Review

Hypoxia-Inducible Factors: Master Regulators of Cancer Progression

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Abstract

Intratumoral hypoxia (reduced O₂ availability) is a common finding in human cancer and leads to increased activity of hypoxia-inducible factors (HIFs), which regulate the expression of genes that contribute to angiogenesis, metabolic reprogramming, extracellular matrix remodeling, epithelial-mesenchymal transition, motility, invasion, metastasis, cancer stem cell maintenance, immune evasion, and resistance to chemotherapy and radiation therapy. Conventional anti-cancer therapies target well-oxygenated and proliferating cancer cells, whereas there are no approved therapies that target hypoxic cancer cells, despite growing clinical and experimental evidence indicating that intratumoral hypoxia is a critical microenvironmental factor driving cancer progression. In this review, our current understanding of the consequences of HIF activity and the translational potential of targeting HIFs for cancer therapy are discussed.

Keywords: oxygen biology; tumor microenvironment

Over 580,000 Americans died of cancer in 2015 because of invasion, metastasis, immune evasion, and drug resistance, which comprise the lethal cancer phenotype. Because 580,000 is a large number, especially when counting human lives, it is reasonable to ask whether current evaluation and treatment of cancer patients is missing something extremely important.

The Thesis

Consider a histological section of the highly aggressive brain cancer glioblastoma multiforme (Figure 1A). We can divide the section into three zones using blood vessels as central points of reference (presented in schematic form in Figure 1B, left). Zone I consists of well-oxygenated cancer cells located in proximity to a blood vessel. Zone III is an area of necrosis, where cancer cells have died of asphyxiation: neoplasia has upset the precise balance between O_2 supply and demand that is fundamental to all healthy tissue, leading to progressively lower O_2 levels as distance from the vessel increases, until available O_2 becomes insufficient to maintain cell viability. Zone II consists of viable but hypoxic cancer cells. These are the most dangerous cells in the cancer and are the focus of this review. Radiation therapy, conventional cytotoxic chemotherapy, and many of the newer targeted cancer therapies are most effective at killing well-oxygenated, proliferating cells in Zone I, whereas there is a greater likelihood that hypoxic cells in Zone II will survive therapy, invade, metastasize, evade the immune system and kill the patient. If this is true, then effective cancer therapy might require a dual approach: (a) conventional therapies designed to kill well-oxygenated cancer cells; and (b) novel therapies designed to kill hypoxic cancer cells.

2

3 **The Target**

4 Consider the tumor section ([Figure 1A](#)) once again. The section has been stained with a
5 monoclonal antibody against hypoxia-inducible factor (HIF) 1 α . When cancer cells are
6 subjected to decreased O₂ availability, they induce HIF-dependent transcriptional
7 activation of target genes, whose protein products mediate cancer progression ([Figure](#)
8 [1B](#), right, and [Table 1](#)). HIF-1 is a heterodimeric transcription factor composed of O₂-
9 regulated HIF-1 α and constitutively-expressed HIF-1 β subunits, which are basic-helix-
10 loop-helix-PAS domain proteins [1]. Two other family members, HIF-2 α and HIF-3 α ,
11 also dimerize with HIF-1 β [1,2]. In well-oxygenated (normoxic) cells near vessels, HIF- α
12 subunits are modified by enzymes that use O₂ and α -ketoglutarate as substrates to
13 hydroxylate proline and asparagine residues (P402, P564, and N804 in human HIF-
14 1 α). Hydroxylation blocks recruitment of the co-activator P300 and induces interaction
15 with the VHL tumor suppressor protein, which recruits an E3 ubiquitin–ligase that
16 targets HIF- α subunits for proteasomal degradation [1]. Under hypoxic conditions,
17 hydroxylation is inhibited, leading to HIF- α accumulation, heterodimerization with HIF-
18 1 β , and transcriptional activation of target genes ([Figure 2](#)). Immunohistochemical
19 analysis of tumor biopsies ([Figure 1A](#)) has revealed that increased HIF-1 α levels are an
20 independent predictor of mortality in cancers of the bladder, brain, breast, cervix, colon,
21 endometrium, esophagus, head and neck, larynx, oropharynx, ovary, liver, lung,
22 pancreas, prostate, and stomach as well as melanoma and acute lymphoid and myeloid
23 leukemias [3–7].

