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REVIEW

The impact of hypoxia in hepatocellular carcinoma metastasis

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Abstract **Hypoxia is a common phenomenon in hepatocellular carcinoma (HCC). Hypoxia stabilizes transcription factor, hypoxia-inducible factor (HIF), to activate gene transcription. Expression of HIF is closely associated with metastasis and poor prognosis in HCC. HIF mediates expression of genes that are involved in every step of HCC metastasis including epithelial-mesenchymal transition, invasion of the extracellular matrix, intravasation, extravasation, and secondary growth of the metastases. Because HIF is the central regulator of HCC metastasis, HIF inhibitors are attractive tools when used alone or as combined treatment to curb HCC metastasis. This review will summarize the current findings on the impact of hypoxia/HIF in HCC, with a particular focus on cancer metastasis.**

Keywords hypoxia; hepatocellular carcinoma (HCC); metastasis; hypoxia-inducible factor (HIF)

Introduction

Hepatocellular carcinoma (HCC), the major form of primary liver cancer, is the fifth most common cancer in the world. HCC is particularly prevalent in South-east Asia including regions of China where chronic hepatitis B virus infection rate is high. HCC is the second most lethal cancer and the 5-year survival of HCC patients is less than 10%. Most HCC patients present symptoms at advanced stages when metastasis has already occurred. Metastasis not only limits the treatment option for HCC, it is the main cause of liver and organ failure, as well as tumor recurrence. Currently, there is no promising curative therapy for advanced or metastatic HCC. Only sorafenib has been shown to extend the survival rate of advanced-staged HCC patients for about 3 months [1,2]. Other palliative therapies for HCC include hepatic artery ligation, transcatheter arterial embolization (TAE), and transcatheter arterial chemoembolization (TACE). Although the principle of these palliative therapies is to restrict blood supply to suppress tumor growth, these therapies may result in a catastrophic physiological condition, hypoxia. Furthermore, due to the rapid-growing nature of HCC, hypoxia is also frequently found in regions of the tumor that are far away from functional blood vessels. Hypoxia further triggers a series of pro-metastatic molecular events in HCC and exacerbates the prognosis. Therefore, the molecular mechanisms driven by hypoxia have become attractive research interests. Understanding the roles of hypoxia and HCC metastasis may facilitate the intervention of new therapeutic strategies for advanced HCC. This review will summarize the molecular biology of hypoxia, the roles of hypoxia in HCC metastasis, and the potentials of therapeutic strategies targeting hypoxia in HCC.

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Molecular events mediated by hypoxia

The major difference between cancer cell and normal cell is that cancer cell growth is dysregulated and uncontrollable. Rapid cancer growth requires increasing consumption of oxygen (O_2). The drastic increase of O_2 demand cannot be met by the growth of functional vasculature leading to oxygen deprivation, hypoxia, in regions of the tumor (Fig. 1). In the case of HCC, hypoxia can also be created by conventional palliative therapies such as TAE/TACE. The median partial pressure of O_2 in human liver cancer is 6 mmHg as compared to 30 mmHg in normal liver [3]. HCC, like other cancer cells, adapts to hypoxia by transcriptional factors, hypoxia-inducible factors (HIFs). HIFs are heterodimers that consist of the constitutively expressed HIF-1β subunit and the O_2-sensitive HIF-1/2α subunit. Under normoxia, prolyl hydroxylases (PHDs) use O_2 as a substrate and hydroxylate the proline residues of 402 and 564 of HIF-1α (Fig. 2). Von Hippel-Lindau (VHL) recognizes the hydroxylated HIF-1α and targets HIF-1α for proteosomal degradation (Fig. 2).

Under hypoxia, HIF-1α can no longer be hydroxylated and therefore is stabilized (Fig. 2). HIF-1α then dimerizes with HIF-1β and binds to the hypoxia response elements (HRE) that encompass the consensus DNA sequence 5′-G/ACGTG-3′ to initiate transcription (Fig. 2). A HIF-1α homolog, HIF-2α, is regulated by O_2 by a similar mechanism. HIF-1α and HIF-2α share many common target genes but also have their unique sets of target genes [4].

