

Review

Research Progress of Warburg Effect in Hepatocellular Carcinoma

Yanguang Yang^{1,2,†}, Yuting Gao^{1,2,†}, Yajun Xiong^{1,†}, Yi Gong^{1,2}, Junlan Lu^{1,2},
Yuman Zhang^{1,2}, Dan Wang¹, Zhihan Liu¹, Xinli Shi^{1,*}

¹Laboratory of Integrated Medicine Tumor Immunology, Shanxi University of Chinese Medicine, 030000 Taiyuan, Shanxi, China

²Department of Pathobiology and Immunology, Hebei University of Chinese Medicine, 050200 Shijiazhuang, Hebei, China

*Correspondence: sxlsunshine@sina.com (Xinli Shi)

†These authors contributed equally.

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Abstract

The Warburg effect, also called aerobic glycolysis, refers to tumor cells that metabolize glucose through glycolysis even in the presence of oxygen. This rapid breakdown of glucose fuels the fast development, growth, and migration of tumor cells. Lactate, the final product of aerobic glycolysis, contributes to an acidic environment within the tumor, promoting the formation of an immunosuppressive microenvironment and accelerating tumor progression by impeding anti-tumor immunity. Numerous studies have confirmed the critical role of aerobic glycolysis in the occurrence and development of hepatocellular carcinoma by influencing tumor cells proliferation, invasion, metastasis, apoptosis, immune escape, angiogenesis, and more. Clinical trials have shown that inhibitors of rate-limiting enzymes in the glycolysis pathway can enhance the effectiveness of sorafenib, a targeted drug for hepatocellular carcinoma, by reducing drug resistance. Additionally, active components of traditional Chinese medicine and specific compound prescriptions are gaining attention for their potential to target and regulate aerobic glycolysis in hepatocellular carcinoma. Therefore, inhibiting the aerobic glycolysis pathway holds promise as a therapeutic strategy for treating liver tumors. This manuscript aims to review the role, research directions, and clinical studies of aerobic glycolysis in hepatocellular carcinoma.

Keywords: Warburg effect; hepatocellular carcinoma; tumor microenvironment

1. Introduction

Liver cancer, one of the most common primary malignant cancers, ranks third in cancer-related mortality [1]. Hepatocellular carcinoma (HCC), accounting for 75%–85% of cases, is the predominant type [1]. Currently, surgical resection and radiofrequency ablation remain the mainstay of treatment for early-stage liver cancer, while interventional therapy and radiotherapy are typically used for intermediate and advanced stages. However, due to the high incidence of drug resistance, the prognosis for HCC remains poor [2]. Studies have shown that atezolizumab plus bevacizumab improves recurrence-free and progression-free survival compared to traditional sorafenib treatment [3,4]. Although immunotherapy has offered new hope for patients, its curative effect is limited, and patient survival times are still short. Therefore, exploring new therapeutic targets for HCC is crucial for developing more effective treatment strategies.

Over the years, many studies have shown that metabolic reprogramming, a significant characteristic of tumors, has gained increasing attention in understanding tumor development. One form of energy metabolism, glycolysis, provides an energy source for tumors [5,6]. Notably, the liver plays a crucial role in glucose metabolism by regulating glycogen synthesis and breakdown. In the 1920s, Otto Heinrich Warburg made a groundbreaking discovery,

observing abnormal glucose metabolism in rat hepatoma cells. Even in the presence of sufficient oxygen, these cells prefer to obtain energy through glycolysis rather than the more efficient mitochondrial oxidative phosphorylation (OXPHOS) pathway, a phenomenon known as the Warburg effect [7]. The main hallmarks of aerobic glycolysis in tumor cells are increased glucose uptake and lactate release. Adenosine triphosphate (ATP) is the main energy source in the body. Despite its inefficiency in ATP production, aerobic glycolysis generates 50% to 70% of ATP in various tumors [8]. Additionally, lactate excretion fosters an acidic tumor microenvironment (TME) linked to tumor progression by facilitating invasion and metastasis [9]. Therefore, targeting tumor aerobic glycolysis holds promise for treating patients with HCC.

Given the key role of aerobic glycolysis in HCC growth, invasion, and treatment. This paper aims to review its characteristics, its relationship with the TME, and the current research and clinical applications of glycolysis in HCC.

2. Aerobic Glycolysis in HCC

There is a growing consensus suggesting that in HCC, aerobic glycolysis not only exists but is also markedly enhanced [10,11]. This phenomenon is ubiquitous in HCC and contributes to various aspects of tumor progression.



