Review

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Zizhuo Wang , Qing Li , Bin Liang *

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Review

Hypoxia as a Target for Combination with Transarterial Chemoembolization in Hepatocellular Carcinoma

Zizhuo Wang [1,†], Qing Li [2,†] and Bin Liang [1,*]

[1] Department of Radiology, Hubei Key Laboratory of Molecular Imaging, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Road, Wuhan 430022, China.
[2] Department of Radiology, The First Affiliated Hospital of Soochow University, Suzhou 215006, China.
* Correspondence: bliang@hust.edu.cn; Tel.: +86 27 85726432
† These authors contributed equally to this work.

Abstract: Hypoxia is a hallmark of solid tumors, including hepatocellular carcinoma (HCC). Hypoxia has proved to be involved in multiple tumor biological processes and associated with malignant progression and resistance to therapy. Transarterial chemoembolization (TACE) is a well-established locoregional therapy for patients with unresectable HCC. However, TACE-induced hypoxia regulates tumor angiogenesis, energy metabolism, epithelial-mesenchymal transition (EMT), and immune processes through hypoxia-inducible factor 1 (HIF-1), which may have adverse effects on the therapeutic efficacy of TACE. Hypoxia has emerged as a promising target for combination with TACE in treatment of HCC. This review summarizes impact of hypoxia on HCC tumor biology, adverse effects of TACE-induced hypoxia on its therapeutic efficacy, highlighting the therapeutic potential of hypoxia-targeted therapy in combination with TACE for HCC.

Keywords: hypoxia; tumor microenvironment; hypoxia-inducible factor; hepatocellular carcinoma; transarterial chemoembolization

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies and a leading cause of cancer deaths worldwide [1]. Based on the Barcelona-Clinic Liver Cancer (BCLC) staging system，HCC is divided into very early, early, intermediate, advanced and terminal stages. Accordingly, current treatment options for HCC include liver resection, liver transplantation, ablation, transarterial chemoembolization (TACE), systemic therapies, and best supportive care [2]. The former three therapies as potentially curative treatment are only suitable for the very early and early stages. However，the majority of HCC patients particularly in China are diagnosed at the intermediate or advanced stages, and the TACE and systemic therapies have been the mainstay of treatment for HCC [3,4].

TACE is a well-established locoregional therapy for patients with unresectable HCC. The concept of TACE is to induce a comprehensive effect of cytotoxicity and ischemia through the intraarterial infusion of chemotherapeutic agents followed by embolization of tumor-feeding arteries [5]. However, recent studies have shed light on the implications of TACE-induced hypoxia in liver tumors. Hypoxia, as an integral characteristic of solid tumors, has proved to be involved in multiple tumor biological processes and associated with malignant progression and resistance to conventional chemotherapy and radiotherapy [6]. Similar results were reported in HCC after TACE. The hypoxic microenvironment induced by TACE results in activation of hypoxia-inducible factors (HIFs) and overexpression of vascular endothelial growth factor (VEGF) in residual tumors [7–13]. Accordingly, anti-angiogenic therapy has been combined with TACE to treat HCC [14]. However, given the fact that angiogenesis is one of tumor biological responses to hypoxia, directly hypoxia-targeted therapy may represent a more effective strategy than anti-angiogenic therapy for HCC. In this review, we will

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describe the relationship between hypoxia and HCC, with a focus on the implications of TACE-induced hypoxia for efficacy and the therapeutic potential of the combination of TACE with hypoxia-targeted therapy against HCC.

2. The Role of Hypoxia in HCC

Hypoxia is one of the fundamental characteristics of the tumor microenvironment (TME) of the majority of substantial tumors, including HCC [15]. The activation of HIFs is one of the primary reasons cancer cells can thrive in hypoxic environments [16]. Within the HIF family, HIF-1 is the most active in regulating the vast majority of hypoxic adaptive changes in tumors [17]. HIF-1 is a heterodimeric protein composed of two subunits: HIF-1α and HIF-1β. Under normoxic conditions, HIF-1α is degraded, maintaining basal levels. However, hypoxia inhibits proteasomal degradation and hydroxylation of HIF-1α. This stabilization allows them to translocate into the nucleus, where they dimerize with a constitutive β subunit to form functional transcription factors under hypoxia [16,18]. Then, HIFs promote cancer progression by affecting various factors such as angiogenesis, extracellular matrix (ECM) remodeling, metastasis, immune evasion and suppression, and aberrant glucose metabolism [16]. The key downstream proteins and cells involved in this process include VEGF, transforming growth factor (TGF-β), N-cadherin, vimentin, Snail, programmed death-ligand 1 (PD-L1), matrix metalloproteinases (MMPs), cellular Myc (c-Myc), P53, and cancer stem cells (CSCs). As one of the most hypoxic malignancies, increasing evidence suggests that hypoxia influences the biological processes and treatment outcomes of HCC [19].

2.1. Hypoxia and Hepatocarcinogenesis

Hypoxia is intimately involved in the process of hepatocarcinogenesis, including the progression from hepatitis to cirrhosis and eventually to liver cancer. The fibrosis resulting from chronic hepatitis and cirrhosis disrupts the normal vascular system, leading to a reduction in hepatic blood supply, consequently inducing hypoxia. Furthermore, exposure of vascular endothelial cells in tumor or precancerous tissues to various high-risk oncogenic factors results in the alteration of endothelial cell morphology and subsequent dysfunction of tumor vasculature, thereby causing hypoxia. Additionally, the rapid proliferation and high metabolism of cancer cells lead to an energy demand that exceeds oxygen supply, exacerbating hypoxia [20].

Conversely, this hypoxia also contributes to hepatocarcinogenesis and progression. Mechanistically, the hypoxic environment fosters genetic instability and the emergence of aggressive cancer cell variants, in which somatic mutations are the most common [21]. First, DNA strand breaks, including both double-strand breaks (DSBs) and single-strand breaks (SSBs), occur during hypoxia and can lead to gene mutations [22]. Furthermore, hypoxia can affect DNA damage repair checkpoints, including ataxia telangiectasia mutated (ATM) and RAD3-related (ATR) checkpoints, promoting cancer formation [23]. Likewise, the mismatch repair (MMR) pathway becomes dysregulated under hypoxia, potentially leading to carcinogenesis [24]. Another, hypoxic conditions can enhance the efficiency of induced pluripotent stem cell (iPSC) generation, and maintain stem cell self-renewal by facilitating the reprogramming process, thus increasing the likelihood of cellular carcinogenesis [25]. These processes contribute to the formation of cancer cell variants with metastatic potential and proliferative capacity.

