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MINIREVIEW

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HIF-1 — A BIG CHAPTER IN THE CANCER TALE

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Approximately 1.0–1.5% of the genome is transcriptionally regulated by hypoxia, and hypoxia-inducible factor (HIF)-1α is the transcription factor modulating many of these genes. Cancer cells are able to survive hypoxic environments and hypoxia itself can activate adaptive cellular responses that contribute to tumor progression. Many HIF-1α-mediated biological effects are beneficial for tumor progression, including metabolic shift toward glycolysis, inhibition of fatty acid β-oxidation, production of cellular reactive oxygen species and altering expression of tumor suppressor genes. HIF-1 promotes selective mitochondrial autophagy, resistance to T cell mediated lysis of cancer cells, induction of pluripotent cancer stem cells, epithelial-mesenchymal and epithelial-mesenchymal-endothelial transitions beneficial for tumor growth and progression, loss of E-cadherin. HIF-1 also induces production of signal molecules and cytokines by carcinoma-associated fibroblasts and upregulation of certain microRNAs important for cancer progression. This minireview focuses on the HIF-1 promoting role in tumor initiation and progression and HIF-1 targeting. HIF-1 pathway downregulation seems to be promising in future cancer treatment.
Key Words: **adaptive cellular response, cancer stemness, hypoxia, hypoxia-inducible factor 1, tumor progression.**

Hypoxia-inducible factor (HIF)-1 plays an essential role in cellular and systemic oxygen homeostasis [1]. Approximately 1.0–1.5% of the genome is transcriptionally regulated by hypoxia, and HIF-1α is the transcription factor modulating many of these genes [2]. Under normoxic conditions, HIF-1α has a half-life in cytoplasm of < 5 min, but hypoxic conditions stabilize HIF-1α, which escapes degradation and translocates to the nucleus [2]. In the nucleus, dimerisation of HIF-1α with HIF-1β under hypoxic conditions results in the formation of the HIF-1 transcription factor, which binds to hypoxia response elements and activates the transcription of O_2-dependent genes [3]. Insufficient oxygen supply produces stressful environments for normal cells and can promote cell death in hypoxic regions by either apoptosis or necrosis [3].

Poorly vascularized and perfused tumor microareas in many aggressive cancers have limited access not only to oxygen but also to glucose. Core regions are also associated with acidic pH since these tumor cells change their metabolism towards increased glycolysis, resulting in increased lactic acid production. Abnormal vascularization of solid tumors results in the development of microenvironments deprived of oxygen and nutrients that harbour slowly growing and metabolically stressed cells. Such cells display enhanced resistance to standard chemotherapeutic agents and repopulate tumors after therapy [4].

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Abbreviations used: ALL – acute lymphoblastic leukemia; BNIP3 – BCL2/adenovirus E1B 19 kDa interacting protein 3; BNIP3L – BNIP3-like; CAF – carcinoma-associated fibroblast; CSC – cancer stem cell; CTGF – connective tissue growth factor; EMT – epithelial-mesenchymal transition; FAO – fatty acid β-oxidation; GPER – G protein-coupled estrogen receptor; HIF-1 – hypoxia-inducible factor 1; LCAD – long-chain acyl-CoA dehydrogenase; MCAD – medium-chain acyl-CoA dehydrogenase; miR – microRNA; NK – natural killer; ROS – reactive oxygen species; TAZ – transcriptional co-activator with PDZ-binding motif; VEGF – vascular endothelial growth factor.

Cancer cells are able to survive hypoxic environments and hypoxia itself can activate adaptive cellular responses that contribute to tumor progression [5]. Many HIF-1α-mediated biological effects are beneficial for tumor progression, including shifting metabolism toward glycolysis, induction of angiogenesis, regulation of apoptosis, induction of migration to escape hostile hypoxic environments, and therapy resistance [5, 6].