Numerous studies have confirmed that aerobic glycolysis plays a role in tumor cell proliferation, invasion, metastasis, apoptosis, cell cycle, immune escape, angiogenesis, and more [12,13]. Enhanced aerobic glycolysis is often associated with a poor prognosis for HCC patients [14]. It is well-established that tumor tissues generally exhibit lower glucose levels and higher lactate levels compared to normal tissues [15]. Similar enhancement of aerobic glycolysis has been observed in various other cancers, including cervical cancer [16], breast cancer [17], pancreatic cancer [18], lung cancer [19], gastric cancer [20], and colorectal cancer [21].

While normal cells primarily rely on mitochondrial OXPHOS for energy generation from glucose, tumor cells often depend heavily on glycolysis. However, it is important to note that aerobic glycolysis provides only a portion of the energy needed by most tumor cells [15]. In most normal cells, around 90% of ATP is derived from OXPHOS, with the remaining 10% coming from aerobic glycolysis. While less efficient in ATP production, tumor cells can obtain up to 60% of their ATP through aerobic glycolysis [22]. One study suggested that tumor cells choose the less efficient aerobic glycolysis for two reasons: faster ATP production compared to OXPHOS and the generation of downstream biomacromolecules needed for cell proliferation, achieved by consuming more glucose [23]. This unique ATP production pathway allows tumor cells to take up more glucose, ultimately meeting their specific energy needs during development [6]. In conclusion, the selection of aerobic glycolysis during HCC development appears to be driven by its ability to meet the unique metabolic demands of tumor cells.

Increased glucose uptake, achieved through the glucose transporters (GLUTs) family that facilitates transport from the extracellular space to the cytoplasm, is a well-established characteristic of HCC [24]. GLUTs are frequently upregulated in various cancers, including HCC, colorectal cancer, breast cancer, lung adenocarcinoma, squamous cell carcinoma, ovarian cancer, and glioblastoma, due to mechanisms like gene expression changes, protein relocation, or stability alterations [25]. Specifically, high GLUT1 expression promotes increased glucose uptake, sustains and accelerates aerobic glycolysis, generates substantial lactate, and influences angiogenesis, migration, and immune escape in HCC [26]. Interestingly, GLUT1 expression has been reported to be negatively correlated with GLUT2 expression. While GLUT2 protein levels were reported to be lower in human HCC compared to normal liver tissue and negatively associated with malignant tumors in previous studies [26], its overexpression has also been documented in HCC samples [27]. Similarly, GLUT3 overexpression has been linked to decreased overall survival in HCC patients [28]. In conclusion, GLUTs play a crucial role in maintaining the energy demands of tumor cells across various biological processes, making them potentially promising targets for anticancer therapies.

It is now understood that three key enzymes limit the rate of aerobic glycolysis in HCC: hexokinases (HKs), phosphofructokinases (PFKs), and pyruvate kinases (PKs). Notably, the overexpression of these enzymes has been associated with a poor prognosis for HCC patients [29]. The first rate-limiting step occurs when glucose enters tumor cells and is phosphorylated by HKs to form glucose-6-phosphate (G-6-P) [30]. While there are four HK subtypes (HK1, HK2, HK3, and HK4), most normal tissues only express HK1. However, high levels of HK2 in HCC tissues are indicative of a poorer prognosis [31]. Next, PFK1 catalyzes the conversion of fructose-6-phosphate (F-6-P) to fructose-1,6-bisphosphate (F-1,6-BP) using ATP [32]. Elevated PFK1 expression has been shown to promote both aerobic glycolysis and tumor cell proliferation [33]. Finally, PKs, the last rate-limiting enzyme in the aerobic glycolytic process, convert phosphoenolpyruvate (PEP) to ATP and pyruvate. Pyruvate kinase M2 (PKM2), specifically, is significantly upregulated in tumor cells and plays a dual role in both regulating tumor cells metabolism and contributing to poor prognosis [22]. Its expression is controlled by various signaling pathways and transcription factors, with HIF-1 α being the main upstream regulator in HCC [29]. HIF-1 α 's high expression in regulating HCC cells aerobic glycolysis further confirms the presence of the Warburg effect [29,34]. Pyruvate is subsequently converted to lactate by lactate dehydrogenase (LDH), with a recent study showing elevated lactate dehydrogenase A (LDHA) levels in HCC cells and their contribution to tumor progression [35]. Interestingly, lactate itself can be recycled as a substrate within the tricarboxylic acid cycle (TCA cycle) [36]. To prevent excessive lactate buildup, monocarboxylic acid transporters (MCTs) are upregulated to transport lactate out of the cell [37]. This suggests that inhibiting MCTs and promoting intracellular lactate accumulation could be a promising therapeutic strategy for indirectly killing tumor cells in HCC. The whole process of aerobic glycolysis is shown in Fig. 1.