HIF-1α and HIF-2α expression in human HCC

Due to the high frequency of intratumoral hypoxia found in human cancers, HIF-1α and HIF-2α should be frequently detected in cancer biopsies. However, examination and quantitation of HIF-1α and HIF-2α in human clinical examples are particularly challenging due to the extremely short half-life of HIF-1α/2α (~5 min). To detect HIF-1α and HIF-2α in human HCC samples, specimens have to be snap-frozen or fixed immediately after surgical resection. It is therefore hard to precisely assess the amount of HIF-1α and HIF-2α in resected HCC tumors in practical clinical situation. Despite these technical challenges, scattered studies demonstrated successful detection of HIF-1α and HIF-2α in human HCC specimens and their relation with patients' prognosis. Immunohistochemistry (IHC) staining showed that HIF-1α was highly expressed in 24/36 cases of HCC tissue and weakly expressed in 2/36 adjacent non-tumorous liver tissue [5]. No HIF-1α expression was found in the 6 cases of normal liver tissue [5]. Expression of HIF-1α was significantly higher in HCC with microscopic venous invasion [5]. Expression of HIF-1α also correlated with decreased survival rate in HCC patients [6]. A tissue microarray study of 309 HCC specimens further showed that HIF-1α expression was significantly associated with lymph node metastasis [7], which is found in 7.45% of HCC patients and is related to cancer death [8,9]. An independent study showed that HIF-1α expression was detected in liver dysplastic nodules, which are pre-neoplastic lesions of HCC in both diethynitrosamine (DEN)-treated mice and human subjects [10]. Also demonstrated by IHC staining, HIF-2α expression was found in 219/315 cases of HCC tissue [11]. Expression of HIF-2α was found to be higher in HCC tissue than non-cancerous liver tissue in most of the cases. Interestingly, HIF-2α expression was detected in HCC cells and infiltrated macrophages, but not in other

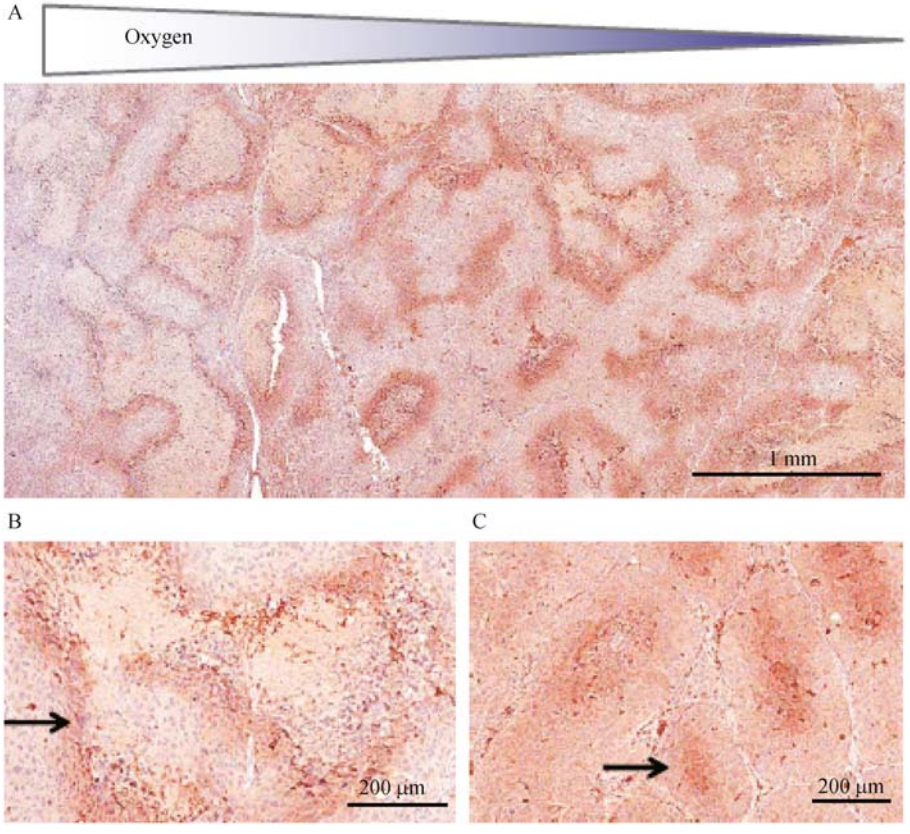

Fig. 1 Hypoxia is found in regions of HCC. Immunohistochemistry staining of pimonidazole shows hypoxic regions of HCC tumor derived from HCC cells, MHCC-97L, that were orthotopically implanted in nude mice. (A) O_2 level reduces from peripheral to central region of the HCC tumors. Hypoxic regions are commonly found in (B) peri-necrotic regions and (C) central regions of HCC.