24

In addition to regulation by O₂ levels, HIF activity is also modulated by somatic mutations in oncogenes and tumor suppressor genes, tumor virus infection, cytokines, and other signaling molecules in the tumor microenvironment [3]. HIF activation represents a final common pathway in cancer progression that has pleiotropic effects upon critical aspects of cancer pathobiology, including angiogenesis, metabolic reprogramming, extracellular matrix (ECM) remodeling, epithelial-mesenchymal transition (EMT), motility, invasion, metastasis, stem cell maintenance, and immune evasion (Figure 1 and Table 1). Below, we provide an overview of our current understanding of the consequences of HIF activity and the translational potential of targeting HIFs for cancer therapy.

Angiogenesis

Solid tumors initially lack vascularization and depend on O₂ diffusion from host vessels. Angiogenesis, the sprouting of new capillaries from pre-existing vessels, is an adaptive response to hypoxia, which is co-opted by cancer cells to stimulate O₂ delivery and support tumor growth. HIFs function as master regulators of angiogenesis by modulating expression of genes involved in the angiogenic response, including: vascular endothelial growth factor A (VEGF-A); stromal-derived factor 1 (SDF-1), which is also known as chemokine (C-X-C motif), ligand 12 (CXCL12); angiopoietin 2 (ANGPT2); placental growth factor (PGF); platelet-derived growth factor B (PDGF-B); and stem cell factor (SCF), which is also known as KIT ligand (KITL) [8]. Signal transduction occurs upon binding of these ligands to their cognate receptors (VEGF-A → VEGFR2, SDF-1 → CXCR4, ANGPT2 → TIE2, PGF → VEGFR1, PDGF-B →

PDGFR- α/β , SCF $\rightarrow$ CKIT), which are expressed on the surface of endothelial cells, pericytes and vascular smooth muscle cells [8].

In rapidly growing tumors, angiogenesis also involves mobilization of bone marrow-derived angiogenic cells (BMDACs), a heterogeneous population of endothelial progenitor cells, mesenchymal stem cells, and myeloid cells, which exert paracrine pro-angiogenic effects [8]. BMDACs express CXCR4, VEGFR2, and CKIT and are mobilized into the circulation and recruited to tumors through HIF-dependent secretion of SDF-1 (CXCL12), VEGF-A, and SCF (KITL) by hypoxic cancer cells [9,10]. Inhibition of HIF activity impairs BMDAC mobilization and tumor vascularization by inhibiting *VEGFA*, *KITL*, and *CXCL12* expression [10,11].

Physiological angiogenesis gives rise to functional vessels through orchestrated expression of multiple angiogenic factors to increase perfusion, whereas tumor angiogenesis generates vessels that are structurally and functionally abnormal due to an improper balance and/or excessive production of angiogenic factors [12]. As a result, tumor perfusion often remains inadequate despite extensive angiogenesis. Inhibition of vascularization by anti-VEGF therapy may further increase intratumoral hypoxia and HIF activity, which promotes resistance mediated by other angiogenic factors (described above) and cancer progression (described below). Thus, HIFs function as master regulators with pleiotropic effects on multiple angiogenic pathways and combination therapy with HIF inhibitors is likely to increase the efficacy of anti-VEGF therapy [13].

