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Hypoxia-inducible factors in hepatocellular carcinoma (Review)

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Abstract. Maintenance of an appropriate oxygen concentration is essential for the function of the liver. However, in many pathological conditions, and particularly in the tumor microenvironment, cells and tissues are frequently in a hypoxic state. In the presence of hypoxia, the cells adapt to the low oxygen levels through the hypoxia-inducible factor (HIF) pathway. Overgrowth of tumor cells restricts the diffusion of oxygen in tumors, leading to insufficient blood supply and the creation of a hypoxic microenvironment, and, as a consequence, activation of the expression of HIFs. HIFs possess a wide range of target genes, which function to control a variety of signaling pathways; thus, HIFs modulate cellular metabolism, immune escape, angiogenesis, metastasis, extracellular matrix remodeling, cancer stem cells and other properties of the tumor. Given their crucial role in the occurrence and development of tumors, HIFs are expected to become new targets of precise treatment of hepatocellular carcinoma.

Contents
1. Introduction
2. Structure and function of HIFs
3. Expression of HIF in HCC and their association with clinical outcomes
4. Relationship between HIF and HCC
5. Metabolism
6. Immune escape
7. Angiogenesis
8. Metastasis
9. Extracellular matrix remodeling
10. Cancer stem cells
11. HIF-1α as a therapeutic target
12. Conclusions and future perspectives

1. Introduction
Hepatocellular carcinoma (HCC) is one of the most common malignant tumors of the digestive system. In 2015, 466,100 patients were newly diagnosed with HCC in China, and the number of deaths caused by HCC deaths was ~422,100 (1). In China, HCC is one of the four major causes of cancer-related deaths. HCC is a hypermetabolic tumor that consumes more oxygen than the surrounding normal tissues. However, the uncontrolled proliferation of HCC cells leads to an insufficient oxygen supply and the rapidly growing tumor not only quickly consumes oxygen but also lacks adequate vascularization, subsequently generating a hypoxic microenvironment. Hypoxia-inducible factors (HIFs) are recognized as crucial transcriptional regulators that are activated under hypoxia (2). A number of recent studies have documented the involvement of HIFs in HCC cell proliferation, angiogenesis, invasion and metastasis (3,4). In addition, progress has been made in the development of HCC therapies involving the targeting HIFs (5). Currently, the research on HIFs is focused on two aspects, the mechanism of transcriptional regulation of HIFs and cancer therapy targeting HIFs. Therefore, the present review examined the processes of regulation and activation of HIFs in HCC, and focused on the progress of research on the function of HIFs in HCC.

2. Structure and function of HIFs
The rapid proliferation of cancer cells leads to the rapid consumption of tissue oxygen. When the rate of oxygen consumption exceeds the rate of oxygen supply by the
circulation, hypoxia develops (2). A hypoxic state activates a series of adaptive responses of cells, which are primarily mediated by HIFs. The human genome encodes three different HIF subtypes: HIF-1α, HIF-2α and HIF-3α (Fig. 1). HIFs are heterodimers composed of a functional α subunit and a stably expressed β subunit (6). The N-terminus of HIFs has a basic helix-loop-helix (bHLH) domain and a Per-ARNT-Sim (PAS) domain that participate in the heterodimerization of the α and β subunits. These domains also mediate HIF binding to the hypoxia response element (HRE) in a target gene promoter. The C-terminus of HIF proteins includes two transactivation domains (TAD), an N-terminal (N)-TAD and a C-terminal (C)-TAD. The N-TAD domain serves an essential function in activating HIF-1α or HIF-2α target genes; N-TAD is the major transactivation domain responsible for HIF-1α or HIF-2α target gene specificity; as a transcriptional activation domain, N-TAD may serve as an important cofactor for interaction sites. Transcriptional cooperation between HIF-1α and certain factors (such as SMAD3/4 and ETS-1) can induce activation of multiple HIF target genes under hypoxic conditions (7). The C-TAD acts to recruit p300/CREB-binding protein (CBP) and other auxiliary transcription factors. In addition, the structure of HIFs includes an oxygen-dependent degradation domain (ODDD), which overlaps with N-TAD, but its function is different from N-TAD. The ODDD serves as the recognition site of the von Hippel-Lindau tumor suppressor protein (pVHL) and is involved in the stabilization of proteins and the regulation of intracellular oxygen concentration. The β subunit is constitutively expressed, it is not regulated by intracellular oxygen concentration, and does not have transcriptional activity alone; only a heterodimer of HIF-α and HIF-β subunits is active. The ODDD contains two proline residues that can be hydroxylated by the prolyl hydroxylase domain (PHD) enzymes. Hydroxylated HIF subtypes are recognized by pVHL, which is ubiquitinated by pVHL-related elogin BC-Cul2 ubiquitin ligase complex. Hydroxylated HIF-1α binds to pVHL, which recruits elogin B, elogin C, cullin-2 and loop cassette 1 to form the E3 ubiquitin ligase complex. Unlike the targeted proteasomal degradation, HIF-1α forms the E3 ubiquitin ligase complex, which ubiquitinates HIF-1α and is ultimately mediated by the 26S proteasome (8-10), whereas HIF-2α is ubiquitinated by the of E2 ubiquitin-binding enzyme; but, both HIF-1α and HIF-2α are subsequently degraded by 26S proteasome (8).