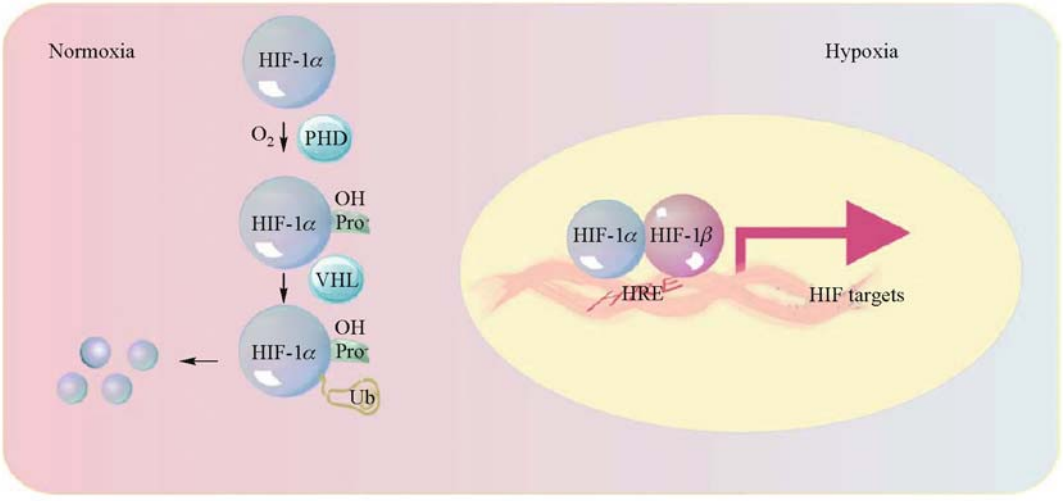

Fig. 2 Molecular biology of hypoxia. In the presence of O_2 (normoxia), PHDs hydroxylate HIF-1α at the proline residues that are recognized by VHL, targeting HIF-1α for ubiquitin-mediated proteosomal degradation. In the absence of O_2 (hypoxia), HIF-1α dimerizes with HIF-1β to initiate gene transcription.

stromal cells within the tumors [12]. Furthermore, expression of HIF-2α significantly correlated with invasive HCC features such as portal vein invasion, capsule infiltration, and intrahepatic metastasis [11,12]. HIF-2α expression was also significantly associated with tumor size, tumor grade, necrosis, and angiogenesis in human HCC [11,12]. HIF-2α expression was found to be associated with shorter survival in HCC patients [11].

Seven-gene signature in human HCC

Recently, a seven-gene signature associated with hypoxia has been reported in HCC and its prognostic relevance was further examined [13]. From 4 independent cohorts of HCC patients, a seven-gene signature has been found to be significantly associated with poor survival and early recurrence in HCC patients. Notably, van Malenstein *et al.* have developed a hypoxia score based on this seven-gene signature and reported that patients with a score of $\leqslant 0.35$ had longer survival period compared to those patients with a score of > 0.35 (307 days versus 1602 days) [13]. These findings implicated that the degree of hypoxia correlated with the aggressiveness of HCC and the survival outcome of patients.

HCC metastasis

HCC metastasis is a multi-step process. HCC metastasis begins with the invasion of HCC cells from the primary HCC tumor to the extracellular matrix (ECM) of the neighboring stroma. HCC cells then disseminate into the circulation system through intravasation. Being able to survive in the circulation system, HCC cells then extravasate, eventually enter and colonize the secondary tissue site. In intrahepatic metastasis, the portal vein transports HCC cells from the primary site to other parts of the liver. In extrahepatic metastasis, hepatic vein transports HCC cells from the primary site in the liver to the systemic blood stream. The heart then pumps the circulating HCC cells to the lung, and eventually to other organs such as bones, brain, and adrenal glands. Each step of HCC metastasis is supported by different pro-metastatic genes. Interestingly, hypoxia/HIF-1α have shown to be central regulators of many pro-metastatic genes and are thus ultimately involved in every step of HCC metastasis (Fig. 3).