Metabolism

Hypoxia elicits multiple adaptive changes in cellular metabolism, which are coordinated by HIF-1 and serve to maintain redox homeostasis by increasing glycolysis and attenuating respiration (Figure 3). Decreased oxidative metabolism is necessary to limit production of reactive oxygen species (ROS) by the mitochondrial electron transport chain (ETC) under hypoxic conditions [14]. HIF-1 enhances glycolysis through transactivation of genes encoding glucose transporters and glycolytic enzymes, thereby increasing flux through the Embden-Meyerhoff pathway [14]. HIF-1 α interacts with pyruvate kinase M2, which functions as a co-activator to amplify transactivation of genes required for conversion of glucose to lactate, including *GLUT1*, *LDHA*, and *PDK1*, which encode glucose transporter 1, lactate dehydrogenase A, and pyruvate dehydrogenase kinase 1 [15]. HIF-1 also stimulates storage of glucose as glycogen by transactivating genes encoding enzymes that catalyze glycogen synthesis [16,17].

Increased flux through the Embden-Meyerhoff pathway leads to increased pyruvate production and NAD⁺ depletion. The pool of NAD⁺ necessary to sustain glycolysis is maintained through expression of LDH-A, which catalyzes the following reaction: $\text{pyruvate} + \text{NADH} \rightarrow \text{lactate} + \text{NAD}^+ + \text{H}^+$. HIF-1 maintains intracellular pH by increasing lactate and proton efflux through expression of monocarboxylate transporter 4 (MCT-4), carbonic anhydrase 9, and Na⁺-H⁺ exchanger-1 [18]. Expression of MCT-1 and LDH-B by non-hypoxic cells allows them to import lactate (produced by hypoxic cells) and convert it to pyruvate for utilization as a substrate for oxidative phosphorylation, thereby

establishing metabolic symbiosis between hypoxic and non-hypoxic cells within a tumor [19].

Pyruvate dehydrogenase (PDH) catalyzes conversion of pyruvate to acetyl CoA for oxidation in the tricarboxylic acid (TCA) cycle. In hypoxic cells, PDK-1 phosphorylates and inactivates PDH, thereby decreasing generation of reducing equivalents (NADH and FADH_2) that are utilized by the ETC to generate ATP [14]. Inefficiency of ETC activity in hypoxic cells results in electron leakage prior to complex IV, which generates superoxide anions [20] and oxidative stress that can lead to cell death [14]. However, mitochondrial ROS production induces HIF-1 α stabilization, which leads to decreased oxidative phosphorylation and ROS production in a classical stimulus-response loop to maintain redox homeostasis. Besides inducing *PDK1* expression, HIF-1 regulates mitochondrial metabolism through several mechanisms: (i) HIF-1 increases ETC efficiency under hypoxic conditions by mediating a subunit switch in cytochrome *c* oxidase (complex IV) in which COX4-1 is replaced by COX4-2 through transactivation of *COX4I2* as well as *LONP1*, which encodes a mitochondrial protease that degrades COX4-1 [14]. (ii) HIF-1 induces mitochondrial-selective autophagy by transactivation of *BNIP3* and *BNIP3L* encoding BCL2 family members [14]. (iii) HIF-1 suppresses mitochondrial biogenesis by transactivation of *MXI1* encoding a negative regulator of the c-MYC $\rightarrow$ PGC-1 β axis [14]. (iv) HIF-1 increases expression of *NDUFA4L2* encoding NADH dehydrogenase (ubiquinone) 1 α subcomplex subunit 4-like 2, which suppresses complex I activity [21]. (v) HIF-1 induces expression of miR-210, a microRNA that inhibits expression of ISCU proteins, which are required for complex I

assembly [22,23]. (vi) HIF-1 suppresses fatty acid oxidation by inhibiting expression of *MCAD* and *LCAD*, which encode medium- and long-chain acyl CoA dehydrogenases, respectively [24]. These studies demonstrate that HIF-1 plays an essential role as a master regulator of mitochondrial metabolism (Figure 3).