2.2. Hypoxia and Angiogenesis

Hypoxia and angiogenesis are closely related processes in the context of cancer development and progression. Under hypoxia, heightened levels of HIF-1α and HIF-2α prompt an increase in angiogenic factor production. Of these, VEGF, as the primary signaling molecule in angiogenesis, ultimately triggers tumor angiogenesis [26]. Additionally, various other proangiogenic factors which are mediated by HIFs also stimulate angiogenesis, such as insulin-like growth factor-2, angiopoietin 2 (ANG2), bone morphogenetic protein (BMP4), basic fibroblast growth factor (BFGF), platelet-derived growth factor (PDGF), placental growth factor (PGF), TGF-β, erythropoietin (EPO),

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plasminogen activator inhibitor-1 (PAI-1), stromal-derived factor 1 (SDF-1), myeloid-derived growth factor (MYDGF), and angiopoietins [27,28]. However, the newly formed blood vessels in tumors are often disorganized and distorted, failing to alleviate the hypoxic environment.

2.3. Hypoxia and Metastasis

Hypoxia, via the HIF-1 pathway, regulates gene expression of effector molecules involved in tumor infiltration and metastasis, thereby promoting these processes. Tumor infiltration is essential for metastasis, both being integral to tumor spread. The first step in tumor cell invasion and metastasis is the epithelial-mesenchymal transition (EMT) [29,30]. EMT is a process where epithelial cells transform into mesenchymal cells with enhanced migratory ability, regulated by HIF-1α and cyclooxygenase-2 (COX-2). The inactivation of epithelial (E)-cadherin, a key cell adhesion protein, weakens cell-cell contacts and increases mobility, thus initiating EMT. Activation of HIF-1 has been shown to downregulate epithelial cadherin (E-cadherin) expression, resulting in weakened interconnections and cancer cell detachment. Additionally, HIF-1 upregulates integrin gene expression, promoting cancer cell adhesion to the extracellular matrix. Furthermore, HIF-1 stimulates cancer cells to release matrix metalloproteinase 2 (MMP2) and urokinase plasminogen activator (uPAR), hastening extracellular matrix degradation within tumor sites by upregulating gene expression. Moreover, HIF-1 triggers the production of autocrine motility factors (AMF), like hepatocyte growth factor (HGF), to enhance tumor cell migration.

Besides, under hypoxia, HIFs transactivate key markers that activate multiple signaling pathways promoting metastasis. These pathways include TGF-β, Wnt/β-Catenin, Notch, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), reactive oxygen species (ROS), and basic fibroblast growth factor-related signaling [31–39].

2.4. Hypoxia and Resistance to Radiotherapy and Chemotherapy

Hypoxic TME remains a significant factor contributing to resistance against radiotherapy and chemotherapy [40]. Cancer cells often outgrow their blood supply, creating hypoxic and necrotic areas more than 100 μm from blood vessels. These areas are more resistant to radiotherapy as it relies on oxygen to generate free radicals that kill cancer cells [20]. Hypoxia induces cell cycle arrest, significantly slowing or halting cell proliferation [18]. Thus, most chemotherapeutic agents, which target actively dividing cells, are less effective against hypoxic cells. Moreover, in HCC, the expression of multidrug resistance-related genes, including multidrug resistance gene 1 (mdr1), P-glycoprotein (a key multidrug resistance transporter), and lung resistance protein, is regulated by hypoxia [41,42]. Additionally, hypoxia also fosters resistant to p53-mediated apoptosis to reduce the efficacy of chemotherapy [43]. Finally, The expression of HIF is significantly upregulated following radiation or chemotherapy, suggesting that HIF pathways actively contribute to radiotherapy and chemotherapy resistance [44].

2.5. Hypoxia and Immune Suppression

Tumor hypoxia is one of the most critical factors influencing the immune response. Hypoxia regulates the tumor immune microenvironment (TIME) in at least three major ways [45] to prompt immune suppression. First, hypoxia affects TIME by hindering the function or infiltration of immune cells, including macrophage-mediated T-cell suppression regulated by HIF-1α [46]. Second, hypoxia recruits immunosuppressive cells such as tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), natural killer (NK) cells, tumor-associated fibroblasts (CAFs), and cytotoxic T lymphocytes (CTLs) [47,48] to block the immune response. Third, hypoxia upregulates regulatory molecules, which further prevents the activation of immune effector cells, including triggering receptor-1 (TREM-1) expressed on myeloid cells is upregulated by HIF-1α in TAMs, which increases cytotoxic CD8+ T cell death [46], impairs cellular activity, and delays or prevents apoptosis of HCC cells.

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In antitumor immunotherapy, hypoxia contributes to drug resistance by inducing membrane proteins that increase drug efflux and by regulating key signaling pathways. MDSCs and HIF-1α can also modify the expression of immune checkpoint ligands PD-L1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) [49]. Thus, numerous studies have shown that hypoxia and HIFs enable tumor cells to evade the immune response.

2.6. Hypoxia and Abnormal Glucose Metabolism

Hypoxia significantly influences abnormal glucose metabolism in cancer cells, promoting the Warburg effect, where cells favor glycolysis over mitochondrial ATP synthesis even in the presence of oxygen [50]. HIFs are the main transcription factors involved in cell adaptation to hypoxia [51], driving this metabolic shift by upregulating glycolytic enzymes such as hexokinase 2 (HK2), lactate dehydrogenase A (LDHA), aldolase A (ALDOA), phosphoglycerate kinase 1 (PGK1), and pyruvate kinase M2 (PKM2). HK2, the initial rate-limiting enzyme of glycolysis, is an isoform of hexokinase that is overexpressed in HCC. HK2 is integral to the compensatory pathway of ATP generation, supplying energy to hypoxic cells [52]. In addition, HIFs increase pyruvate dehydrogenase kinase 1 (PDK1) and lactate dehydrogenase A (LDHA) to shift metabolism from oxidative phosphorylation to glycolysis [53]. In hypoxia, HIF-1α and fat-mass and obesity-associated protein (FTO) mediate N6-methyladenosine (m6A) modifications, boosting ALDOA expression to promote glycolysis [54].