PHDs are key enzymes of this degradation process, which uses oxygen and 2-ketoglutarate as substrates, and Fe²⁺ and ascrobate as co-factors of dioxygenase (Fig. 2). The activity of HIFs can also be suppressed by the HIF-1 inhibitor, such as factor inhibiting HIF-1α (FIH-1). The catalytic effect of FIH-1 is similar to that of PHD, which also requires oxygen and 2-ketoglutarate as substrates. Factor inhibiting HIF-1α (FIH) is an asparaginyl hydroxylase that catalyzes the hydroxylation of asparagine 803 (Asn803) on C-TAD, preventing HIF-1α from interacting with p300/CBP and inhibiting its transcriptional activity. However, both PHDs and HIFs are oxygen-dependent and, therefore, are inactive under hypoxic conditions, forming stable aggregates of HIF subtypes in the cytoplasm (11,12). Additionally, PHD activity can be inhibited by numerous important metabolites, including reactive oxygen species (ROS), nitric oxide (NO), succinate and fumarate (13). By contrast, cysteine may enhance PHD2 activity by inhibiting autooxidation (14).

HIF expression can also be regulated by other factors, including growth factors such as platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1), insulin and heregulin (Fig. 2). The Akt/HIF-1α/PDGF-BB autocrine signaling loop is formed under hypoxic conditions to increase the chemosensitivity of liver cancer cells (15). Previous studies have shown that IGF-1 affects HIF-1α and HIF-2α protein synthesis (16,17). Insulin regulates HIF-1α by a ROS-sensitive activation of Spl in 3T3-L1 preadipocytes (18); this is a novel transcriptional mechanism by which insulin is involved in Spl. Heregulin stimulates HIF-1α synthesis via a rapamycin-dependent manner (19). Acetyltransferases can acetylate the lysine residue at position 532 of HIF-1α, enhancing the binding ability of pVHL to HIF-1α and, ultimately, promoting its degradation (20). Receptor for activated protein C kinase 1 (RACK1) and heat shock protein 90 (Hsp90) compete to bind to the PAS region of HIF-1α; RACK1 enhances the binding of HIF-1α to E3 ligase and promotes degradation, whereas Hsp90 stabilizes HIF-1α and prevents its degradation (Fig. 2) (21).

The expression and activity of HIF-2α are also regulated by certain non-oxygen-dependent pathways, such as small ubiquitin-related modifier (SUMO) modification. SUMO modification is the main mechanism of HIF-2α degradation under hypoxia, which can negatively regulate the expression of HIF-2α. HIF-2α binds covalently to SUMO-2 via Lys394, resulting in its modification by SUMO. SUMO-modified HIF-2α is degraded by a mechanism involving SUMO-dependent E3 ubiquitin-protein ligase RNF4 and pVHL (Fig. 2) (22).

Although numerous studies have focused on HIF-1α and HIF-2α, our understanding of the role of HIF-3α in cancer cells is limited (23). It has been reported that HIF-3α can also be activated under hypoxic conditions and regulate the transcription and protein stability of HIF-1α (24-26). In addition, HIF-3α can activate the transcription of a set of specific target genes, which partially overlaps with genes upregulated by HIF-1α and HIF-2α, but their role remains to be demonstrated in future studies (27-29).

3. Expression of HIFs in HCC and their association with clinical outcomes

A large number of clinical studies have demonstrated a relationship between HIFs and metastasis, recurrence, vascular proliferation and prognosis of patients with HCC (Table I). The data indicate that the expression of HIF-1α in HCC tissues was higher compared with that in corresponding adjacent tissues. Overexpression of HIF-1α is associated with poor prognosis in patients with HCC; however, some recent studies have not reported that expression of HIF-2α or HIF-3α in HCC is associated with prognosis (Table I).

