathway could promote gluconeogenesis in the liver.[136] Molecules can treat HCC by inhibiting PI3K/AKT activation, such as MiR-612.[137]

The Wnt/β-catenin pathway has been reported to occur in both early and late stages of HCC[138,139] and suppress mitochondrial respiration and promotes glycolysis.[140] HIF-1α can stimulate Wnt/β-catenin signaling via the
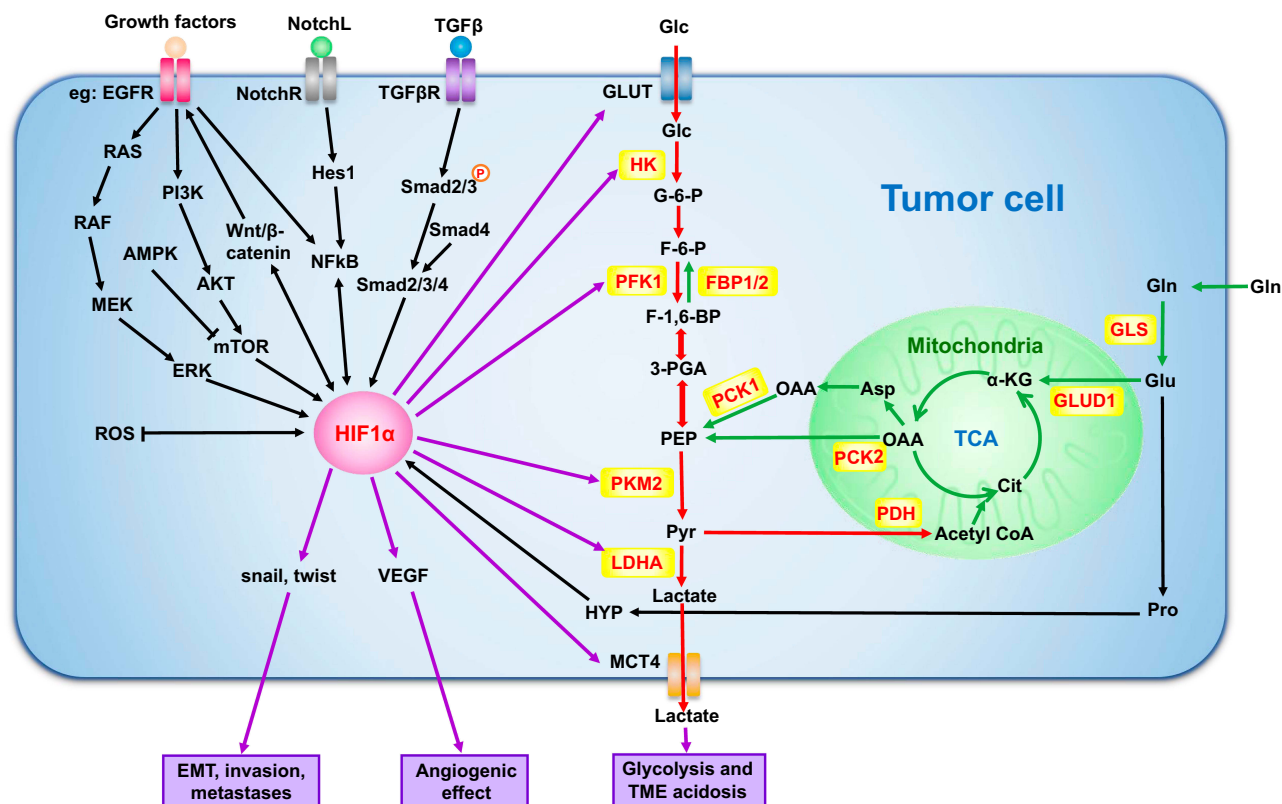
Figure 3 Mechanisms of glycolysis regulation by HIF-1α and gluconeogenesis in tumor cells. HIF-1α can directly regulate glycolysis-related enzymes to affect tumor cell glucose metabolism. It can also affect tumor growth and metastasis by regulating the level of active oxygen, EMT and angiogenesis. In addition, cytokines, signaling pathways and glutamine, which play an important role in tumor, can also regulate the biological activity of tumor cells by influencing HIF-1α. Black arrows: pathways regulating HIF-1α; Red arrows: glycolysis-related processes; Green arrows: gluconeogenesis-related processes.