Hypoxia triggers the activation of genes and miRs to maintain metabolic homeostasis via HIF-mediated transcriptional induction [55]. HIF-1 increases the expression of solute carrier family 2 member 1 (SLC2A1) and solute carrier family 2 member 3 (SLC2A3), which encode glucose transporters glucose transporter 1 (GLUT1) and glucose transporter 3 (GLUT3), respectively, enhancing glucose uptake [53]. The transcriptional coactivator ALL1-fused gene from chromosome 9 protein (AF9), eukaryotic translation initiation factor 3 subunit A (eIF3a), and membrane-associated protein 17 (MAP17) further support glycolysis and the Warburg effect [56–58]. Additionally, the hepatitis B virus X-interacting protein (HBXIP)/HIF-1α/methyltransferase-like 3 (METTL3) axis [59], long non-coding RNA RAET1K (lncRNA-RAET1K)/miR-100-5p/HIF-1α axis [60] and HOTAIR/miR-130a-3p/HIF-1α axis [61] have been found to be associated with abnormal glucose metabolism and may serve as therapeutic targets in HCC.

3. TACE of HCC

TACE is the recommended first-line therapy for patients with intermediate-stage disease, and is, by far, the most common technique used to treat unresectable HCC [2,5]. The rationale for TACE comes from previous findings that normal hepatic tissue receives most of its blood supply from the portal vein, whereas hepatic malignancies receive most of their blood supply from the hepatic artery [62]. Therefore, it is reasonable to employ the hepatic artery as an approach to target the tumor while preserving the normal liver tissue. TACE treatment consists of transcatheter intraarterial delivery of chemotherapy combined with embolization of the tumor-feeding arteries, which will result in the comprehensive effects of cytotoxicity and ischemia against the tumor [5].

TACE can be technically divided into conventional TACE and drug-eluting beads TACE (DEB-TACE). The conventional TACE involves infusion of single or multiple chemotherapeutic agents with or without ethiodized oil followed by embolization with particles such as gelatin sponge, polyvinyl alcohol, or calibrated microspheres [63]. In this way, high concentrations of chemotherapeutic agents can be directly delivered to the tumor bed. Additionally, embolization of the tumor-feeding arteries can not only induce tumor ischemic and hypoxic necrosis, but also enhance the cytotoxic effect of chemotherapy by reducing drug washout, prolonging interaction time between drugs and tumor cells, improving drug penetration within the tumor [64]. Previous two randomized controlled trials demonstrated conventional TACE resulted in higher overall survival compared to best supportive care [65,66]. The therapeutic efficacy of conventional TACE on HCC has been confirmed by subsequent clinical studies [67].

In the contrast, the DEB-TACE is defined as administration of calibrated microspheres onto which chemotherapeutic medication is loaded or adsorbed with the intention of sustained in vivo

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drug release [68]. Drug-eluting microspheres have the ability to load chemotherapeutic agents (e.g., doxorubicin, epirubicin and idarubicin) and release them in a controlled and sustained mode. Accordingly, DEB-TACE allows higher doses of chemotherapy with lower systemic exposure, along with permanent embolization. In a randomized phase II trial, DEB-TACE yielded improved radiologic tumor response and toxicity compared to conventional TACE [69]. However, the superiority of DEB-TACE over conventional TACE has never been demonstrated in terms of overall survival (OS) in clinical trials [70–73]. Therefore, there is currently insufficient evidence to recommend DEB-TACE over conventional TACE. Developing novel microspheres with superior drug loading mechanisms and loading other drugs (e.g., drugs targeting hypoxic cells) or formulations (e.g., oncolytic viruses and immunostimulants), will be the focus of future research in the DEB-TACE field.

4. TAE-induced Hypoxia and Its Implications for Tumor Biological Processes

Transarterial embolization (TAE) is an important component of TACE procedures. However, recent studies have raised question concerning the precise effect of embolization on liver tumors. Research has found that diversity in the degree and duration of hypoxia may have different effects on tumor cells. Severe or sustained hypoxia induces cell death, whereas mild or transient hypoxia may lead to a series of adaptive responses in tumor cells, such as activating signaling pathways that regulate cell survival, glucose metabolism conversion, angiogenesis, infiltration and metastasis, and drug resistance, thereby allowing the tumor cells to survival or even evolution [74]. Theoretically, embolization of tumor-feeding artery results in ischemia of the tumor and subsequent tumor necrosis. Unfortunately, due to the complexity of blood supply for HCC and the limitations of interventional embolization techniques, embolization of the tumor-feeding vessels may be incomplete. This may result in part of the tumor continuing to survive and even evolve in a hypoxic microenvironment because of the reduced blood supply.

Central to these processes is the role of HIF-1α. Previous research has indicated a notable increase in HIF-1α, VEGF, hexokinase II, COX-2 and PD-L1 levels in tumors following TAE [7,13,75–78]. In two previous animal studies, elevated expression of HIF-1α was reported in liver tumors after TAE [75,79]. Subsequent animal study used a modified Clark-type microelectrode research system to measure pO2 and found that TAE rapidly reduced the tumor oxygenation [8]. The study also found the positive HIF-1α staining was detected predominately in viable tumor cells in the tumor peripheral zone, which displayed a distribution pattern similar to that observed in hypoxic arears marked by pimonidazole [8] These studies suggest that TAE of liver tumors resulted in HIF-1α overexpression as a result of intratumoral hypoxia generated by the procedure.

Previous two clinical studies measured VEGF levels in serum and plasma in HCC patients and found that the VEGF levels increased significantly after TAE [9,10]. Similar results were observed in another clinical study comparing VEGF expression in tumor specimens between HCC patients pretreated with TACE and without TACE [11]. Subsequent animal studies confirmed that TAE-induced hypoxia resulted in increased VEGF expression, promoting neovascularization of residual tumors [12,13]. Another initial clinical study found that expression of hexokinase II mRNA was increased in tumor tissue in some HCC patients pretreated with TAE, and the hexokinase II mRNA expression was significantly correlated with HIF-1α protein expression. In addition, both HIF-1α and hexokinase II protein expressions were co-localized in the cancer cells adjacent to necrotic areas. This study suggest that HCC may switch the metabolic profile to glycolysis through HIF-1α [7]. In addition, a recent clinical study demonstrated upregulation of HIF-1α and COX-2 proteins together with epithelial-to-mesenchymal transition (EMT) alteration in HCC tissues following TACE treatment, which was associated with a negative correlation to overall survival [77]. Also, a preclinical study demonstrated that hypoxia selectively upregulated programmed death-ligand 1 (PD-L1) on myeloid-derived suppressor cells (MDSCs) via HIF-1α. Blocking PD-L1 under hypoxia enhanced MDSC-mediated T cell activation by modulating MDSCs cytokine production IL-6 and IL-10 [78]. Taken together, these results suggest that hypoxia after TAE of liver tumors is involved in tumor

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angiogenesis, energy metabolism, EMT and immune processes, which may have adverse effects on the therapeutic efficacy of TAE.