4. Relationship between HIF and HCC

A number of previous studies have demonstrated a complex relationship between HIF and HCC (30,31). The relationship between HIFs and HCC include, metabolism, immune escape, angiogenesis, metastasis, extracellular matrix (ECM) remodeling and activity of cancer stem cells (CSCs) (Fig. 3).
The rapid proliferation of cancer cells requires a large amount of energy, resulting in increased consumption of oxygen, which leads to the generation of a hypoxic environment in the tumor tissue. Under hypoxia, tumor cells undergo a transition from aerobic to anaerobic metabolism. This difference in metabolism between normal and cancer cells was first identified in 1920 (32). Normal cells under physiologic oxygen concentration convert glucose into pyruvate, which is further metabolized in the mitochondria via the tricarboxylic acid cycle and oxidative phosphorylation. In these cells, the availability of oxygen inhibits the rate of glycolysis (Pasteur effect), enables mitochondrial respiration, increases ATP levels and inhibits the activity of phosphofructokinase (PFK) responsible for glycolysis (33). Under hypoxic conditions, the final product of anaerobic glycolysis is pyruvic acid, which is subsequently metabolized to lactic acid. In comparison with non-malignant tissues, tumor cells rely more on the use of glycolysis to support their energy needs, even when oxygen is available, a phenomenon called the Warburg effect (34). Tumor cells are known to produce energy by generating ATP in anaerobic glycolysis, a process mainly regulated by HIF-1α (35,36). HIF-1α accelerates the glycolysis pathway of cancer cells by activating related target genes and transcription products. This activation may occur through three distinct mechanisms.

The first mechanism, the metabolism of HCC, is often related to the Warburg effect, involves HIF-1 activation of key enzymes involved in glucose metabolism and glycolysis (37,38). Overexpression of HIF-1α in cancer cells increases the activities of several isoforms of glucose transporters, including GLUT1 and GLUT3, which facilitate the uptake of glucose into cancer cells (39). HIF-1α also induces the expression of several enzymes involved in the glycolytic process, such as aldolase-A (ALD-A) and aldolase-C, which catalyze the conversion of fructose-1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, respectively. In addition, HIF-1α activates the transcription of genes encoding pyruvate dehydrogenase (PDH) and pyruvate dehydrogenase kinase 1 (PDK1), which in turn reduces the availability of intracellular acetyl-CoA and, consequently, inhibits the tricarboxylic acid cycle (39). This results in a decrease in the production of NADH and FADH2, which are essential for the production of ATP through oxidative phosphorylation. Additionally, HIF-1α enhances the expression of glycolytic enzymes, such as phosphofructokinase (PFK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), by activating their transcription (40). These mechanisms lead to an increase in the production of ATP through glycolysis, even in the absence of oxygen, a phenomenon called the Warburg effect (40).

In the second mechanism, HIF-1α induces the overexpression of several glycolytic enzymes, such as GAPDH, hexokinase, lactate dehydrogenase, and phosphofructokinase, which increase the rate of glycolysis and the production of lactic acid. This leads to an increase in the extracellular acidification of the tumor microenvironment, a phenomenon known as the Warburg effect (41). In addition, HIF-1α activates the transcription of genes encoding glycolytic enzymes, such as PFKL and GAPDH, which are involved in the regulation of the glycolytic pathway (42). This results in an increase in the production of ATP through glycolysis, even in the absence of oxygen, which is essential for the survival and proliferation of cancer cells.