Abbreviations: GLS, glutaminase; GLUD1, glutamate dehydrogenase; αKG, α-ketoglutarate; TCA, tricarboxylic acid; GLUT1, glucose transporter1; PFK1, phosphofructo-kinase; PKM2, pyruvate kinase M2; LDHA, lactate dehydrogenase A; MCT4, monocarboxylate transporter4; VEGF, vascular endothelial growth factor; EMT, epithelial-to-mesenchymal transition; HIF-1α, hypoxia-inducible factor-1α; ICN, the intracellular domain of Notch; Glc, glucose; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; F-1,6-BP, fructose-1,6-bisphosphate; 3-PGA, glyceraldehyde-3-phosphate; PEP, phosphoenolpyruvate; Pyr, pyruvate; Cit, citrate; OAA, oxaloacetic acid; Asp, asparagine; Glu, glutamate; Gln, glutamine; Pro, proline; HYP, hydroxyproline; NotchL, Notch ligand; NotchR, Notch receptor.

coactivator BCL9 in HCC.[141] Wnt signaling further drives HCC proliferation through MYC, frizzled (FZD), Glypican-3 (GPC3), EGFR and CTNNBIP1.[138,142-144] 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.[145,146] 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).[142,143,147,148] PROX1 was also found to be a target for treating HCC sorafenib tolerance.[147]

Transforming growth factor-β1 (TGF-β1) is a common cytokine that regulates a variety of cellular processes.[149] In advanced tumor, TGF-β1 acts as an oncogenic factor and induces tumor proliferation, EMT invasion and metastasis.[149,150] 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.[31] TGF-β1 promotes tumor progression by reducing mitochondrial respiration and enhancing glutamine anaplerosis and the pentose phosphate pathway (PPP) cycle.[151] TGF-β1 and its mediated signaling pathway can still induce HIF- α to participate in the process of metabolic reprogramming under normoxic conditions.[152]

The EGFR/MEK/ERK/HIF-1α/VEGFA cycle regulates glucose metabolism and promotes HCC proliferation, angiogenesis and metastasis.[153,154] VEGFs and their cognate receptors (VEGFRs) are critical in the regulation of vessel formation in angiogenesis.[155] HIF-1α can induce angiogenesis by binding to the VEGF gene promoter and upregulating VEGF expression.[156] In addition, TGF-β1 can induce VEGF expression via Smad and HIF-2α.[152]

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.[157] Notch/Hes1 signaling could induce glycolysis by inactivation of p53 and activation of the NF-kB pathway.[126,158] As a target gene of NF-kB, the transcriptional activity of HIF-1α was significantly increased by activated Notch1.[158] In turn, HIF-1α was reported to upregulate the expression and function of Notch in HCC.[159,160] Notch can promote the proliferation of hepatoma cells through the PI3K-Akt, mTOR and Ras pathways.[126] Moreover, Notch inhibits hepatoma cells apoptosis by downregulation of ROS production via the NICD1/Hey1/PINK1 pathway and inactivation of p53.[36,126] Notch promotes EMT, invasion and metastasis in HCC through NICD/snail and Wnt3a pathway.[157,161-163] However, there is a dispute that blocking Notch promotes HCC progression and metastasis by accelerating proliferation of kclTAMs via Wnt signaling and IL-10 production through c-MYC.[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.[165–167] Activation of AMPK/mTOR by glycochenodeoxycholate can also promote HCC invasion and migration by activating autophagy.[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.[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.[165–167,170,171] Among them HSF1 also participates in the promotion of gluconeogenesis.[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.[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.[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.[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.[17,18] Increasing local oxygen pressure and reversing hypoxia can use nanotechnology to introduce O_2 into the tumor or generate oxygen at the tumor site by increasing the decomposition of endogenous hydrogen peroxide and light-triggered water splitting.[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.[176–178] Drugs that inhibit the expression and accumulation of HIF-1α transcriptional activity and protein accumulation include cardenolides and ezn-2208.[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.[180,181] Semaxanib causes low HIF-1 DNA-binding activity by inhibiting PI3K activity and AKT phosphorylation.[182] HIF-1a 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