5. Hypoxia-Targeted Therapy for HCC

Tumor hypoxia has emerged as an attractive therapeutic area due to its essential role for cancer. Based on the therapeutic mechanism of action, four general strategies were developed in the past 20 years.

5.1. Targeting HIF and HIF-Related Hypoxia Signaling

HIF-1α/HIF-2α inhibitors can be categorized as indirect or direct. Indirect HIF inhibitors regulate upstream and downstream effectors in the HIF pathway, while direct inhibitors decrease HIF mRNA expression, protein synthesis, or DNA binding. Several recent HIF inhibitors that show promising potential in treating HCC, such as RO7070179 (EZN-2968) [80], CT-707 [81], PT-2385 [82], meloxicam [83], various natural agents [84–88] (e.g., camptothecin analogs, curcumin, sanguinarine, resveratrol, ginsenosides).

5.2. Prodrugs Activated by Hypoxia

Hypoxia-activated prodrugs (HAPs), which are inactive compounds that are converted into active drugs via enzymatic or metabolic processes under hypoxic conditions especially hypoxic tumor cells within the body [89]. HAPs may be able to bypass drug resistance mechanisms that are commonly associated with traditional chemotherapy while minimizing damage to healthy tissue [89,90]. Several bioreductive prodrugs that have reached clinical trials for thetreatment of HCC, such as tirapazamine (TPZ) [91], TH-302 (evofosfamide) [92], CEN-209 (SN30000), Myo-inositol trispyrophosphate (ITPP) [93], PR-104 [94].

5.3. Hypoxia-Selective Gene Therapy

Antisense gene therapy targeting HIF-1, as one of the gene therapies, refers to constructing recombinant plasmids of antisense HIF-1α, transferring these plasmids into hypoxic cells, and transcribing antisense RNA to exert the inhibitory effect of HIF-1α [95]. Oncolytic adenovirus (Ovs) that target hypoxic tumors selectively infects tumor cells by utilizing internal gene mutation or metabolic reprogramming of tumor cells, and then replicate in tumor cells to kill target cells, or indirectly kill tumors by stimulating the immune system's antitumor response [96,97]. In addition, genetic engineering approaches can be used to construct high-affinity NK (haNK) cells which can improve tolerance against acute hypoxia, and maintain the functions of NK cells to kill cancer cells [98]. Hypoxia-directed enzyme prodrug gene therapy is using anaerobes to transfer functional genes to the tumor hypoxia zone [99]. However, current studies have shown that the HIF specific strategy of using hypoxia-selective gene therapy is controversial.

5.4. Target Other Hypoxia-Associated Biological Processes and Pathways

Target other hypoxia-associated biological processes and pathways such as PI3K/Akt/mTOR axis [100], VEGF [101], biotherapy targeting anoxic tumor bacteria [102,103], targeting hypoxia-associated metabolic dysregulation [104,105].

6. Combination of Hypoxia-Targeted Therapy and TACE for HCC

While hypoxia-targeted treatment has demonstrated potential in vitro studies, its effectiveness in clinical trials is frequently constrained. The researchers suggest that this limitation may stem from the heterogeneity in tumor types and levels of hypoxia within the tumor. It is hypothesized that combining hypoxic tumor-targeting drugs with interventional embolization could lead to mutually beneficial and synergistic anti-tumor outcomes in the treatment of liver cancer. This section aims to summarize the available drugs or pathways that target hypoxic tumor cells in combination with

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TACE for the treatment of HCC. The combination of hypoxia-targeted therapy and TACE for HCC are summarized in Tables 1–4.

6.1. Combination of HIF-Related Pathway Inhibitors and TACE

6.1.1. HIF-1

Melatonin is an endogenous hormone secreted by the pineal gland [106]. The anti-tumor mechanism of melatonin is primarily to inhibit tumor angiogenesis by inhibiting the HIF-1α/VEGF signaling pathway [107,108], and also to inhibit tumor metastasis by targeting MMP-2 and MMP-9 to reduce the permeability of the vessel wall [109,110]. As early as 2002, TACE in combination with melatonin (MLT) was used to treat patients with advanced primary HCC [111]. The results demonstrated that MLT reduced hepatic impairment following TACE and enhanced immune activity of patients. Recently, melatonin was loaded on a temperature-sensitive nano gel, p(N-isopropyl-acrylamide-co-butyl methylacrylate) (PIB-M), for tumor embolism in VX2 rabbit models by Chen et al.[112]. The results of the study confirm that melatonin can inhibit the growth and migration of HepG2 and LM3 cells by targeting HIF-1α, MMP-2, MMP-9, and E-cadherin in vitro. Furthermore, the concentration of melatonin in the tumor after embolization remains at a high level for the following three days, which suggests that this sustained effect on the tumor cells and TME may be achieved.

A number of natural compounds have been demonstrated to inhibit HIF-1α and VEGF. The combination with TACE has been shown to have promising evidence of targeting hypoxia, indicating potential for clinical translation in the treatment of hepatocellular carcinoma. 10-Hydroxycamptothecin (HCPT), a camptothecin analog, is a naturally occurring alkaloid with antitumor activity. Camptothecin analogs can mediate S-phase cytotoxic effects, through the induction of a stable DNA-Topo I complex which can lead to DNA linkage breaks. The ability to inhibit HIF-1 has also been found. Our preliminary research [113] found that the levels of HIF-1, VEGF, and microvessel density were comparable following intrahepatic arterial infusion of distilled water and HCPT+TACE ($P > .05$), indicating that HCPT significantly inhibits the expression of HIF-1 and angiogenesis in postembolization hepatic tumors. This finding suggests that HCPT may have a broader application in this clinical setting. Curcumin is a highly polyphenolic molecule that has been reported to inhibit the viability of cancer cells and is commonly used to prevent or treat a variety of diseases. Dai et al. [114] observed that the levels of tumor HIF-1α, VEGF, and MVD were significantly reduced in the liposomal curcumin combined with TAE in their initial study. Further investigations [115] revealed that, in addition to the aforementioned findings, curcumin liposome was capable of inhibiting survivin levels, and significantly inhibited cell viability and promoted apoptosis in the G1 phase by regulating apoptosis-related molecules. The ginsenoside Rg3 was found to inhibit the nuclear localization of HIF-1α by binding to HIF-1α [116]. Ginsenoside Rg3 in combination with TAE has been shown to significantly decrease CD31, VEGF expression and levels of the anti-apoptotic Bcl-2 at both mRNA and protein levels, while significantly increase pro-apoptotic gene caspase-3 and Bax expression in the VX2 rabbit tumor models [117]. A subsequent prospective controlled clinical trial [118] demonstrated that the combination of ginsenoside Rg3 and TACE provided a greater survival benefit than TACE alone in patients with HCC. Additionally, the trial indicated that Rg3 alleviated some of the adverse syndromes and blood anomalies associated with TACE.