In the third mechanism, HIF-1α regulates the expression of genes involved in the Warburg effect, such as GLUT1 and GLUT3, which increase the uptake of glucose into cancer cells (43). HIF-1α also induces the expression of genes encoding glycolytic enzymes, such as PFKL and GAPDH, which increase the rate of glycolysis and the production of lactic acid. This leads to an increase in the extracellular acidification of the tumor microenvironment, a phenomenon known as the Warburg effect (44). In addition, HIF-1α activates the transcription of genes encoding glycolytic enzymes, such as PFKL and GAPDH, which are involved in the regulation of the glycolytic pathway (45). These mechanisms lead to an increase in the production of ATP through glycolysis, even in the absence of oxygen, which is essential for the survival and proliferation of cancer cells.
reported that in late-stage HCC, a large number of triggering receptor expressed on myeloid cells 1 (TREM-1)-positive TAMs indirectly affect the cytotoxic function of CD8+ T cells and trigger their apoptosis (51). A previous study demonstrated that specific scavenging of macrophages with chlorophosphate liposomes resulted in significant suppression of tumor growth and angiogenesis (52). The role of TAMs was also documented in a study in which their inhibition delayed the growth of HCC in nude mice (53). The role of macrophages in HCC was also underscored by the demonstration that expression of hypoxia-induced high mobility group box-1 protein (HMGBl) promotes tumor invasion and metastasis in animal models of HCC by regulating macrophage-derived IL-6 (54). A previous study demonstrated that hypoxia promotes the immunosuppressive phenotype of HCC cell lines through upregulation of HIF1-dependent C-C motif chemokine 20 (CCL20) expression, and CCL20 significantly induces indoleamine 2,3-dioxygenase (IDO) expression in monocyte-derived macrophages (55). This study also showed a link between elevated CCL20 levels and poor survival in patients with liver cancer, suggesting a link between microenvironment of immunosuppressive hypoxic tumors and promotion of metastasis (55).
<table>
<thead>
<tr>
<th>HIF</th>
<th>Author, year</th>
<th>Result</th>
<th>Conclusion</th>
<th>Patient samples (n)</th>
<th>(Refs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIF-1α</td>
<td>Wada et al., 2006</td>
<td>Positive expression of HIF-1α in HCC tissues was higher compared with that in normal tissues</td>
<td>Overexpression of HIF-1α indicates poor prognosis in patients with HCC</td>
<td>HCC (69); normal (0)</td>
<td>(126)</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>Dai et al., 2009</td>
<td>Positive expression of HIF-1α in HCC tissues was high</td>
<td>Overexpression of HIF-1α indicates poor prognosis in patients with HCC</td>
<td>HCC (110); high HIF-1α (42), low HIF-1α (68); corresponding adjacent tissue (416)</td>
<td>(127)</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>Xia et al., 2012</td>
<td>Positive expression of HIF-1α in HCC tissues was high</td>
<td>Overexpression of HIF-1α indicates poor prognosis in patients with HCC</td>
<td>HCC (953); high HIF-1α (475), low HIF-1α (478); corresponding adjacent tissue (44)</td>
<td>(128)</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>Wang et al., 2014</td>
<td>Positive expression of HIF-1α in HCC tissues was higher compared with that in normal tissues</td>
<td>Overexpression of HIF-1α indicates poor prognosis in patients with HCC</td>
<td>HCC (69); high HIF-1α (30), low HIF-1α (39); corresponding adjacent tissue (44)</td>
<td>(129)</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>Liu et al., 2014</td>
<td>Positive expression of HIF-1α in HCC tissues was higher than that in normal tissues</td>
<td>Overexpression of HIF-1α indicates poor prognosis in patients with HCC</td>
<td>HCC (101); normal (0); corresponding adjacent tissue (127)</td>
<td>(130)</td>
</tr>
<tr>
<td>HIF-2α</td>
<td>Bangoura et al., 2007</td>
<td>Expression of HIF-2α in HCC tissues was higher than in normal tissues</td>
<td>Overexpression of HIF-2α indicates poor prognosis in patients with HCC</td>
<td>HCC (315); corresponding adjacent tissue (192); normal tissue (22)</td>
<td>(131)</td>
</tr>
<tr>
<td>HIF-2α</td>
<td>Sun et al., 2013</td>
<td>Expression of HIF-2α in HCC tissues was low</td>
<td>Expression of HIF-2α is not related to prognosis in patients with HCC</td>
<td>HCC (85); normal (0); corresponding adjacent tissue (85)</td>
<td>(132)</td>
</tr>
<tr>
<td>HIF-2α</td>
<td>Yao et al., 2015</td>
<td>Positive correlation between expression of HIF-2α and vascular invasion of HCC tissues</td>
<td>Expression of HIF-2α is not related to prognosis</td>
<td>HCC (1066); normal (0); corresponding adjacent tissue (84)</td>
<td>(133)</td>
</tr>
<tr>
<td>HIF-2α</td>
<td>Yang et al., 2016</td>
<td>Protein level of HIF-2α in HCC tissues was lower compared with that in adjacent tissues</td>
<td>Expression of HIF-2α is not related to prognosis</td>
<td>HCC (206); corresponding adjacent tissue (84); chronic hepatitis tissue (84)</td>
<td>(134)</td>
</tr>
<tr>
<td>HIF-3α</td>
<td>Liu et al., 2016</td>
<td>Expression of HIF-3α in HCC tissues was lower compared with that in chronic hepatitis tissues</td>
<td>Expression of HIF-3α is not related to prognosis</td>
<td>HCC (126); corresponding adjacent tissue (84)</td>
<td>(135)</td>
</tr>
</tbody>
</table>

HIF, hypoxia-inducible factor; HCC, hepatocellular carcinoma; MMP, matrix metalloproteinase.
7. Angiogenesis