3. The Relationship between Aerobic Glycolysis and TME

A large amount of lactate produced by tumor cells not only acidifies the TME but also promotes tumor angiogenesis, metastasis, drug resistance, and immunosuppression [38]. The TME, the immediate environment supporting cell survival, consists of cellular components (T cells, regulatory T cells (Tregs), natural killer (NK) cells, tumor-associated macrophages (TAMs)) and non-cellular components (chemokines, cytokines, and other molecules). This acidic TME, also known as tumor acidosis, results from the accumulation of lactate and H⁺ ions. Thus, the interplay between aerobic glycolysis and the TME warrants further investigation.

Recently, the link between aerobic glycolysis and cytokines has gained increasing attention. Glycolysis pro-

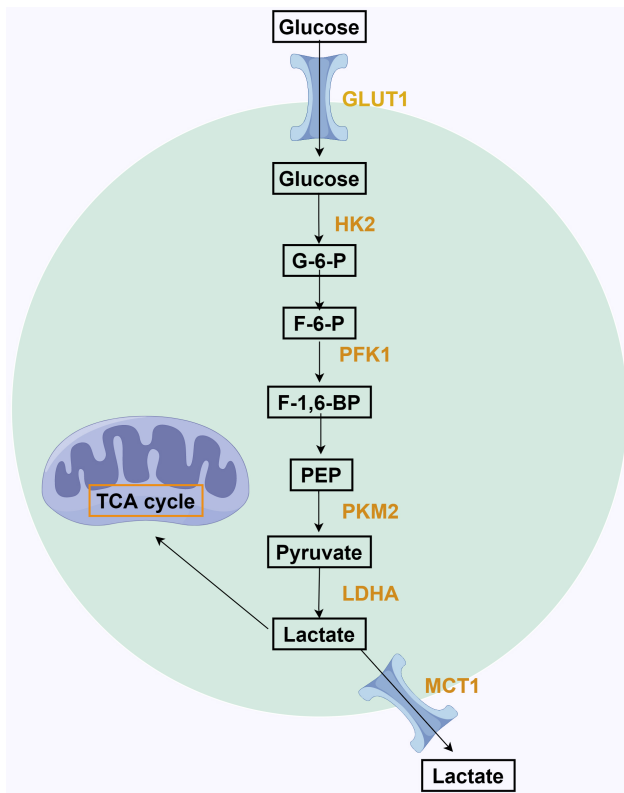


Fig. 1. The process of aerobic glycolysis in tumor cells. Glucose entry into tumor cells is catalyzed by hexokinases 2 (HK2) to form Gmur6murp, which is then generated by phosphofructokinases 1 (PFK1) catalysis of Fmur6murp. Then, phosphoenolpyruvate (PEP) produces pyruvate through pyruvate kinase M2 (PKM2), and pyruvate forms lactate under the action of lactate dehydrogenase A (LDHA). Finally, lactate is excreted out of the cell through monocarboxylic acid transporters 1 (MCT1). TCA, tricarboxylic acid; F-1,6-BP, fructose-1,6-bisphosphate; F-6-P, fructose-6-phosphate; G-6-P, glucose-6-phosphate.

motes the production of the effector molecule interferon- γ (IFN- γ), and a study showed reduced IFN- γ expression in T cells with impaired LDHA activity under T helper 1 (Th1) differentiation conditions [39]. High levels of H^+ and lactate, resulting from inhibited glycolysis and IFN- γ production, can impair immune cell function [40]. Compared with the naive T cells cultured without exogenous cytokines, interleukin-2 (IL-2) stimulated the messenger RNA (mRNA) expression level of key molecule, including *SLC2A1* and *LDHA* [41]. *SLC2A1* encodes the glucose transporter GLUT1, while *LDHA* encodes the main subtypes of LDH [41]. Interleukin-1 β (IL-1 β), a key member of the IL-1 family, plays a crucial role in inflammation, immune regulation, and glycolysis regulation. Recent studies indicate that IL-1 β promotes glycolysis by enhancing glucose uptake and the expression of the glycolytic enzyme HK2 [42,43]. Similarly, IL-1 β augmented glucose uptake via GLUT3 in immature rat ovarian cells [44]. Transforming growth factor β (TGF- β) has been shown to induce tu-

mor cell migration and invasion while also altering *LDH* gene expression and favoring LDHA expression to promote aerobic glycolysis in HCC cell lines [45]. Overexpression of CCL2 in glioma cells leads to increased p-AKT, which in turn increases the expression of the glycolytic transporter GLUT1, promoting aerobic glycolysis [46]. Tumor necrosis factor- α (TNF- α) and IL-17, primarily produced during inflammation, synergistically enhance aerobic glycolysis and lactate production by activating the NF- κ B pathway and promoting GLUT1 and HK2 expression, thereby contributing to tumor initiation and progression [47].