Hypoxia and cell motility in HCC

Epithelial-mesenchymal transition (EMT) describes a phenomenon when cells lose the epithelial property and gain mesenchymal property and this involves loss of cell-cell contact and cell polarity. EMT increases cell migratory and invasive ability and is associated with the first step of cancer metastasis. Loss of E-cadherin and gain of vimentin are typical hallmarks of EMT. Furthermore, the expressions of the transcription repressors of E-cadherin, including SNAIL, TWIST, TCF3, ZEB1, and ZEB2, increase during EMT and serve as markers of EMT. Multiple studies have demonstrated that hypoxia induces EMT, as marked by loss of E-cadherin, increase of vimentin, SNAIL, and TWIST in different HCC cell lines [6,14]. HIF-1α expression was shown to inversely correlate with E-cadherin and positively correlate with N-

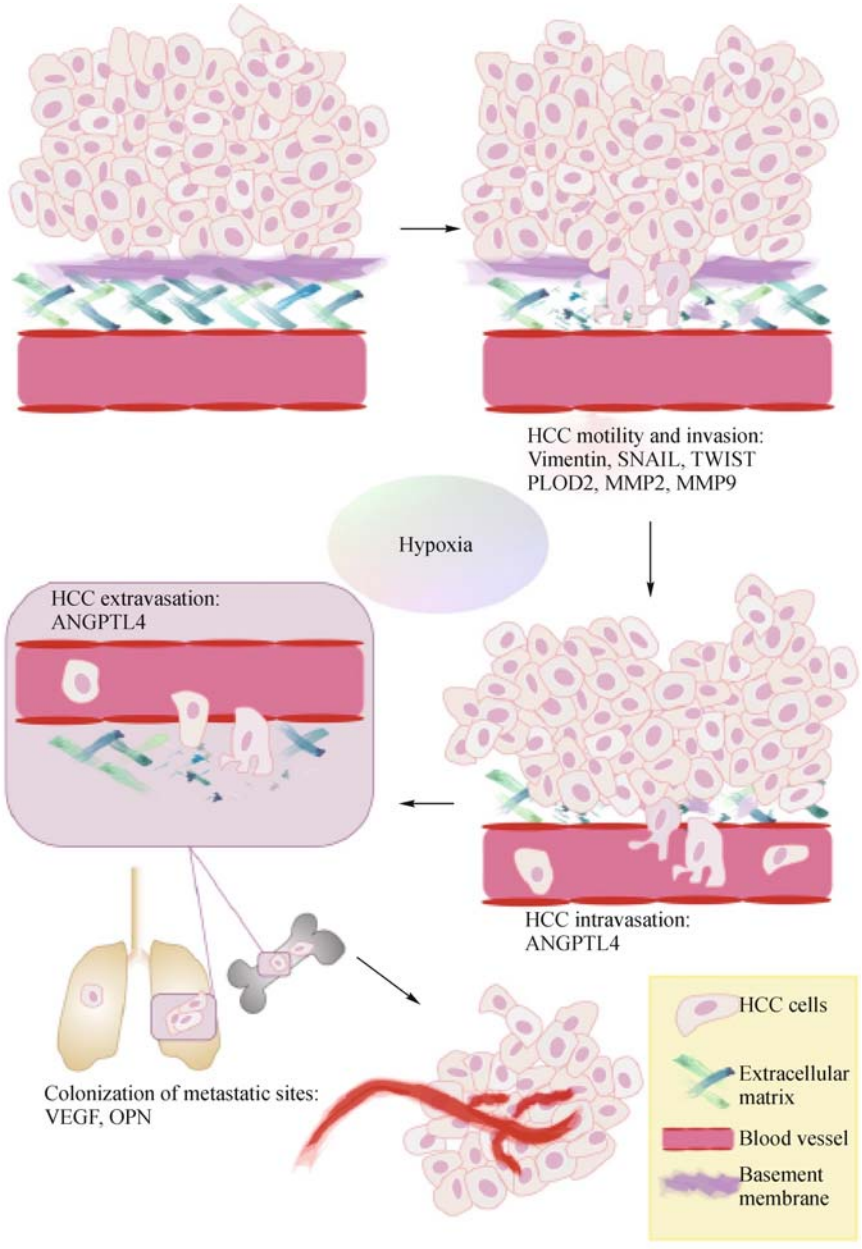

Fig. 3 Hypoxia and HCC metastasis. Hypoxia regulates every step of HCC metastasis by inducing genes involved in cell motility and invasion, intravasation, extravasation, and colonization of the metastatic sites.