The rapid growth of tumors necessitates the *de novo* formation of a large number of blood vessels to transport oxygen and nutrients. Angiogenesis is a complex process that involves the degradation of the extracellular matrix, the activation, proliferation, and migration of vascular endothelial cells, and the establishment of a new vascular network (56). The most important signaling molecule in this process is VEGF (Fig. 3), which specifically promotes the proliferation and migration of vascular endothelial cells. Compared with the normal vascular system, the blood vessels of tumors are leaky, distorted and disordered. Inhibition of the expression of HIF-1α in endothelial cells suppresses tumor growth, whereas inhibiting the expression of HIF-2α enhances the formation of blood vessels supplying the tumor (57). However, these blood vessels are disordered and do not correct the hypoxic state of the tumor microenvironment. This phenomenon is caused by differential regulation of NO homeostasis, which in turn regulates vascular endothelial growth factor expression in the NO-dependent feedback loop (57). HIF-1α is a major regulator of VEGF expression. The HIF-1α/p300/CBP complex binds to the HREs in five regions of the VEGF promoter. Under hypoxia, high levels of accumulated HIF-1α upregulates the expression of a series of angiogenic factors, such as VEGF, and enhances the stability of VEGF mRNA, ultimately activating tumor angiogenesis (58,59). Lee et al (60) used acridine flavin to inhibit the heterodimerization of HIF-1α and HIF-1β and revealed that
This result provided additional evidence for the role of HIF-1α in the activation of VEGF. Another study demonstrated that the levels of HIF-1α, as well as VEGF protein and mRNA, detected after 20 weeks of HCC were significantly higher than before 20 weeks in an experimental rat HCC model, suggesting that HIF-1α and VEGF may have important functions during HCC development (61). Sorafenib, an inhibitor of multiple kinases, has been tested in clinical trials of HCC carcinoma, and the mechanism of its action has been reported to be closely related to anti-angiogenesis (62); it can effectively inhibit the expression of HIF-1α, thereby reducing the expression of VEGF and, ultimately, leading to a decrease in angiogenesis in tumors. In addition to VEGF, many other signaling molecules are also highly expressed under hypoxic conditions via HIF-dependent mechanisms, including angiopoietin 2 (ANG2), placental growth factor (PGF), PDGF-β and stromal-derived factor 1 (SDF-1); all of these growth factors promote angiogenesis in tumors (63). ANG-like protein 4 (ANGL4) has also been identified as a gene target of HIF-1α (64); ANGL4 affects HCC angiogenesis and metastasis by modulating the expression of vascular cell adhesion molecule and integrin β1.

In contrast to HIF-1α, HIF-2α is only expressed during normal development of blood vessels and lungs (65). It has also been detected in tumor vascular endothelial cells, tumor cells and TAMs (66); and hypoxia-inducible expression of HIF-2α has been reported in the brain, lung, heart, liver, duodenum, pancreas and kidney of mice (67). HIF-2α mainly acts on angiogenesis-related genes, including VEGF, erythropoietin (EPO), VEGF receptor 2 (VEGFR2), angiogenin, and tyrosine-protein kinase receptor TIE-2 (68,69); experiments using different tumor cell lines and animal models have demonstrated that HIF-2α activates tumor angiogenesis by upregulating VEGF. Additionally, HIF-2α forms a complex with transcription-assisted activator ETS proto-oncogene 1 (ETS-1), and binds to HRE4 on the promoter of VEGFR2, activating its expression (70).

8. Metastasis

Intrahepatic and extrahepatic metastasis is the major contributor to poor prognosis in patients with HCC. Invasion and metastasis of tumors is a complex process in which the first step involves EMT. In the process of EMT, polar epithelial cells transform into mobile stromal cells, gaining the ability to migrate to distant sites. HIF-1α is a crucial regulator of EMT under hypoxic conditions, acting through seven distinct mechanisms detailed in the subsections below (Fig. 3).

Snail homolog 1 (SNAI1) and SMAD-interacting protein 1 (SIP1) signaling pathways. Inactivation of epithelial (E)-cadherin, a protein essential for cell adhesion, results in the weakening of cell-cell contacts and increased mobility, initiating EMT. HIF-1α inhibits the expression of E-cadherin by upregulating SNAI1 and SIP1, transcriptional inhibitors of E-cadherin (71). HIF-1α regulates SNAI1 by binding to two HREs on the SNAI1 promoter, affecting the expression of E-cadherin, as well as N-cadherin and vimentin, activating EMT in HCC cells and promoting HCC invasion and metastasis (72).