In HCC, aerobic glycolysis not only occurs in tumor cells but also extends to immune cells within the TME [48]. Damaged immune cells with compromised function contribute to disease progression [49]. T cell function in the TME is intricately linked to glucose availability, extracellular lactate accumulation, and interactions with tumor cells. High glucose uptake in tumor cells often leaves T cells starved, leading to dysfunction [48,50]. Notably, T cells glucose transporter GLUT1 is downregulated in tumors, hindering the effector function of CD4 $^+$ T cells [51]. Similar to T cells, activated NK cells rely on aerobic glycolysis for energy during proliferation and effector functions. These cells differentiate into two subsets based on CD56 receptor expression: cytotoxic CD56 dim cells and IFN- γ -producing CD56 bright cells [52]. However, the acidic environment created by excessive lactate production from glycolysis significantly reduces the cytotoxicity of NK cells, impairing their anti-tumor activity [40]. Notably, CD56 bright cells exhibit higher metabolic activity, GLUT1 expression, and glucose uptake compared to CD56 dim cells [52]. Interestingly, the aldose reductase inhibitor fidarestat enhances NK cell glycolysis by reducing the expression levels of aldose-keto reductase family 1 member B10 (AKR1B10), thereby inhibiting HCC cells growth [53]. In HCC, the proportion of regulatory T cells within the TME is reportedly increased [54]. These tumor-infiltrating Tregs typically exhibit increased surface expression of GLUT1 and glycolysis-related genes, leading to higher glucose uptake and enhanced aerobic glycolysis [55]. Interestingly, Tregs in high-glycolysis liver tumors display higher PD-1 expression compared to effector T cells. Under low-glucose conditions, Tregs actively uptake lactate via MCT1, promoting PD-1 expression, while effector T cells downregulate their programmed death 1 (PD-1) levels [56]. TAMs play a complex role in HCC, influencing immunosuppression, angiogenesis, tumor invasion and metastasis, metabolic support, and drug resistance [57]. In HCC, TAMs release exosomes containing lncMMPA, not cytokines or microRNAs, which upregulate GLUT1, HK2, and LDHA expression in HCC cells, ultimately increasing their aerobic glycolysis and proliferation [58]. Additionally, fibronectin-1 (FN-1) activates TLR4 signaling in macrophages, leading to increased PKM2 expression and subsequent production of IL-1 β , TNF- α , and

Table 1. Research direction of aerobic glycolysis.

Intervention means	Target	Role	Reference
BAY-876	GLUT1	antagonist of GLUT1 receptor	[61]
Benzamides and Rapafucins	GLUT1	inhibit the expression of GLUT1	[62]
WZB117	GLUT1	a GLUT1 inhibitor	[63]
Napabucasin	GLUT1	reduces the expression of CD47 and GLUT1	[64]
Sirolimus combined with Huai Er	PI3K/Akt/mTOR-HIF-1 α	inhibits the expression of LDHA and GLUT1	[65]
Down-regulation of BTF3	GLUT1	inhibits the expression of GLUT1	[66]
STF-31	GLUT1	directly binds to GLUT1	[67]
2-DG	HK2	inhibits the expression of HK2	[68]
3-BrPA	HK2	inhibits the expression of HK2	[69,70]
NaBu	HK2 and c-Myc	inhibits the expression of HK2	[71]
miR-202	HK2	inhibits the expression of HK2	[72]
Metformin	HIF-1 α /PFKFB3/PFK1	inhibit the expression of PFK1	[73]
Vitamin K ₃ and vitamin K ₅	PFK1 and HKs	inhibits the expression of HKs and PFK1	[74]
TRIM35	PKM2	blockades the phosphorylation levels of PKM2 Y105	[75]
SETD5	PKM2	inhibits the expression of PKM2	[76]
CaMKK β knockdown	PKM2 and PI3K/AKT	inhibits the expression of PKM2	[77]
TKP	PKM2	inhibits the expression of PKM2	[78]
MiR-342-3p	MCT1	inhibits the expression of MCT1	[79]
Syrosingopine	MCT1 and MCT4	inhibits the expression of MCT1 and MCT4	[80]
AZD3965	MCT1	inhibits the expression of MCT1	[81–83]
AR-C155858	MCT1	inhibits the expression of MCT1	[84]

Abbreviations: BTF3, basic transcription factor 3; 2-DG, 2-Deoxyglucose; 3-BrPA, 3-bromopyruvate; NaBu, sodium butyrate; TRIM35, Tripartite motif-containing protein 35; SETD5, SET domain-containing 5; CaMKK β , Calcium ions/calmodulins stimulate protein kinase kinases β ; TKP, protease from *Trichosanthes kirilowii*; c-Myc, *MYC* oncogene.

programmed death-ligand 1 (PD-L1), further contributing to immune suppression [59]. In conclusion, targeting immune cells within the TME and regulating their aerobic glycolysis presents a promising therapeutic strategy for HCC.