Induction of HIF-1α has two effects in tumor cells: i. HIF-1α triggers the phosphorylation of Src which subsequently phosphorylates the tyrosine residue Y705 of STAT3 (signal transducer and activator of transcription 3); ii. HIF-1α activates autophagy by a mechanism implicating the increased expression of BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3)/BNIP3-like (BNIP3L) and the dissociation of the beclin 1-BCL2 (B cell lymphoma 2) complex [7].

By upregulating the expression of BNIP3 and BNIP3L, HIF-1 promotes selective mitochondrial autophagy [7]. Autophagy is typically a stress adaptation mechanism in response to a variety of stress stimuli including starvation, hypoxia, endoplasmic reticulum stress, and oxidative stress that trigger a pro-survival pathway avoiding cell death. In cancer therapy, autophagy is activated as an adaptive response to promote cell survival. Autophagy ensures cell metabolism, supplies tumor cell survival and prevents cancer cells from accumulating dysfunctions [8]. During cancer progression, autophagy can be induced by different stresses, particularly hypoxia, nutrient deprivation, or extracellular matrix detachment. Recent evidence has demonstrated that tumor cells can escape natural killer (NK)-mediated immune surveillance by activating autophagy under hypoxia [7].

An interesting recent report has proved that lymphoid effectors not only provide lytic signals but also promote autophagy in the remaining target cells, a process called cell-mediated autophagy [7]. Thus, cell-mediated autophagy has been reported in dif-

ferent human epithelial tumors after interaction with immune cells at a high ratio of effectors to targets. Importantly, it has been shown that cell-mediated autophagy not only acts as a mechanism of resistance to immune cell-mediated lysis but also limits the cytotoxic activity of stress factors such as γ-radiation [7]. BNIP3L and BNIP3 also play roles in the mediation of mitophagy allowing the cells to get rid of dysfunctional mitochondria and promote virus-specific NK cell survival during the contraction phase of the response to murine cytomegalovirus. It has implications for the development of effective antiviral and anticancer treatments, as well as vaccination strategies targeting the autophagy pathway in NK cells [9].

In breast cancer in response to reduced O_2 availability, HIF-1 mediates the transcriptional activation of genes encoding proteins that are required for many important steps in progression, such as angiogenesis, cancer stem cell (CSC) maintenance, cell motility, epithelial-mesenchymal transition (EMT), extracellular matrix remodeling, metabolic reprogramming, metastasis, and resistance to therapy [10]. HIF-1 binds directly to gene encoding the transcriptional coactivator with PDZ-binding motif (TAZ), an effector of Hippo signaling. TAZ interacts with DNA binding proteins to activate transcription of target genes, such as *CTGF* (encoding connective tissue growth factor), which promote EMT and the breast cancer stem-cell phenotype bidirectional functional interactions between HIF-1α and TAZ that synergistically drive the expression of downstream target genes in hypoxic breast cancer cells [10].

Carcinoma-associated fibroblasts (CAFs) play a pivotal role in cancer progression by contributing to invasion, metastasis and angiogenesis [11]. Hypoxia upregulates HIF-1α, G protein-coupled estrogen receptor (GPER) and smooth muscle actin α expression in CAFs, and induces the secretion of interleukin-6, vascular endothelial growth factor (VEGF), and CTGF in CAFs [12]. As a biological counterpart of these findings, conditioned medium from hypoxic CAFs promotes tube formation in human umbilical vein endothelial cells in a HIF-1α/GPER dependent manner. The functional cooperation between HIF-1α and GPER in CAFs was also evidenced in the hypoxia-induced cell migration, which involves a further target of the HIF-1α/GPER signaling like CTGF [13].

HIFs may promote a CSC state, whereas the loss of HIFs induces the production of cellular reactive oxygen species (ROS) and activation of p53 and $p16^{INK4A}$ proteins, which lead to tumor cell death and senescence. Hypoxic conditions enhance the generation of induced pluripotent stem cells. During reprogramming of somatic cells into a PSC state, cells attain a metabolic state typically observed in embryonic stem cells. The embryonic stem cells and induced pluripotent stem cells share similar bioenergetic metabolisms, including decreased mitochondrial number and activity, and induced anaerobic glycolysis [14].