TGF-β signaling pathway. The TGF-β signaling pathway is widely involved in embryonic development, tissue and organ formation, cell proliferation, apoptosis, differentiation and migration. TGF-β has a dual function in the development of tumors. TGF-β signaling pathway induces EMT, facilitating the invasion and metastasis of tumors (73). It has been also demonstrated that hypoxia is an important stimulator of EMT by activating HIFs (74). Under hypoxic conditions, HIF expression in hepatocytes promotes TGF-β signaling; HIF and TGF-β signaling contribute to the mechanism of hypoxia-stimulated hepatocyte EMT (74). It has been reported that the TGF-β1 pathway serves an important role in the regulation of liver cancer by regulating SMAD4, SMAD2/3, cleaved Notch1, and β-catenin proteins (75).

Notch signaling pathway. The Notch signaling pathway regulates embryonic development and differentiation, and proliferation and apoptosis of mature cells. Notch signaling induces EMT primarily by two mechanisms. The first one involves the upregulation of SNAI1 achieved by Notch-mediated recruitment of HIF-1α and the resulting increase in lysyl oxidase (LOX), which stabilizes SNAI1, thus promoting EMT (76). The second mechanism relies on the interaction of Notch with the TGF-β1/SMAD pathway, which also activates EMT (77). Although the molecular mechanisms underlying hypoxia and Notch pathway activation are not clear, there is indeed a link between them. Hypoxia activates Notch-responsive promoters and increases expression of Notch direct downstream genes; the Notch intracellular domain interacts with HIF-1α, and after activation of Notch under hypoxic conditions, HIF-1α is recruited to the Notch reactive promoter (78).

NF-κB signaling pathway. The presence of a bi-directional correlation between HIF and NF-κB has also been reported, in which NF-κB can induce HIF and HIF can also regulate NF-κB (79). Cancer is characterized by the presence of hypoxia and inflammation. Hypoxia has been demonstrated to promote inflammation through the regulation of gene expression by oxygen-sensitive transcriptional regulators, including HIF and NF-κB (80). The basis for this association includes the regulation of the components of the NF-κB pathway and the transcriptional regulation of HIF-1 under hypoxia (81).

Wnt signaling pathway. Wnt regulates the growth, proliferation, invasion and metastasis of cancer cells. Under hypoxic conditions, an increase of Wnt3a upregulates the expression of β-catenin and promotes EMT (82). A previous study reported that the Wnt/β-catenin signaling pathway enhances the transcriptional activity of HIF-1α and inhibits the apoptosis of HCC, as well as inducing EMT and triggering HCC metastasis (83). In addition, hypoxia promotes HCC cell migration and angiogenesis by regulating the expression of B-cell CLL/lymphoma 9 (BCL9), which activates Wnt/β-catenin signaling pathway (84).

PI3K/AKT signaling pathway. PI3K/AKT signaling is crucially involved in tumor development. Hypoxia induces the expression of tufetin1 (TUF1) in a HIF-1α-dependent manner (85). In turn, TUF1 activates the Ca²⁺/PI3K/AKT
pathway, promoting HCC cell growth, metastasis and EMT in vitro and in vivo.

ROS signaling pathway. Hypoxia significantly promotes the progression of EMT and is associated with activation of the non-canonical Hedgehog (Hh) signaling pathway. HIF-1α knockdown attenuates hypoxia-induced membrane-spanning protein SMO and glioma-associated oncogene 1 (GLI1) expression and inhibits EMT progression. In addition, SMO inhibitors or GLI1 small interfering (si)RNA can also reverse hypoxia-driven EM in vitro and under hypoxic conditions. It is suggested that non-canonical Hh signaling serves an important role in hypoxia-induced EMT. Hypoxia increases reactive oxygen species (ROS) production, and ROS inhibitors (NACs) block GLI1-dependent EMT processes under hypoxic conditions.