Several other anticancer drugs have also been found to improve the efficacy of TACE by influencing hypoxia signaling. The antitumor effects of arsenic trioxide (ATO)-loaded CSM-TACE have been investigated in VX2 HCC rabbit models. The results indicated that the expression of twist, N-calmodulin, waveform protein, and MMP-9 was decreased in the combined treatment group, while the expression of E-calmodulin was increased [119]. In models of VX2 liver xenograft tumor, the combination of rapamycin with TACE has also been demonstrated to exhibit anti-tumor neovascularization activity and to inhibit the expression levels of NOS, HIF-1a, VEGF, Bcl-2, and Bax. Arterial infusion of rapamycin was found to be more effective than intravenous injection, and large doses were observed to present better efficacy [120]. In addition, AMD3100, chemokine (C-X-C motif)

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receptor 4 (CXCR4) antagonist, was shown to enhance therapeutic efficacy of TACE (doxorubicinlipiodol emulsion) in rats with HCC. AMD3100 was found to reduce TACE-induced MVD in HCC tissues by decreasing the expression of HIF-1α and VEGF. Furthermore, it has been shown to promote apoptosis and reduce cell proliferation in HCC [121]. Repeated TACE-induced hepatic hypoxia was found to exacerbate the progression of fibrosis in the peritumoral liver tissue, which was associated with increased expression of carbon tetrachloride, HIF-1α, TGF-β-1, and VEGF. The progression of fibrosis and the deterioration of liver function subsequent to TACE may be mitigated and slowed by the HIF-1α inhibitor LW6 [122].

6.1.2. HIF-2

In the context of HIF, HIF-1α is primarily implicated in the acute hypoxic process of the tumor. In contrast, HIF-2α is actively involved in the chronic hypoxic process and exhibits a stronger prognostic correlation with TACE relative to HIF-1α [123]. Furthermore, it has been demonstrated that sorafenib downregulates HIF-1α expression, shifting the hypoxic response from HIF-1α- to HIF-2α-dependent pathway. This results in the up-regulation of HIF-2α, which renders hypoxic HCC cells insensitive to sorafenib and induces the expression of VEGF and cyclin D1 [124]. The role of HIF-2 may be underestimated, which may explain why trials of HIF-2 inhibitors in combination with TACE are relatively rare. A recent study [125] utilized multifunctional polyvinyl alcohol (PVA)/hyaluronic acid (HA)-based microspheres loaded with doxorubicin (DOX) and PT-2385, a potent HIF-2α inhibitor, to improve the treatment of HCC. The results showed that PT/DOX-MS can block tumor cells in the G2/M phase. The introduction of PT-2385 effectively suppresses the expression levels of HIF-2α in hypoxic HCC cells, thereby downregulating the expression levels of Cyclin D1, VEGF, and TGF-α. Additionally, the combination of DOX and PT-2385 can jointly inhibit the expression of VEGF. This suggests that HIF-2α may be an ideal target for TACE therapy.

Table 1. Summarize of the therapeutic strategies of TACE combined with HIF-related pathway inhibitors for HCC.

Item	Year	Refs.	Mechanism	Targets affected	Cancer hallmark affected
HIF-1	2023	[112]	Melatonin could inhibit tumor cell proliferation and migration by targeting HIF-1α and VEGF-A.	HIF-1α	↓ HIF-1α ↓ VEGF-A ↓ MMP-2 ↓ MMP-9 ↑ E-cadherin
	2020	[119]	HIF-1α, VEGF and microvessel density were decreased by the CSM-ATO.	HIF-1α	↓ HIF-1α ↓ VEGF ↓ MVD ↓ Twist ↓ N-cadherin ↓ Vimentin ↓ MMP-9 ↑ E-cadherin

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2021	[120]	Arterial instillation of rapamycin+TACE in treatment of rabbit hepatic xenograft tumors could reduce tumor neovascularization and inhibit iNOS, HIF-1α, VEGF, Bcl-2 protein expression.	HIF-1α	↓ iNOS ↓ HIF-1α ↓ VEGF ↓ Bcl-2
2022	[121]	CXCR4 antagonist AMD3100 enhanced therapeutic efficacy of TACE in rats with HCC via promoting the HCC cell apoptosis, reducing cell proliferation, and inhibiting MVD.	HIF-1α CXCR4	↓ HIF-1α ↓ VEGF ↓ CXCR4 ↓ MVD ↓ Proliferation ↑ Apoptosis
2015	[122]	HIF-1α inhibitor LW6 attenuated the hypoxia-induced fibrosis progression in vivo. HIF-1α by HIF-1α-siRNA significantly decreased expression of TGF-β1 and VEGF in hypoxic hepatocytes.	HIF-1α	↓ HIF-1α ↓ VEGF ↓ TGF-β1 ↓ Collagen I ↓ α-SMA ↓ Fibrosis
2010	[113]	10-hydroxycamptothecin is a HIF-1α inhibitor.	HIF-1α	↓ HIF-1α ↓ VEGF ↓ MVD
2015	[114]	Liposomal curcumin could block HIF-1α-mediated angiogenesis.	HIF-1α	↓ HIF-1α ↓ VEGF ↓ MVD
2019	[115]	Curcumin liposome suppressed the HIF-1α and survivin levels and inhibited the angiogenesis in VX2 rabbits after TAE.	HIF-1α	↓ HIF-1α ↓ VEGF ↓ MVD ↓ Survivin ↓ Proliferation ↑ Apoptosis
2013	[117]	Ginsenoside Rg3 combined with TAE could effectively inhibit tumor growth by inhibiting tumor angiogenesis and inducing cancer cell apoptosis.	VEGF	↓ VEGF ↓ CD31 ↓ Angiogenesis ↑ Caspase-3 ↑ Bax

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	2016	[118]	The combination of ginsenoside Rg3 and TACE provided a greater survival benefit than TACE alone in patients with HCC.	VEGF	↑ Overall survival ↑ Time to progression ↑ Time to untreatable progression ↑ Disease control rate
HIF-2	2022	[125]	PT-2385 could effectively inhibit the expression level of HIF-2α in hypoxic HCC cells, thereby down-regulating the expression levels of Cyclin D1, VEGF and TGF-α.	HIF-2α	↓ HIF-2α ↓ VEGF ↓ TGF-α ↓ Cyclin D1