cadherin in human HCC biopsies [6]. Furthermore, HCC cells exhibit mesenchymal morphology and demonstrated increased migratory and invasive abilities upon hypoxia exposure [6]. Treatment of cobalt chloride ($CoCl_2$), a metal chelator that inhibits the activity of PHDs resulting in HIF-1α stabilization, also induced EMT and promoted HCC cell migration and invasion [6]. Two HREs were found in the promoters of SNAIL and were confirmed to be functional HRE by luciferase reporter assay in HCC cell line [6]. SNAIL was further shown to be significantly correlated with HIF-1α and HIF-2α expression in HCC [6].

Hypoxia and cell invasion in HCC

Extracellular matrix modifying enzymes

The major component of the ECM is collagen I, a fibrous collagen. The synthesis and modification of collagen I rely on multiple enzymes. Procollagen-lysine, 2-oxoglutarate 5-dioxygenase (PLOD) catalyzes the hydroxylation of lysine residues in collagen molecules, thereby allowing carbohydrate to attach to and stabilize the collagen [15]. This modification of collagen prevents collagen degradation,

hence facilitates collagen accumulation which is directly associated with fibrosis [15]. A recent study shows that fibrosis mediates metastasis [16]. Dense collagen fiber matrix provides tracks for cancer cell movement, thereby directs and enhances cancer cell motility [17]. The PLOD family has three members, namely PLOD1-3. Hypoxia induces different members of PLOD family in different cancer cell types. PLOD1 and PLOD2 have been shown to be induced by HIF-1α in breast cancer cell lines. PLOD2 is the only member that was induced by hypoxia in HCC cell lines, Huh7 and HepG2 [18]. High expression of PLOD2 was associated with lower disease-free survival rate in HCC patients, as compared to the patients with low expression of PLOD2 [18]. PLOD2 overexpression correlated with larger HCC tumor size and the presence of macroscopic intrahepatic metastasis [18].

The matrix metallopeptidase (MMP) family members are the major proteases that degrade the ECM. MMPs are initially produced as an enzymatically inactive form (zymogen). MMPs become active when it is cleaved intracellularly by furin or extracellularly by other MMPs or serine proteinases [19]. MMPs are inhibited by a family of proteins called tissue inhibitor of metalloproteinases which are also present in cancers (TIMPs) [20]. TIMPs bind and inhibit MMPs in a 1:1 stochiometric ratio. The MMP family is divided into six subgroups and each has its specific substrate preference. MMP2 and MMP9 belong to the gelatinase subgroup that mainly degrades gelatin and collagen IV in the ECM. MMP2 and MMP9 are critical to the early step of metastasis as degradation of the ECM facilitates cell invasion into the basement membranes of the neighboring stroma and the blood vessels. Furthermore, degradation of ECM liberates the availability of bioactive molecules such as VEGF that further promotes metastasis. MMP2 and MMP9 were shown to be regulated by HIF-1α and HIF-2α, respectively [21,22]. MMP2 neutralizing antibodies reduced angiogenesis and HCC invasion in capillary tube formation assay and matrigel-coated transwell assay, respectively [23]. Early study demonstrated that MMP9 mRNA was frequently upregulated in 15/16 human HCC specimens. Higher MMP9 expression was found in HCCs with tumor capsular infiltration than those without. Many studies showed that MMP2 expression was not altered in HCC relative to non-tumorous liver tissue [24,25]. However, serum and tissue expression of an MMP2 inhibitor, TIMP2, was found to be lower in HCC patients with metastasis than those without, suggesting that MMP2 activity might be elevated in HCC and contribute to metastasis [25]. High MMP2 expression was found to be correlated with lymph node metastasis in HCC patients [7] and localized in the invasive edge of metastatic HCC tissue.