In hypoxic HCC cells, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4 (NOX4) expression was found to increase at mRNA and protein levels. siRNA-mediated knockdown of NOX4 expression abolishes hypoxia-induced ROS production and hypoxia-induced GLI1-dependent EMT. Hypoxia triggers ROS-mediated GLI1-dependent EMT progression by inducing NOX4 expression. Non-canonical Hh pathway regulates HIF-1α/NOX4/ROS signaling pathway under hypoxic conditions to regulate EMT processes in HCC cells (86). A relationship has also been identified between HIF signaling and p53 family members. A previous study reported that due to the binding of p53 protein to HIF-1α, p53 is stabilized, and hypoxia induction of transcriptionally active wild-type p53 gene is achieved (87). Conversely, p53 and p73 interact with HIF-1α, suppressing its activity, thereby inhibiting the migration and metastasis of tumor cells (88,89). A number of studies have demonstrated that microRNAs (miRNAs) are also closely related to the migration and metastasis of tumors, and their effects involve the activity of HIF-1α. For example, miRNA (miR)-130b and miR-21 can activate EMT through the PTEN/AKT/HIF-1α pathway and enhance HCC metastasis (90,91). Hypoxia-induced downregulation of miR-204, which acts as a post-transcriptional regulator of vasodilator-stimulated phosphoprotein (VASP) expression, promotes intrahepatic metastasis of HCC (92). miR-199a-5p (93), miR-592 (94) and miR-3662 regulate the Warburg effect and HCC progression (95). Circular RNA circ-EPHB4 derived from ephrin B4 protein (rAGAP) is a protein comprising small ubiquitin-related signaling. Recombinant analgesic-antineoplastic peptide (rAGAP) is a protein comprising small ubiquitin-related modifiers linked to ubiquitin-histidine tags. rAGAP inhibits the AKT/P3K pathway, suppressing angiogenesis and tumor progression (109). Circular RNA circ-EPHB4 derived from the gene coding for a member of the ephrin (Eph) receptor tyrosine kinase family, EphB4, prevents tumor growth by modulating the HIF-1α and AKT/P3K signaling (110). The drug salidroside significantly increases the sensitivity of HCC to platinum and inhibits hypoxia-induced EMT by blocking the HIF-1α signaling (111). Rapamycin counteracts the process of EMT and angiogenesis, thus inhibiting the development and progression of tumors. Studies on HIF and stem cells focused on the role of HIF in hematopoietic stem cells (105); based on data suggesting the involvement of HIF in the function of hematopoietic stem cells, studies have demonstrated that the HIF signaling pathway serves an important role in the induction and maintenance of CSC and EMT phenotypes, and regulates its function by regulating multiple complex signaling molecules within the tumor microenvironment (106). A recent study reported that hypoxia significantly enhances stem cell-related properties of HCC cells, an effect that can be abolished by the knockdown of HIF-1α or HIF-2α (3). Additionally, HIF-1α-specific small interfering RNA treatment markedly reduces the expression of CD133 in CSCs at the RNA and protein levels (107). Importantly, EMT activation can induce CSC characteristics. Notch1 mediates the process of EMT-induced CSCs by direct interaction with HIF-1α; upregulation of the intracellular expression of Notch by HIF-1α can activate EMT and induce HCC cells to acquire the features of CSC in vitro (108).

10. Cancer stem cells

CSCs have an important function in the initiation, development, recurrence and metastasis of tumors. Studies on HIF and stem cells focused on the role of HIF in hematopoietic stem cells (105); based on data suggesting the involvement of HIF in the function of hematopoietic stem cells, studies have demonstrated that the HIF signaling pathway serve an important role in the induction and maintenance of CSC and EMT phenotypes, and regulates its function by regulating multiple complex signaling molecules within the tumor microenvironment (106). A recent study reported that hypoxia significantly enhancing stem cell-related properties of HCC cells, an effect that can be abolished by the knockdown of HIF-1α or HIF-2α (3). Additionally, HIF-1α-specific small interfering RNA treatment markedly reduces the expression of CD133 in CSCs at the RNA and protein levels (107). Importantly, EMT activation can induce CSC characteristics. Notch1 mediates the process of EMT-induced CSCs by direct interaction with HIF-1α; upregulation of the intracellular expression of Notch by HIF-1α can activate EMT and induce HCC cells to acquire the features of CSC in vitro (108).
growth and lung metastasis in a rat model of HCC (112). Ruscogenin reduces the expression of MMP-2, MMP-9, urokinase plasminogen activator, VEGF and HIF-1α by interfering with the PI3K/AKT/mTOR signaling pathway, resulting in an inhibition of tumor growth (113). The dietary phytochemical sulfonamide prevents angiogenesis of HCC by inhibiting STAT3/HIF-1α/VEGF signaling transduction (114). N1-guanyl-1,7-diaminohexane (GC7) enhances the sensitivity of HCC to doxorubicin by reversing the EMT signaling pathway induced by HIF-1α (115). Everolimus suppresses tumor growth and angiogenesis by blocking AKT/mTOR signaling pathway in vitro by promoting cell apoptosis and inhibiting endothelial cell proliferation (116). Finally, Huangia polysaccharide TP-1 is a naturally occurring bioactive macromolecule, found in Huangia fungus, prevents tumor growth and metastasis by downregulating HIF-1α-VEGF and AUF-1/AEG-1 signal transduction pathways (117). ii) Drugs inhibiting the expression of HIF-1α mRNA. Two compounds, RO70179 and EZN-2968, have been demonstrated to markedly reduce the expression of HIF-1α in HCC tissues (118). iii) Drugs inhibiting the synthesis of HIF-1α protein. Topotecan, an inhibitor of topoisomerase, has been reported to block the entry of the ribosome on HIF-1α mRNA, preventing translation of the protein (119). Additionally, vorinostat, a histone deacetylase inhibitor, decreases interaction between acetyl-Hsp90 and HIF-1α, inhibiting HIF-α nuclear translocation (120). iv) Drugs promoting the degradation of HIF-1α protein. A previous study has reported that evoamidein in combination with vorinostat accelerated the degradation of HIF-1α in HCC cells under hypoxic conditions (121). v) Drugs inhibiting HIF-1α stabilization. Curcumin can induce the clearance of ROS by upregulating nuclear factor E2-related factor 2 (Nrf2) and glutathione (GSH), which inhibit the stabilization of HIF-1α, and, in turn, suppress the expression of connective tissue growth factor (CTGF), providing a protective effect on HCC (122). vi) Drugs blocking the binding of HIF-1α to target genes; for example, doxorubicin (115). vii) Drugs inhibiting HIF-1α-mediated transcriptional activation; for example, bortezomib (123). viii) Drugs used for systemic therapy. A previous study demonstrated that inhibition of HIF-1α by systemic therapy with digoxin significantly delayed the development of HCC (124). In addition, metformin was reported to enhance the potential of regorafenib by regulating the levels of HIV TAT-interactive protein (TIP30) and HIF-2α, and inhibits the recurrence and metastasis of HCC after hepatectomy (125).