In tumor, tissue hypoxia plays a critical role in cancer cell maintenance. In particular, Twist1 expression is directly triggered by HIF-1 while the transactivation function of Twist1 has been implicated in EMT [15].

CSCs usually represent a small percentage of cells residing in a tumor mass. Nevertheless, since CSCs have the abilities for self-renewal and differentiation into secondary tumors, it is believed that CSCs are responsible for resistance of cancer cells to conventional chemo-/radiation therapy. Recent experimental evidence suggests that the EMT process indeed contributes to stem-like properties of cancer cells [15].

Since cancer stemness is maintained by HIFs, the HIF-inducible Twist1 may be directly involved in regulating expression of stemness genes, promoting synergetic EMT, and self-renewal capability. Twist1 activation provides several advantages to cancer cells. At the individual cellular level, Twist1-mediated EMT enhances cancer cell ability to move and invade [15]. The Twist1-mediated stemness provides cancer cells with the capability of self-renewal and, more importantly, resistance to conventional anti-cancer chemotherapy. At the multicellular tumor level, Twist1 can extend EMT one step further, to the epithelial-mesenchymal-endothelial transition. In this concept, Twist1-overexpressing tumor cells can trans-differentiate into endothelial cells and directly contribute to the angiogenesis process [15].

The recent discovery that a subset of cellular microRNAs (miRs) are upregulated during hypoxia, where they function to promote tumor development, highlights the importance of hypoxia-induced miRs as targets for continued investigation. Under hypoxic conditions, miR-210 becomes highly upregulated in response to HIFs. HIF-1α drives miR-210 overexpression and the resultant alteration of cellular processes, including cell cycle regulation, mitochondria function, apoptosis, angiogenesis and metastasis [16].

The migration of tumor cells is mediated by loss of E-cadherin, which results in a more invasive phenotype, dissemination of the tumor cells by increased vascular permeability and survival in the bloodstream through resistance to apoptosis as well as adhesion at the premetastatic niche, all of them being controlled by factors under the influence of HIF-1 [17].

HIF-1α inhibits fatty acid β-oxidation (FAO) by inhibiting two FAO enzymes, the medium-chain acyl-CoA dehydrogenase (MCAD) and long-chain acyl-CoA dehydrogenase (LCAD) that catalyze the first step of FAO in mitochondria. Both MCAD- and LCAD-regulated FAO facilitates tumor cell proliferation by altering ROS levels and glycolysis. However, deficiency of LCAD-induced unsaturated fatty acid oxidation affects largely cancer progression by regulating the PTEN pathway [18] and enabling rapid growth and division of cancer cells.

Recently, Krtolica et al. [19] assayed the efficacy of OMX-4.80P (long-acting oxygen carrier in circulation and tumor) that downregulates HIF-1 pathway in intracranial glioblastoma models in nude mice,

immunocompetent rats and in spontaneous canine brain tumors. The preclinical data demonstrate that hypoxia reduction and HIF-1 pathway downregulation dramatically enhance response to radiation therapy leading to tumor cures.

In the recent study, 63% of examined 261 invasive breast cancers from an African population showed strong HIF-1α expression, which was significantly associated with other factors of poor prognosis like VEGF expression and increased angiogenesis, high tumor cell proliferation by Ki-67 rate, p53 expression, as well as high histological tumor grade [20]. Nuclear expression of HIF-1α was found in 97% cases of endometrial cancer patients. Higher HIF-1α expression was associated with higher risk of recurrence [21]. Immunohistochemical studies demonstrated that 40% of examined primary pancreatic cancers were HIF-1α positive and the status of HIF-1α was significantly correlated with metastatic status, VEGF expression and intratumoral microvessel density [22]. Overexpression of HIF-1α was found to be an indicator of poor prognosis for patients with gastric cancer and was significantly correlated with histology, depth of invasion, VEGF, and microvessel density [23, 24].