4. Research Direction of Aerobic Glycolysis

Unlike normal cells, tumor cells, including HCC cells, primarily rely on aerobic glycolysis for energy production. This phenomenon, known as the Warburg effect, allows HCC cells to thrive and proliferate even under hypoxic conditions [60]. Several key players are involved in this process: two transporter families (GLUTs, and MCTs) and three rate-limiting enzymes (HKs, PFKs, and PKs). In HCC, all of these exhibit varying degrees of upregulation, contributing to enhanced glycolysis. Targeting their overexpression through different mechanisms presents a promising strategy for inhibiting aerobic glycolysis and treating HCC, as summarized in Table 1 (Ref. [61–84]).

4.1 Targeting Glucose Transporter GLUT1

GLUT1, a crucial protein for glucose uptake in tumor cells, is upregulated in HCC [85]. BAY-876, a GLUT1 antagonist, significantly inhibited tumor growth in HCC models [61]. Direct treatment of HCC tumors with microcrystalline BAY-876 reduced glucose uptake by the cancer cells [61]. Meanwhile, several other strategies target GLUT1 to inhibit tumor growth. Benzamides and rapafucins can inter-

fere with GLUT1 expression, preventing or reducing tumor cell proliferation [62]. WZB117, another GLUT1 inhibitor, suppresses tumor growth in mouse models by downregulating GLUT1 [63]. Similarly, knocking down GLUT1 expression restrains aerobic glycolysis and inhibits prostate cancer growth [86]. Importantly, combining GLUT1 inhibitors with conventional therapies significantly enhances their efficacy [87–89]. Interestingly, signal transducer and activator of transcription 3 (STAT3) directly regulates the transcription of both CD47 and GLUT1 (SLC2A1). HCC cells and patients typically show high SLC2A1 expression, while SLC2A2 expression is comparatively low [90]. Napabucasin, a STAT3 inhibitor, reduces tumor growth by decreasing CD47 and GLUT1 expression in subcutaneous and orthotopic mouse models [64]. Additionally, combining sirolimus and Huai Er can inhibit aerobic glycolysis by downregulating LDHA and GLUT1 through reduced HIF-1 α expression in HCC [65]. Moreover, the protein RRAD promotes aerobic glycolysis by upregulating GLUT1, while RRAD knockout inhibits it [91]. Furthermore, basic transcription factor 3 (BTF3) expression is elevated in HCC and positively correlates with GLUT1 expression. Down-regulating BTF3 decreases GLUT1 protein levels in HCC cells [66]. Dehydroascorbic acid competes with glucose for GLUT1 usage in xenotransplantation models. Its entry into tumor cells via GLUT1 increases reactive oxygen species (ROS) and decreases glucose uptake, ultimately inhibiting

aerobic glycolysis and killing tumor cells [92]. Similar effects are observed with STF-31, a compound that directly binds to GLUT1 and reduces glucose uptake in renal cell carcinoma (RCC) without harming normal tissues [67]. In conclusion, current research on GLUT1 primarily focuses on inhibiting its expression or function to block glucose uptake in tumor cells, making it a promising target for HCC therapy.