12. Conclusions and future perspectives

The expression of HIF-1α in HCC is significantly higher compared with expression in normal liver cells. HIF-1α is a crucial regulator of the adaptation of HCC cells to the hypoxic microenvironment and can affect the proliferation, growth, invasion, metastasis, angiogenesis, apoptosis and drug resistance of HCC cells by modulating the expression of multiple target genes. A number of studies have demonstrated the feasibility of using HIF-1α as a therapeutic target, which suggested that interventions modifying the activity of HIF-1α by direct or indirect ways may become effective for the treatment of HCC. Despite the growing number of studies on HIF-1α and identification of many HIF-1α inhibitors, their therapeutic application has not moved beyond the pre-clinical stage. Clinical use of these inhibitors faces multiple problems which have to be solved urgently. They include limitations in the specificity of HIF-1α inhibitors and lack of definitive cytotoxicity of HIF-1α inhibitors toward cancer cells. Therefore, compounds need to be developed and screened for clinical application. In the case of YC-1 and other similarly well-investigated inhibitors, further research on their pharmacology and toxicology is still needed. Although gene therapy targeting HIF-1α brings new hope to the treatment of HCC, finding the target gene is only the first step in the long road to clinical application. How to construct a safe and efficient vector, how to search for specific transcriptional regulatory elements in HCC, and how to rationally apply a combined therapy targeting multiple genes are critical questions that must be conclusively answered. Therefore, studies on the function of HIF-1 in HCC have to be expanded, necessitating additional time before the targeted therapy of HIF-1α for HCC can be implemented clinically. In addition, the understanding of the function of HIF-2 and HIF-3 in HCC has only begun to emerge, although it is already documented that HIF-2α affects HCC energy metabolism, angiogenesis, cell proliferation and tumor growth. Other studies have provided information regarding the stability, transcriptional activity and role of HIF-2α in HCC growth and progression, but the exact role in HCC remains unclear. It is generally believed that HIF-2α can be activated in most hypoxic solid tumors, but whether its activation promotes or inhibits tumor growth depends on the biological environment of the tumor. HIF-2α can participate in modulating the progression of HCC through different signaling pathways. However, the specific role of HIF-2α in HCC is still controversial, and definite conclusions have can only be provided by additional experiments. Thus, in-depth analysis of the function of HIF-2α in HCC may help to better understand the mechanism of development and metastasis of this tumor type and to improve the treatment methods. In conclusion, significant additional research effort is necessary to achieve an in-depth understanding of the role of HIFs in HCC.

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YG, ZX, DH and QX conceived and designed the review. YG, ZX, LY, YG, QZ, LH, DH and QX were involved in the collection and collation of references. YG and ZX collected and assembled the data presented in Table I. YG and ZX drew the figures. YG and ZX wrote the manuscript. All authors approved the final manuscript.

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