Hypoxia, intravasation, and extravasation in HCC

Angiopoietin-like 4 (ANGPTL4) is a secretory protein that plays an important role in intravasation and extravasation. ANGPTL4 disrupts the cell-cell junctions between endothelial cells (ECs) and allows cancer cells to enter and exit the blood stream and permeate the lung capillaries [26]. This finding was first demonstrated by an experiment in which breast cancer cells were able to invade through the EC monolayers that were exposed to media containing ANGPTL4 [26]. It was later discovered that ANGPTL4 is a direct target of HIF-1α and HIF-2α [27]. More breast cancer cells were able to invade through EC monolayers that were exposed to media generated by hypoxic cells than normoxic cells [27]. Furthermore, conditioned medium from hypoxic cells was able to decrease transendothelial electrical resistance of EC monolayer which indicates a disruption of EC-EC interaction [27]. Such hypoxia-induced effect is dependent on HIF-1α, HIF-2α, and ANGPTL4 in breast cancer [27]. However, in HCC, ANGPTL4 was only regulated by HIF-1α but not HIF-2α [14]. Short interference RNA against HIF-1α but not HIF-2α suppressed hypoxia-induced ANGPTL4 expression in multiple HCC cell lines such as MHCC97L and SMMC-7721 [14]. IHC study also showed that ANGPTL4 protein expression significantly correlated with HIF-1α expression in HCC tissue [14]. Functionally, secreted ANGPTL4 was shown to be important for transendothelial migration of HCC cells. Secreted ANGPTL4 in HCC patients correlated significantly with intrahepatic metastasis and lung metastasis [14].

Hypoxia and colonization in HCC

Circulating cancer cells may reside dormant or may not even survive in the secondary organs. Being able to adapt and propagate at a foreign soil is a rate-limiting step in cancer metastasis. Hypoxia regulates several secretory factors that facilitate cancer growth at the secondary organs.

Vascular endothelial growth factor (VEGF) is a well-characterized HIF direct target. VEGF binds to its receptor, VEGFR, to activate the downstream signaling network responsible for angiogenesis (the growth of new blood vessels from preexisting blood vessels) and vasculogenesis (the growth of *de novo* blood vessels from endothelial cells). VEGF and VEGFR play multiple roles in cancer metastasis. VEGF promotes angiogenesis in the secondary organs. Furthermore, VEGF/VEGFR is implicated in metastatic niche formation. An elegant study has clearly demonstrated that VEGFR1 + bone marrow derived cells (BMDCs) can be detected at the secondary organs prior to cancer cell arrival [28]. VEGFR1 + BMDCs create a favorable metastatic microenvironment for cancer cell adherence and growth by expressing MMP9, fibronectin, and SDF-1 [28]. In HCC, an *in situ*-hybridization study showed that strong VEGF signal was found in HCC particularly in the necrotic regions [29]. VEGF was induced by hypoxia in three HCC cell lines Huh7, SkHepG1, and HepG2 [29]. Luciferase reporter assay performed in HepG2 cell line further confirmed that HRE found in the promoter of VEGF is functional [29].

Interestingly, monoclonal neutralizing antibodies against VEGFR1 and/or VEGFR2 profoundly suppressed HCC formation and spontaneous lung metastasis in a chemical (diethylnitrosamine, DEN)-induced HCC mouse model [30].