6.2. Combination of Hypoxia-Activated Prodrugs and TACE

6.2.1. Tirapazamine

Tirapazamine (TPZ), a bioreductive agent, is preferentially toxic to hypoxic cells. Under hypoxic conditions, TPZ is metabolized by an intracellular reductase to form a highly reactive radical species capable of inducing DNA single- and double-strand breaks and chromosome aberrations, resulting in cell death. In the presence of oxygen, the TPZ radical is oxidized back to the parent molecule, thereby largely preventing radical-induced damage [126]. In 2011, Sonoda et al. [127] found that the combination of intraperitoneal TPZ and TAE with gelatin microspheres significantly reduced the tumor growth rate compared with TAE or TPZ treatment alone in the rabbit VX2 model. Subsequently, another study by Lin et al. [128] reported that the combination of intravenous TPZ and hepatic arterial ligation had synergistic tumor-killing activity against hepatocellular carcinoma (HCC) in HBx transgenic mice. The safety findings of the toxicological study by Liu et al. [129] involving rats supported the clinical usage of intraarterial injection of TPZ in combination with embolization. Several follow-ups Phase I trials [130,131] demonstrated the safety and tolerability of intraarterial TPZ with TAE/TACE for HCC, yielding promising tumor responses. Li et al. [8] prepared TPZ-loaded CalliSpheres microspheres (CSMTPZs) and found that CSMTPZ therapy exhibited advantages in terms of hypoxia-selected cytotoxicity, tumor apoptosis and necrosis, animal survival, and safety over the conventional combination of TPZ and TAE in the rabbit VX2 model.

6.2.2. TH-302

TH-302 (evofosfamide) is a 2-nitroimidazole triggered HAP of the cytotoxin bromo-isophosphoramide mustard [132]. The dinitroimidazole structure will be fragmented with an alkylating agent dibromoisophosphoramide mustard that selectively binds to the DNA and kills the tumor cells. Thus, it exerts little activity in the normoxic zone and fewer side effects on the normal tissues. TH-302 has shown broad-spectrum anticancer efficacy in multiple human cell lines and xenograft models [92,133–137]. In the rabbit VX2 model, Duran et al. [138] combined the cTACE and TH-302 by mixing the doxorubicin/Lipiodol emulsion and TH-302 followed by embolization with 100-300 μm bland beads. The results indicate that cTACE+TH-302 induced smaller tumor volumes, lower tumor growth rates, higher necrotic fractions, and exhibited no additional toxicity profile compared to cTACE. Another rabbit VX2 model by Ma et al. [139] involved the preparation of TH-302 loaded poly (lactic-co-glycolic acid) (PLGA)-based TACE microspheres. The results demonstrated that the TH-302 loaded microspheres exhibited sustained drug release in the liver

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tissue and superior anti-tumor efficacy in comparison to TH-302 injection and TH-302+lipiodol. Furthermore, no significant toxicity was observed throughout the treatment period.

Table 2. Summarize of the therapeutic strategies of TACE combined with hypoxia-activated prodrugs for HCC.

Item	Year	Refs.	Mechanism	Targets affected	Cancer hallmark affected
Tirapazamine	2011	[127]	The combination of TPZ i.p. and GMS i.a. enhanced the antitumor effect of TPZ.	Hypoxic tumor	↓ Tumor growth
	2016	[128]	At levels below the threshold oxygen levels created by HAL, TPZ was activated and killed the hypoxic cells, but spared the normoxic cells.	Hypoxic tumor	↑ Necrosis ↑ Apoptosis
	2021	[129]	The safety findings of this toxicological study involving rats supported the clinical usage of IA injection of TPZ in combination with embolization.	Hypoxic tumor	ALT Total bilirubin Histopathology
	2021	[131]	TPZ may be synergistic with TAE.	Hypoxic tumor	Tumor responses were evaluated using mRECIST criteria
	2022	[130]	TPZ i.a., in combination with TAE, was well tolerated and showed promising efficacy signals in intermediate-stage HCC.	Hypoxic tumor	Using the modified Response Evaluation Criteria in Solid Tumors
	2022	[8]	TPZ may exert synergistic tumor-killing activity with TAE for liver cancer.	Hypoxic tumor	↑ Necrosis ↑ Apoptosis
TH302	2017	[138]	Evofosfamide in combination with cTACE enhanced anticancer effects.	Hypoxic tumor	↓ Ki-67 ↑ γ-H2A.X ↑ annexin V ↑ caspase-3 ↑ Apoptosis
	2020	[139]	TH-302 is a hypoxia-activated prodrug targeting the intra-tumoral hypoxic environment.	Hypoxic tumor	↑ Necrosis ↑ Apoptosis

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6.3. Combination of Gene Therapy and TACE

6.3.1. Hypoxia Pathway-Related Gene Therapy

RNA modulation is a common modality for HIF-related gene therapy. Previous studies have proposed the use of RNA interference (RNAi) of HIF-1α to enhance the efficacy of TAE in the treatment of HCC. RNAi is a process whereby the expression of specific genes is silenced by the introduction of small interfering RNAs (siRNAs), endogenous miRs, and other short double-stranded RNAs [140]. The study by Chen et al. [141] has confirmed that RNAi of HIF-1α improves the efficacy of TAE in the treatment of HCC. Its resulting HIF-1α silencing effectively inhibits the increase in VEGF expression and MVD after TAE, inhibits liver tumor growth, and reduces the number of lung metastases. The results of their subsequent study [142], which employed ultrasound-guided HIF-1α RNAi, demonstrated an improvement in the efficacy of TACE in the treatment of HCC, thereby further confirming previous findings. Moreover, the study by Ni et al. [143] yielded comparable outcomes. In this study, the iodized oil emulsion was prepared by combining lipiodol with a siRNA transfection compound, which was then delivered via the hepatic artery during the TAE procedure. In the rabbit VX2 model by Guo et al. [144], the TAE with drug-free microspheres combined with intraarterial transfecting HIF-1α shRNA on HCC demonstrated superior anti-tumor efficacy compared to monotherapy.