HIF-1α overexpression was more frequent in patients with hepatic metastases in peritoneal cavity. The patients with HIF-1α overexpression had a shorter disease-free survival and overall survival than patients with weak expression [25]. High HIF-1α expression is associated with the development and progression of hepatocellular carcinoma [26]. Oxygen-regulated component of HIF-1 (HIF-1α) is overexpressed in clusters of leukemic cells in the bone marrow specimens of childhood acute lymphoblastic leukemia (ALL) and absent in biopsies of normal bone marrow [27]. Half of HIF-1α-positive ALL biopsies exhibited VEGF coexpression. In all ALL specimens, HIF-1α immunostaining was concentrated primarily within the nuclei of leukemic cells.

Considering the multiple roles of HIF-1 in tumor progression and metastasis, there has been great interest in developing inhibitors targeting this pathway. Most of the reported HIF-1α inhibitors were originally discovered for targeting other endogenous molecules and later their HIF-1α inhibitory activity was recognized through some empirical testing [28]. In particular, cardiac glycosides were identified as inhibitors of HIF-1α. Digoxin decreases cell proliferation and viability in liver cancer cell line [29].

Cisplatin downregulates the level of the regulatable α subunit of HIF-1, HIF-1α, in cisplatin-sensitive ovarian cancer cells through enhancing HIF-1α degradation but does not downregulate HIF-1α in their cisplatin-resistant counterparts [30]. Pharmacological promotion of HIF-1α degradation enhances response to cisplatin in both cisplatin-sensitive and cisplatin-resistant ovarian cancer cells. These findings suggest that the HIF-1α-regulated cancer metabolism pathway could be a novel target for overcoming cisplatin resistance in ovarian cancer.

EZN-2698, an antisense oligonucleotide against HIF-1α, is being tested in a Phase I clinical trial with advanced solid tumors [28, 31]. Temsirolimus and everolimus have passed Phase III clinical trials and FDA approval for treatment of metastatic renal cell carcinoma [28]. Combination treatment with everolimus, letrozole and trastuzumab is well tolerated and effective in estrogen receptor$^+$, HER2$^+$ and/or mutant solid tumors, including breast and cervical cancer [32]. Everolimus has already received regulatory approval in combination with exemestane for the treatment of aromatase inhibitor-refractory metastatic hormone receptor-positive breast cancer [33].

Another HIF inhibitor, topotecan, is in clinical trial in advanced, retractory non-small cell lung cancer [31]. Trafalis et al. [34] in Phase II study investigated the efficacy and safety of DNA topoisomerase I inhibitor irinotecan plus bevacizumab, a monoclonal antibody against VEGF (BEVIRI) in patients with relapsed chemoresistant small cell lung cancer. BEVIRI combination in relapsed chemoresistant small cell lung cancer demonstrates promising efficacy and low toxicity compared to historical controls [34]. In NAPOLI-1, global, randomized Phase III study evaluating nal-IRI — a nanoliposomal irinotecan — with or without fluorouracil and leucovorin in 417 patients with metastatic pancreatic cancer previously treated with gemcitabine-based therapy, overall survival benefit for nal-IRI plus fluorouracil and leucovorin over fluorouracil and leucovorin with a similar safety profile has been demonstrated [35]. Combination chemotherapy with oral S-1 and biweekly 24-hour infusions of irinotecan plus bevacizumab appears to be highly active and well tolerated both as first-line and second-line chemotherapy for metastatic colorectal cancer [36]. Inhibition of HIF-1α by PX-478 enhances the antitumor effect of gemcitabine by inducing immunogenic cell death in pancreatic ductal adenocarcinoma [37].

In summary, blocking hypoxia and HIF-1 pathway is beneficial in targeting solid cancers. Nevertheless, there have been several approved drugs that affect the HIF-1α pathway and could serve as adjuvant therapy for certain types of cancers along with the existing treatments. Future developments would be directed towards the drugs that are more specific for HIF-1 inhibition [28].

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