Osteopontin (OPN) is a glycoprotein that is involved in cell-matrix interaction. It facilitates the attachment of osteoclasts to the mineralized bone matrix. OPN is highly expressed in different cancer tissue [31]. Breast cancer cells that metastasized to the bone had increased OPN expression than those that metastasized to the adrenal medulla [32]. Stable silencing OPN in breast cancer cells suppressed their attachment to the human bone marrow endothelial cells [33]. These data suggest that OPN may facilitate adherence and subsequent growth particularly in the bone as secondary organs. An array study comparing HCC samples with or without intra-hepatic metastasis first revealed OPN as a gene necessary for HCC metastasis [32]. OPN also predicted advanced tumor stage and tumor recurrence of HCC and was closely associated with decreased patient survival [34]. A neutralizing antibody against OPN suppressed the invasive ability of HCC cell lines, SK-Hep-1 and Hep3B [35]. OPN neutralizing antibody also blocked lung metastasis in nude mice that were subcutaneously injected with HCC-LM3 cells, a highly metastatic clone of MHCC-97 cells [36]. A subsequent study using lentiviral-mediated miRNA against OPN yielded a consistent observation and further revealed that OPN promoted metastasis through activation of MEK/ERK1/2 pathway [37]. Furthermore, recombinant OPN activated MMP-2 and nuclear accumulation of NF-κB in HCC-LM3 [37]. Interestingly, hypoxia induced the expression of OPN in head and neck squamous cell carcinoma cell lines and NIH3T3 cells [38]. Hypoxia-induced expression of OPN is mediated through AKT [38]. These studies focused on the roles of OPN in pulmonary metastasis. OPN particularly benefits bone metastasis in breast cancer model and bone is also a common metastatic site for HCC. Although the question of whether OPN participates in HCC metastasis to bone is interesting, there is currently no *in vivo* model to evaluate HCC metastasis of bone. Therefore, further exploration of new *in vivo* metastatic HCC model is warranted for better understanding of the molecular mechanisms of HCC metastasis beyond lung as secondary organs.

HIF-interacting partners in HCC

Many proteins participate in the stabilization or activation of HIF-1α and are shown to be important players in HIF-1α-mediated HCC metastasis.

P28^{Gank} (Gankyrin) is a component of the 26S proteasome and acts as a chaperone during the assembly of the 26S proteasome. P28^{Gank} is known to be an oncoprotein in HCC [39,40]. Overexpression of P28^{Gank} in HCC cells activated the transcriptional activity of HIF-1α and subsequently promoted EMT and angiogenesis [41]. EMT was induced by P28^{Gank} as marked by the decrease of E-cadherin expression, increase of vimentin, N-cadherin, and TWIST expression in HCC cells [41]. Knockdown of HIF-1α abolished P28^{Gank}-mediated EMT [41]. P28^{Gank} also increased VEGF and MMP2 expression [41]. Reversely, silencing of P28^{Gank} suppressed EMT and HCC cell motility [41]. Overexpression of P28^{Gank} in HCC cells promoted lung metastasis and angiogenesis in orthotopic tumor implantation model [41]. Interestingly, inhibition of AKT suppressed HIF-1α protein expression and P28^{Gank}-induced HCC metastasis [41]. Demonstrated in HCC specimens, P28^{Gank} protein expression elevated from normal liver, to non-invasive primary HCC, and to invasive HCC (with vascular invasion and tumor thrombus) [41].

Prospero-related homeobox 1 (PROX1) is a member of the homeobox transcription factor family. Co-immunoprecipitation experiment showed that PROX1 interacts with the HIF-1 complex in HEK293T cells and HCC cells [42]. PROX activated HIF-1α transcription and further stabilized HIF-1α, thereby mediating EMT in HCC cells [42]. Overexpression of PROX in HCC cell lines resulted in decrease of E-cadherin and increase of vimentin expression while knockdown of PROX resulted in increase of E-cadherin and decrease of vimentin expression [42]. Re-expression of HIF-1α restored EMT in the PROX knockdown cells and promoted HIF-1α-mediated HCC metastasis *in vitro* and *in vivo* [42]. The tissue microarray study showed that high PROX1 expression was significantly associated with decreased overall survival rate and shortened time to tumor recurrence in two independent cohorts of HCC patients [42].

These two studies mentioned above have unraveled the molecular mechanisms that regulate HIF-1α-mediated EMT in HCC.

Inhibitor of DNA binding 1 (ID-1) is a helix-loop-helix (HLH) protein that forms heterodimers with other HLH family of transcription factors. ID-1 has no DNA binding activity hence acts as a transcriptional inhibitor on its interacting HLH proteins [36]. ID-1 can stabilize HIF-1α in HCC cells and subsequently enhance VEGF secretion. In capillary tube formation assay, conditioned medium generated by ID-1 overexpressing HCC cells induced HUVEC (human umbilical vein endothelial cell) elongation and tube formation [35]. Antisense ID-1 reduced HCC tumor burden and angiogenesis in nude mice. Furthermore, metastatic HCC tissue (53/59 cases, intrahepatic and extrahepatic) showed strong ID-1 staining when compared to primary HCC tissue (25/59 cases), indicating that ID-1 was implicated in HCC metastasis [35]. ID-1 expression was significantly associated with VEGF expression in HCC [35]. This study revealed a novel mechanism which drives HIF-1α-mediated angiogenesis in HCC.