The combination of TACE with HIF-related gene knockdown represents a promising therapeutic approach for the treatment of HCC. Liu et al. [145] found that the myocardial infarction associated transcript (MIAT)/microRNA (miR)-203a/HIF-1α axis could affect the efficacy of TAE. MIAT and HIF-1α were highly expressed and miR-203a was lowly expressed in hypoxia-stimulated hepatocellular carcinoma cells after TACE. MIAT gene regulated the miR-203a/HIF-1α axis, and MIAT knockdown enhanced TAE-mediated antitumor effects by upregulating MIR-203a and downregulating HIF-1α. In addition, the lentiviral delivery (LV-H721) of the CRISPR/Cas9 protein and an HIF-1α-specific sgRNA resulted in highly efficient HIF-1α modification. A study employed CRISPR/Cas9-mediated knockdown of HIF-1α [146]. The results demonstrated that lentiviral delivery of CRISPR/Cas9 protein and HIF-1α-specific sgRNA was an effective method for modifying HIF-1α. Furthermore, the combination of CRISPR/Cas9-mediated HIF-1α knockdown and TAE was found to significantly suppress tumors and prolong the survival time of HCC mice.

6.3.2. Hypoxia-Targeted Oncolytic Virus

In 2008, Altomonte et al. [147] proposed a novel method of treatment for HCC in rats, termed viroembolization. This approach involved the co-administration of recombinant vesicular stomatitis virus (VSV) with degradable starch microspheres (DSM), which were injected through the hepatic artery. The researchers observed that viral embolization induced apoptosis in tumor margins that survived embolization, significantly reducing intratumoral CD31 staining. Additionally, the procedure prevented neointimal formation after embolization, recruited NK cells and CD8+ T cells to infiltrate, and led to massive tumor necrosis. The study by Sun et al. [148] demonstrated for the first time that portal infusion of adeno-associated viral vectors expressing antisense HIF1-α downregulated HIF-1a and its downstream effectors, including vascular endothelial growth factor, GLUT1, and LDHA, and enhanced the inhibitory effect of TAE on the growth of HCC in rats. Zhang et al. [149] synthesized a hypoxia-replicative oncolytic adenovirus (HYAD) and constructed VX2 HCC rabbit models by HYAD perfusion combined with polyvinyl alcohol (PVA) embolization. The results showed that in vitro experiments demonstrated that HYAD was expressed and replicated in the presence of HIF-1α expression or hypoxia. In the in vivo experiments in the VX2 model, HYAD perfusion combined with PVA embolization resulted in the highest expression and the longest expression duration compared with HYAD perfusion alone, wild adenovirus type 5 (WT) perfusion combined with PVA embolization, and WT perfusion alone.

In another aspect, data from a rabbit VX2 tumor model found that transarterial viroembolization (TAVE) has been demonstrated the most effective modality with more homogeneous oncolytic virus distribution and therapeutic efficacy compared to other routes of administration via transarterial

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viral infusion, commonly used intratumoral injection and intravenous injection. TAVE is the optimal and safe therapy for the treatment of immune-refractory HCC, and the synergistic effect achieves significant tumor response, standby effect, survival benefit and anti-tumor immune memory, providing an innovative therapeutic approach for clinical practice [150]. It has to be acknowledged that the combination of TACE and oncolytic virus shows great promise, whether targeting hypoxic tumor cells or not.

Table 3. Summarize of the therapeutic strategies of TACE combined with gene therapy for HCC.

Item	Year	Refs.	Mechanism	Targets affected	Cancer hallmark affected
Hypoxia pathway-related gene therapy	2012	[141]	HIF-1α RNAi visibly reduced the expression of HIF-1α and vascular endothelial growth factor, suppressed tumor angiogenesis, and attenuated metastasis.	HIF-1α	↓ HIF-1α ↓ VEGF ↓ MVD
	2015	[142]	HIF-1α RNAi could downregulate the levels of HIF-1α and VEGF, inhibit tumor angiogenesis, and lessen metastases.	HIF-1α	↓ HIF-1α ↓ VEGF ↓ MVD
	2017	[143]	HIF-1α-siRNA could inhibit the expression levels of HIF-1α and VEGF effectively.	HIF-1α	↓ HIF-1α ↓ VEGF
	2020	[144]	HIF-1α shRNA could decrease the formation of blood vessels, slow tumor growth, reduce tumor size and promote tumor cell apoptosis.	HIF-1α	↓ HIF-1α ↓ VEGF ↓ CD34
	2020	[145]	MIAT knockdown potentiated the therapeutic effect of TAE in liver cancer by regulating the miR-203a/HIF-1α axis in vitro and in vivo.	MIAT/ miR-203a/ HIF-1α	↑ miR-203a ↓ HIF-1α
	2018	[146]	The combination of CRISPR/Cas9-mediated HIF-1a knockdown and TAE was found to significantly suppress tumors.	HIF-1α	↓ HIF-1α ↓ CD31 ↓ Invasiveness ↓ Migration ↓ Proliferation ↑ Apoptosis
Hypoxia-targeted oncolytic virus	2008	[147]	Viral embolization induced apoptosis in tumor margins that survived embolization, significantly reducing intratumoral CD31 staining.	Hypoxic tumor	↓ CD31 ↓ Proliferation ↑ Apoptosis ↑ Necrosis
	2009	[148]	Intraportal delivery of adeno-associated	HIF-1α	↓ HIF-1α

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		viral vectors expressing antisense HIF-α augmented TAE to combat hepatocellular carcinoma.		↓ VEGF ↓ GLUT1 ↓ LDHA ↓ Proliferation ↑ Apoptosis
2019	[149]	Adenovirus expression protein E1A has the properties of promoting apoptosis, inhibiting invasion, and inhibiting metastasis.	Hypoxic tumor	↓ Proliferation ↓ Migration ↑ Apoptosis
2023	[150]	TAVE modified the immune cell densities for immune-excluded liver cancer, partially destroyed vessel metastases, and established antitumor immune memory.	Tumor cells	↓ Proliferation ↑ Apoptosis ↑ Necrosis

6.4. Combination of Other or Novel Therapies and TACE

6.4.1. Metabolic Reprogram

Metabolic reprogramming combined with TACE is an emerging cancer treatment strategy that enhances efficacy by simultaneously disrupting tumor metabolism and blocking tumor blood supply. 3-Bromopyruvate (3-BrPA) is one of the early promising anti-glycolytic drugs. Early studies have found that 3-BrPA combined with TAE/TACE shows limited efficacy [151,152].

A study was conducted by Wilkins et al. [153] to assess the efficacy of TAE in combination with two cinnamic acid derivatives, caffeic acid (CA) or ferulic acid (FA), in a rat model of N1-S1 HCC. The results demonstrated that CA and FA resulted in a significant reduction of lactate efflux from N1S1 tumor cells by more than 90% ($P < 0.01$). Additionally, pathological assessment revealed the absence of residual live tumor cells in the TAE+CA and TAE+FA groups, accompanied by a notable reduction in tumor volume compared to TAE.