4.2 Targeting Hexokinase HK2

HK2 inhibition offers a promising strategy for treating human liver cancer by hindering tumor cells aerobic glycolysis and inducing cell death [93]. 2-Deoxyglucose (2-DG) is a widely studied HK2 inhibitor that has been reported to inhibit glycolysis by inhibiting hexokinase [68]. 2-DG, similar to glucose, catalyzes the production of 2-deoxy-D-glucose-6-phosphate (2-DG-6P) through HK2, which inhibits the activity of HK2 and indirectly inhibits aerobic glycolysis, unlike G-6-P, which is produced by glucose [94]. In rat HCC models, 2-DG was shown to reduce PKM2 and LDHA expression, leading to decreased aerobic glycolysis and tumor cell death [95]. Combining 2-DG with sorafenib demonstrated superior antitumor effects compared to sorafenib alone, suggesting its potential for synergistic action with other anticancer drugs [96]. Besides, this combination synergistically inhibited the development of sorafenib-resistant HCC cells by targeting ATP production [97]. Mitochondrial fusion protein mitofusin-1 (MFN1) downregulation, linked to metastasis and poor HCC prognosis, is significantly inhibited by 2-DG treatment, demonstrating its potential in combating metastasis [98]. Moreover, 2-DG fusion nanoparticles such as (2-DG)-encapsulated poly (lactic-co-glycolic acid) (PLGA) nanoparticles (2DG-PLGA-NPs) exhibit potent antitumor effects by enhancing T cells transport, cytotoxicity, and antitumor immunity, leading to HCC growth suppression in mice [99]. 2-DG also demonstrates strong cytotoxicity in prostate cancer cell lines. Phase II clinical trials using 45 mg/kg of 2-DG showed promising results in inhibiting tumor cell metabolism and achieving antitumor effects in patients with prostate cancer and other solid tumors [100]. 3-bromopyruvate (3-BrPA), another inhibitor of HK2, is also often used to inhibit aerobic glycolysis [69]. The combination of 3-BrPA and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) was found to significantly reduce the production of ATP and the excretion of lactate in HCC cell lines, including SMMC-7721 and HepG2 cells [70]. Other studies have found that 3-BrPA can promote the apoptosis of Hep3B and SK-Hep1 cells by inducing endoplasmic reticulum stress [101]. Further study demonstrated that 3-BrPA inhibited hypoxia-induced mitochondrial stability and promoted tumor cell apoptosis, leading to its ability to inhibit tumor progression in an MH3-induced HCC mouse model [102]. Both 3-BrPA and sodium citrate (SCT) inhibited tumor progression in mice with transplanted tumors by in-

hibiting aerobic glycolysis and inducing tumor cells apoptosis [103]. 3-BrPA has shown strong anti-tumor effects by inhibiting aerobic glycolysis and reducing ATP production in other cancer treatments, including malignant peripheral nerve sheath tumors, pancreatic cancer, and breast cancer [104–106]. Similar conclusions support the view that HK2 knockdown, when combined with metformin or sorafenib, significantly increases HCC cell death and slows tumor growth compared to metformin or sorafenib alone [93]. Sodium butyrate (NaBu) has been reported to inhibit HK2 expression through the c-Myc signal pathway, reducing aerobic glycolysis of HCC cells and enhancing the anti-tumor effect of sorafenib [71]. Besides, miR-202 can suppress aerobic glycolysis and cell proliferation by regulating HK2 expression in HCC [72].

4.3 Targeting PFK1

PFK1 constitutes the majority of PFKs isoforms within the human liver [107]. HIF-1 α , a pivotal regulator of glycolytic metabolism, actively participates in HCC by modulating the expression of key glycolytic enzymes, including HK2, PFK1, PKM2, and LDHA [108]. Metformin has been demonstrated to suppress HCC cell proliferation through downregulation of the HIF-1 α /PFKFB3/PFK1 pathway, leading to reduced PFK1 expression and subsequent inhibition of glycolysis [73]. Interestingly, vitamins K₃ and K₅ exhibit potential as glycolytic enzyme inhibitors, targeting HKs and PFK1 during aerobic glycolysis in MCF-7 cells [74]. In addition, studies suggest that Tat-activating regulatory DNA-binding protein (TARDBP) downregulates the expression of the microRNA 520 (miR-520) family, while miR-520 itself inhibits PFKs expression, ultimately leading to aerobic glycolysis suppression [109]. Although current research on PFK1 in HCC remains limited, its irreplaceable role within glycolysis underscores its potential as a promising therapeutic target for future HCC treatment.

4.4 Targeting Pyruvate Kinase PKM2

The high expression of PKM2 in HCC tissues suggests a poor prognosis [110]. Some studies have shown that PKM2 knockdown suppresses the migration of HCC cells and prevents the development of tumors in HCC xenografted mice [111]. Both *in vivo* and *in vitro*, guanosine triphosphate binding protein 4 (GTPBP4) induces PKM2 expression by modifying protein ubiquitination, promoting the development of aerobic glycolysis and liver cancer [112]. Similarly, heat shock protein 90 (HSP90) binds to PKM2, increasing its abundance and enhancing the effect of aerobic glycolysis, thereby promoting HCC cells development [13]. Overwhelming evidence currently supports that inhibiting PKM2 expression is an effective treatment strategy for HCC. Tripartite motif-containing protein 35 (TRIM35) has been reported to block the Y105 phosphorylation of PKM2, thereby inhibiting aerobic glycol-

Table 2. Traditional Chinese Medicine of aerobic glycolysis.