sorafenib.[183] 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.[184] 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 detective 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.[185,186] 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.[187] 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.[67] 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.[188–190] Patients with large HCC showed a marked enhancement of the anticancer activity after TACE with bicarbonate local infusion into tumor.[190] Sodium bicarbonate, with a pKa of 6.1, is sufficient to meet the above requirements.[188] However, published clinical trials have indicated that pH buffer with a pKa of approximately 7 is a more ideal treatment.[189] 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.[191] 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 detective 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,[192] high-frequency negative trials with high toxicity pushed scientists to find other ways.[193] 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).[194] 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.[195,196] The PD-1 inhibitor MEDI4736 resulted in lower hepatotoxicity than CTLA-4 antibody in HCC patients.[193] Although antitumor activity of PD-1 antibody is promising, less than 20% of HCC patients respond to it.[104] 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.[197] 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.[198] 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.[122] The antiviral activity of hepatic T cells has been found to be controlled by LrNK via the PD-1-PD-L1 axis.[116] Moreover, anti-NKG2A mAb was revealed play an important role in unleashing both T and NK cells.[122] 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.[199] 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.[200] New GLUT inhibitors such as rapafucins are being explored.[201] 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.[202] Studies have shown that blockade of HK2 in human hepatoma cells can inhibit the occurrence of tumor and increase cell death.[203] 2-DG is a well-known HK2 inhibitor, that has been reported to inhibit hexokinase by competing with glucose.[204,205] Lonidamin is a mitochondrial HK inhibitor that suppresses the activity of HK1 and HK2.[206] Others such as 3-bromopyruvic acid (3-BrPA), ketoconazole

and posaconazole can also affect tumor metabolism and growth by blocking HK.[207,208]

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.[209] Metformin can target the HIF-1α/PFKFB3/PFK1 pathway in hepatoma cells and reduce hepatoma cell proliferation by inhibiting glycolysis.[210] 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.[211] Recent studies have found that metformin can also induce tumor cell death and increase sensitivity to chemotherapy drugs by inhibiting PKM2 in osteosarcoma.[212]

LDHA is located at the bifurcation point of glycolysis and oxidative phosphorylation. Inhibition of LDHA may be a promising antitumor strategy. The piperidindione derivatives miR-30a-5p, miR-41 and GNE-140 have been indicated to inhibit LDHA in breast and pancreatic cancer.[213,214] 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).[215] 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.[16] FBP also has two isoforms: liver isoform, FBP1 and muscle isoform, FBP2.[216] Studies of increasing gluconeogenesis are mainly focused on PCK1 and FBP1.

PCK1 and PCK2 are downregulated in HCC and suggest a poor prognosis.[16] Bian found that Nur77 could stabilize PCK1 by attenuating its sumoylation and ubiquitination and then suppress HCC.[14] PCK1 was also found to inhibit hepatoma cell proliferation by downregulating cell cycle progression through the AMPK pathway.[217]

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.[13] 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.[15] The second loop is FBP1 and polycomb repressive complex 2 (PRC2). PCR2 can downregulate FBP1, and conversely, FBP1 can interfere with PCR2 functions.[218] Histone deacetylases and FX11 inhibitor stabilize FBP1 in HCC to inhibit of tumor growth and invasion.[219,220] 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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