Pharmacological insight

From a clinical point of view, studying the biology of hypoxia

adaptation provides translational insights to HCC treatment. In addition to the rapid proliferation of HCC cells and the presence of abnormal tumor vasculature, hepatic ischemia induced during TAE/TACE treatment results in hypoxia in HCC tumors. Although TACE has been widely used as a standard treatment for inoperable patients, the overall response rates are inconsistent among different patients [43,44] and the long-term survival benefits are far from satisfactory [45,46]. In fact, it has been suggested that hypoxia induced by TACE might enhance the aggressiveness of HCC tumors, as reflected by the increase of angiogenesis and the accelerated tumor doubling time after the initial remission of TACE, as well as the disappointing tumor recurrence rate following the treatment [45–47]. A recent study using preclinical HCC model has demonstrated that combination of TAE with antisense oligonucleotides against HIF1α inhibits tumor angiogenesis and growth [48]. This finding supports the notion that HIF1α signaling is a promising direction to tackle the hypoxic responses in HCC elicited by TACE.

As discussed in this review, HIF-1α is involved in activating a broad array of signaling pathways that support the metastatic cascades of hypoxic cancer cells [49,50]. Inhibitors targeting hypoxia/HIF are therefore good therapeutic option for patients who have advanced or metastatic HCC or who undergo TAE/TACE. A recent study has found that sorafenib may serve as an HIF inhibitor in HCC [34]. Sorafenib suppresses HIF-1α accumulation and activation in an HCC cell line, PLC [34]. Sorafenib inhibits HIF protein synthesis through suppressing the mTOR/p70S6K/4E-BP1 protein translational pathway [34]. Sorafenib may also reduce VEGF production and suppress the growth and angiogenesis in nude mice bearing the PLC tumors [34]. Of note, this animal study used a high dose of sorafenib (100 mg/kg) as compared to the dose used in pre-clinical study (10–30 mg/kg); it is still unclear whether the pre-clinical dose of sorafenib inhibits HIF activity. Sorafenib is the only drug that has been shown to improve prognosis of advanced HCC patients. Two independent clinical trials have demonstrated that sorafenib could prolong the overall median survival of advanced HCC patient for about 3 months [1,2]. Whether this is related to HIF remains an important question to explore, as sorafenib is a multikinase and can also suppress the Raf/MEK/ERK pathway and receptor tyrosine kinases such as VEGFR-2, VEGFR-3, Flt-3, c-KIT, and PDGFR.

Conclusions

Hypoxia is a common finding in HCC. Compelling evidence has shown that hypoxia is an important regulator of HCC metastasis. Clinical data have also suggested that HIF is overexpressed in human HCCs and is associated with an increased risk of mortality due to HCC metastasis. HIF induces the expression of many pro-metastatic genes and those that have been verified in HCC models, including the EMT-driving genes (vimentin, SNAIL, and TWIST), ECM modifying enzymes (PLOD2, MMP2, MMP9), secretory factors that facilitate the permeability of blood vessels (ANGPTL4 and VEGF), and secretory factors (VEGF and OPN) that promote growth in the secondary organs. The expressions of these genes in human HCC are consistently overexpressed and associated with increased metastasis. Furthermore, several interacting partners of HIF in HCC participate in HCC progression. HIF is considered the central player that confers metastatic potential to hypoxic HCC cells and is therefore an attractive therapeutic target. Intriguingly, sorafenib, the only drug that could prolong the survival of advanced stage HCC patients, coincidentally suppresses HIF. More evidence is warranted to demonstrate whether sorafenib improves the prognosis of HCC patients through HIF inhibition. This evidence will not only improve the current therapeutic regimen of HCC but also open up new avenues for the investigation of novel and existing HIF inhibitors for HCC treatment.

Compliance with ethics guidelines

Carmen Chak-Lui Wong, Alan Ka-Lun Kai, and Irene Oi-Lin Ng declare that they have no conflict of interest. This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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