Intervention means	Target	Role	Reference
DHA	GLUT1	inhibits the expression of YAP1 to reduce the expression levels of SLC2A1	[115]
Ginsenoside Rg3	PI3K/Akt/HK2	inhibits the expression of HK2	[116]
Ginsenoside CK	AKT/mTOR/c-Myc	inhibits the expression of HK2 and PKM2	[117]
QUE	AKT/mTOR	inhibits the expression of HK2	[118]
Astragaloside	miR-2b	inhibits the expression of HK2	[119]
Oroxylin A	HIF-1 α	inhibits the expression of PDK1, LDHA and HK2	[120]
Chrysin	HK2	inhibits the expression of HK2	[121]
Genistein	GLUT1 and HK2	inhibits the expression of HK2 and GLUT1	[122]
Rosmarinic acid	GLUT1 and HK2	inhibits the expression of HK2 and GLUT1	[123]
Dauricine	HK2 and PKM2	reduces the expression of HK2 and PKM2	[124]
Shikonin	PKM2	reduces the expression of PKM2	[112]
PB2	PKM2	reduces the expression of PKM2	[125]

Abbreviations: DHA, dihydroartemisinin; Ginsenoside CK, ginsenoside Compound K; QUE, Quercetin; PB2, proanthocyanidin B2.

ysis in HCC cells and achieving antitumor effects [75]. Similarly, down-regulation of SET domain-containing 5 (SETD5) suppressed PKM2 expression, inhibiting aerobic glycolysis in HCC cells and tumor growth in xenografted mice [76]. Additionally, calcium ions and calmodulins stimulate protein kinase kinases β (CaMKK β) knockdown, which inhibits PKM2 expression, restraining aerobic glycolysis and proliferation of HepG2 cells [77]. Furthermore, TKP, a serine protease from *Trichosanthes kirilowii*, inhibits aerobic glycolysis by reducing PKM2 expression, thus inhibiting the proliferation of Bel-7402 and HepG2 cells [78]. Therefore, down-regulating PKM2 expression remains one of the most promising avenues for HCC treatment.

4.5 Targeting Monocarboxylic Acid Transporter MCT1

It has been reported established that MCT1, a key transporter of lactate excreted from cells, significantly promotes tumor cell death when inhibited, causing lactate accumulation within the cells [37]. Additionally, autophagy induces MCT1 expression by activating the Wnt/ β -catenin pathway and promotes aerobic glycolysis in HCC cells [113]. Furthermore, CD147 enhances both MCT1 expression and lactate output, leading to the activation of the PI3K/Akt/MDM2 pathway, subsequently increasing p53 degradation [114]. Interestingly, up-regulating MCT1 expression can also enhance CD147 expression in hepatocellular carcinoma. MiR-342-3p directly targets MCT1 in HCC, exerting its antitumor effect by limiting lactate transport out of the cell [79]. Moreover, syrosingopine, a dual inhibitor of MCT1 and MCT4, combined with metformin, represents an effective anticancer strategy [80]. Notably, AZD3965, a specific MCT1 inhibitor, has already reached clinical trials for various cancers [81–83]. Similarly, AR-C155858, another MCT1 inhibitor, demonstrates potent anti-tumor effect in breast cancer models [84]. Therefore, the development of MCT1 inhibitors holds significant potential as a future treatment option for HCC.

5. Traditional Chinese Medicine and Aerobic Glycolysis

Recent studies have increasingly shown that traditional Chinese medicine monomers and their extracts can inhibit aerobic glycolysis and exhibit strong anti-tumor effects, as summarized in Table 2 (Ref. [112,115–125]). Notably, dihydroartemisinin (DHA) has emerged as a potential anti-tumor agent by directly targeting aerobic glycolysis metabolism. Several studies, including our previous work, have suggested that DHA inhibits tumor growth by targeting glycolysis metabolism. For example, we demonstrated that DHA suppresses the Warburg effect in HepG2215 cells and HCC mice by reducing SLC2A1 levels through YAP1 inhibition [115]. Similarly, DHA effectively suppressed aerobic glycolysis and ESCC progression by down-regulating PKM2 expression in esophageal squamous cell carcinoma (ESCC) and ESCC cells [126]. Furthermore, DHA inhibited leukemia cell K562 proliferation by suppressing GLUT1 and PKM2 levels, thereby regulating glucose uptake and inhibiting aerobic glycolysis [127]. In LNCaP cells, DHA reduced Akt/mTOR and HIF-1 α activity, leading to decreased expression of GLUT1, HK2, PKM2, and LDH and subsequent inhibition of aerobic glycolysis [128]. DHA was also found to inhibit the NF- κ B signaling pathway to prevent GLUT1 translocation to the plasma membrane, thereby inhibiting the progression of non-small-cell lung cancer (NSCLC) cells via targeting glucose metabolism [129]. Moreover, DHA combined with 2-DG can reportedly induce apoptosis in A549 and PC-9 cells [130].