Bumetanide (BU) is a fast-acting, short-duration loop diuretic. In addition, BU blocks the rate-limiting step of phosphofructokinase in anaerobic glycolysis. A study [154] demonstrated a significant increase in tumor necrosis in N1-S1 HCC treated with TAE in combination with BU, with tumor volume reduced by 18.2 ± 12.2%. The TAE group demonstrated a reduction of 90.4 ± 10.2%, while the TAE+BU group exhibited a 72.2% reduction compared to the TAE-only group ($P < 0.0001$). Additionally, histopathological evaluation revealed the absence of residual tumor tissue in the TAE+BU group.

PKM2 is a splice variant of pyruvate kinase. It has been implicated in the propagation of the Warburg effect in cancer cell metabolism. Martin et al. [155] found that PKM2 is associated with chemotherapy and TACE resistance. Inhibition of PKM2 by gene silencing or the agent shikonin allows for targeted therapeutic reprogramming to overcome resistance, thereby reversing TACE resistance to improve TACE efficacy.

A study [156] evaluated the antitumor effects of the LDHA inhibitor sodium oxalate (Ox) in combination with TACE and the underlying mechanisms using the rabbit VX2 liver tumor model. The results demonstrated that Ox + TACE downregulated VEGF and matrix metalloproteinase-9 (MMP-9) and enhanced the infiltration of CD3+ and CD8+ T cells into tumor tissues, significantly improving the antitumor effect.

6.4.2. Mitochondrial Autophagy

Targeting of mitochondrial fission and mitophagy is a novel therapeutic approach to HCC. Ischemia induces mitochondrial autophagy to maintain nutrients and energy in the short term and

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to limit short-term damage. However, in the long term, this process transforms into a self-destructive process that ultimately leads to cell death. Mitochondrial autophagy removes damaged mitochondria and promotes the survival of HCC cells [157].

Lin et al. [158] demonstrated that the mitochondrial fission and mitophagy, which are mediated by dynamin-related protein 1 (DRP1), were significantly enhanced in surviving HCC cells under hypoxic conditions when subjected to in vitro simulation of the conditions that occur after TACE. Moreover, Mdivi-1, an inhibitor of DRP1, suppressed hypoxic HCC growth in vivo. Therefore, combined blockade of DRP1 is a potential approach to improve the efficacy of TAE/TACE therapy.

Zhong et al. [159] found that TACE induces S100 calcium binding protein A9 (S100A9), a key oncogene involved in post-TACE progression, via the HIF-1α-mediated pathway. S100A9 promotes the growth and metastasis of HCC by causing the deubiquitination and stabilization of PGAM5 and leading to mitochondrial fission and ROS production.

Furthermore, mitophagy has been found to be blocked by the combination of sorafenib with glucose restriction, thus suggesting a promising combination strategy such as transarterial sorafenib-embolization (TASE) for the treatment of unresectable HCC [160].

Table 4. Summarize of the therapeutic strategies of TACE combined with other or novel therapies for HCC.

Item	Year	Refs.	Mechanism	Targets affected	Cancer hallmark affected
Metabolic reprogram	2002	[151]	3-bromopyruvate is a potent inhibitorof cell ATP production.	Tumor cells	↑ Necrosis ×Migration
	2008	[152]	3-BrPA acts as an irreversible inhibitor of glycolytic enzymes.	Tumor cells	↑ Necrosis ×Migration
	2017	[153]	CA or FA enhanced the effectiveness of TAE therapy for HCC in part by blocking lactate efflux.	Anaerobic metabolism/ lactate	↓ ECAR ↓ MCT4 ↑ Necrosis
	2017	[154]	Bumetanide is a glycolytic metabolism pathway inhibitor.	Glycolysis	↑ Necrosis
	2020	[155]	Shikonin, a naphthoquinone, has been shown to inhibit glycolysis through PKM2 specific inhibition.	PKM2 Glycolysis	↑ Necrosis ↓ Migration ↓ Proliferation ↓ Lactat
	2024	[156]	Ox could inhibit LDHA and the Warburg effect.	Aerobic glycolysis	↓ MMP-9 ↓ VEGF ↑ Apoptosis ↑ $CD3^+T$ ↑ $CD8^+T$

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Mitochondrial autophagy	2024	[158]	Blocking DRP1-mediated mitochondrial fission and mitophagy increased the incidence of mitochondrial apoptosis of HCC cells during hypoxia.	Mitochondrial mitophagy	↑ Mitochondrial apoptosis ↑ Apoptosis-inducing factor ↑ Cytochrome c ↓ Mitochondrial membrane potential
	2022	[159]	Depletion or pharmacologic inhibition of S100A9 significantly dampened the growth and metastatic ability of HCC.	Mitochondrial S100A9	↓ Mitochondrial fission ↓ ROS production ↓ EMT programs
	2022	[160]	Sorafenib and glucose restriction exacerbated mitochondrial damage and ultimately caused cell death.	Mitochondrial mitophagy Glycolysis	↓ ATP ↓ OCR ↓ Mitochondrial mitophagy ↑ Necrosis

7. Conclusions

In view of the negative impact of local hypoxia on the therapeutic efficacy of TACE for HCC, hypoxia represents a promising target for combination use with TACE. Previous clinical phase III trials on TPZ for solid tumors showed negative results, which is likely due to the fact that the hypoxia within tumors is not sufficient to effectively activate TPZ to exert its hypoxia-selective cytotoxicity. In contrast, TACE generates sufficient hypoxic microenvironment, which is conducive to the effectiveness of TPZ. Encouraging results have been observed in the phase I trial investigating TPZ with TAE for HCC, and phase II trials are currently underway. Future directions for in this field include a comprehensive investigation of the role of possible interactions between hypoxia and the effect of TAE during the TACE procedure. Additionally, there is a need to develop new formulations of hypoxia-targeted drugs suitable for combination with TACE, such as novel HAPs that can be suspended in lipiodol or loaded onto drug-eluting microspheres at high doses. Moreover, Nanotechnology has been utilized to deliver anti-tumor drugs and embolize tumor blood vessels. Further studies are required to assess the feasibility of use of nanomaterials to deliver hypoxia-targeted drugs and its efficacy and safety as a chemoembolization agent targeting hypoxic tumor cells. Overall, the combination of TACE with hypoxia-targeted therapy constitutes an effective strategy, and continued investigation and innovation in this field will be crucial to improve outcomes for patients with HCC.

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