Some studies have shown that ginsenoside Rg3 combined with sorafenib can significantly inhibit the PI3K/Akt signaling pathway and the expression of HK2 in HCC cells [116]. Similarly, ginsenoside Compound K (CK) effectively inhibits the AKT/mTOR/c-Myc pathway and HK2 and PKM2 expression, leading to aerobic glycolysis inhibition in HepG2 and Huh7 cells and inducing apoptosis [117]. Quercetin (QUE), a bioactive flavonoid, inhibits

tumor growth in HCC xenograft mice by suppressing the AKT/mTOR pathway and HK2 expression [118]. Recent studies have found that astragaloside suppresses HCC cell proliferation by upregulating miR-2b and inhibiting HK2 expression [119]. Additionally, oroxylin A inhibits HIF-1 α expression and the mRNA levels of its downstream molecules PDK1, LDHA, and HK2, thereby inhibiting aerobic glycolysis in HepG2 cells [120]. After chrysin intervention in an HCC mouse model, HK2 expression in tumor tissues was significantly decreased, and tumor progression was inhibited by aerobic glycolysis inhibition [121]. Other studies have shown that genistein and rosmarinic acid can also inhibit HK2 and GLUT1, thereby suppressing HCC cells aerobic glycolysis [122,123]. Dauricine, a natural alkaloid, reduces HK2 and PKM2 expression and enhances the therapeutic effects of cisplatin and sorafenib in HCC cells [124]. Shikonin, a specific PKM2 inhibitor, significantly inhibited tumor growth in HCC model mice by inhibiting aerobic glycolysis [112]. Additionally, PB2, a flavonoid proanthocyanidin B2, directly reduces lactate yield by inhibiting PKM2 expression. Furthermore, sorafenib combined with PB2 can effectively improve SORA chemosensitivity in xenograft mice injected with HCC-LM3 cells [125]. The above studies overlap in their assertion of the potential of TCM in targeting aerobic glycolysis for HCC treatment.

6. Conclusions

Aerobic glycolysis plays a pivotal role throughout HCC development, supplying a significant amount of ATP for both initiation and progression of the disease. By targeting key factors within this pathway, such as GLUT1, HK2, PFK1, PKM2, and MCT1, alongside their regulatory signaling pathways, we can effectively inhibit aerobic glycolysis and impede tumor growth. Consequently, transporters and key enzymes involved in aerobic glycolysis emerge as promising candidates for targeted drug development in tumor therapy.

Our comprehensive literature analysis suggests three highly promising research directions for HCC treatment. The tumor microenvironment plays a crucial role in tumorigenesis. As tumor cells rely on glycolysis for energy, they produce large amounts of lactate that acidify the TME, suppressing immune cell response and promoting tumor progression. Restoring this acidified TME to a normal pH and inhibiting lactate transporter MCT1 in tumor cells (preventing intracellular lactate from entering TME) represent two powerful treatment options. Additionally, inhibiting GLUT1 reduces glucose uptake, hindering glycolysis at its source and starving tumor cells of energy for growth. Finally, suppressing HK2 expression directly decreases the rate of aerobic glycolysis, inhibiting tumor cell growth and development. Collectively, these three approaches offer effective means to inhibit aerobic glycolysis and achieve successful HCC treatment.

While the potential of targeting aerobic glycolysis for HCC treatment shows promise, most supporting evidence currently comes from animal and cell experiments, with limited clinical data available. We acknowledge that our review primarily focused on preclinical studies and lacked in-depth analysis of clinical trials investigating aerobic glycolysis inhibition. Moving forward, we will concentrate on exploring the ongoing clinical advancements in this promising therapeutic approach. In-depth research and development of targeted aerobic glycolysis therapies hold the potential to offer new treatment options for HCC patients. Combining these novel drugs with existing chemotherapy regimens could further enhance therapeutic outcomes. However, it is crucial to consider that inhibiting aerobic glycolysis may also negatively impact the antitumor activity of immune cells. Therefore, significant research efforts are still warranted to optimize this approach and ensure its safe and effective application in HCC patients.

Author Contributions

YY, YGao, and YX performed the research, analyzed the literature data, and wrote the original draft. XS designed the research study, reviewed this paper, and supervised the progress of the research. JL and YGong analyzed the collected literature and made charts. YZ, DW, and ZL performed the acquisition and analysis of data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

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Conflict of Interest

The authors declare no conflict of interest.

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