Decreased entry of pyruvate into the TCA cycle leads to compensatory anaplerosis, whereby α -ketoglutarate, generated through reductive carboxylation of glutamine, is converted to citrate and utilized as a substrate for lipid synthesis [25–27]. This TCA cycle reprogramming is due to HIF-1-dependent expression of SIAH2, an ubiquitin ligase that triggers proteasomal degradation of the E1 subunit of α -ketoglutarate dehydrogenase [28]. Hypoxia also increases levels of glutamine-derived L-2-hydroxyglutarate [29], which is a competitive inhibitor of HIF prolyl hydroxylases, thereby stabilizing HIF-1 α [30].

HIF-1 reprograms lipid metabolism by inducing lipid droplet accumulation, stimulating fatty acid (FA) uptake, and inhibiting mitochondrial FA oxidation in hypoxic cells due to transactivation of genes encoding adipophilin, FA binding protein (FABP) 3 and FABP7 as well as suppression of *MCAD* and *LCAD* expression through inhibition of the c-MYC $\rightarrow$ PGC-1 β axis [24,31]. Thus, HIF-1 inhibits oxidative metabolism of both glucose and FAs in order to maintain redox homeostasis under hypoxic conditions. HIF-1 also participates in serine synthesis and one-carbon metabolism by transactivating *PHGDH* and *SHMT2* encoding phosphoglycerate dehydrogenase and serine

hydroxymethyltransferase 2, which increase NADPH generation and antioxidant defenses in hypoxic cells [32,33]. Taken together, the studies presented in this section demonstrate that HIF-1 functions as a master regulator of mitochondrial redox homeostasis by decreasing oxidant production and increasing antioxidant capacity under hypoxic conditions.

Epithelial-Mesenchymal Transition, Invasion and Metastasis

HIFs coordinately regulate numerous signaling pathways in hypoxic cancer cells, which mediate invasive and metastatic phenotypes, including: (i) conversion of epithelial cells into motile and invasive mesenchymal cells (epithelial–mesenchymal transition, EMT); (ii) basement membrane disruption and extracellular matrix (ECM) remodeling, leading to local tissue invasion and passage into the lumen of lymphatic or blood vessels (intravasation); (iii) inhibition of anoikis, which is cell death due to detachment from the ECM during transit in lymph or blood; (iv) exit from vessels at metastatic sites (extravasation); (v) establishment of pre-metastatic niches, a pre-requisite for metastatic colonization; and (vi) transition from mesenchymal to epithelial phenotype for clonal expansion. Treatment of mice bearing orthotopic breast tumors with a HIF inhibitor, such as acriflavine or digoxin, blocks both hematogenous and lymphatic metastasis of breast cancer cells [34–36].

EMT and Local Invasion

EMT is characterized by loss of cell-cell adhesion and cell polarity [37]. HIF-1 downregulates E-cadherin, a major component of the adherens junctions, by enhancing

expression of SNAIL1, SNAIL2, ZEB1, ZEB2, TWIST, and TCF3 [38], which are transcription factors that repress genes encoding epithelial characteristics and activate genes encoding mesenchymal characteristics. HIFs regulate HGF → MET and RHOA → ROCK1 signaling to promote cancer cell motility [38,39]. A secreted form of glucose phosphate isomerase, which is known as autocrine motility factor, is induced by hypoxia [38]. HIF-dependent expression of the receptor tyrosine kinase AXL increases cell motility and invasion [40]. HIF-1 α indirectly promotes EMT by modulating NOTCH and integrin-linked kinase signaling [41,42].

Degradation of basement membrane and ECM is catalyzed by matrix metalloproteinases (MMPs), including MMP2, MMP9, and MMP14, as well as the urokinase-type plasminogen activator receptor (PLAUR), which are all encoded by HIF target genes [38]. HIFs also orchestrate ECM remodeling by stimulating increased collagen crosslinking, which stiffens the ECM, through increased expression of procollagen prolyl-4-hydroxylases (P4HA1, P4HA2), lysyl hydroxylases (PLOD1, PLOD2), and lysyl oxidases (LOX, LOXL2, and LOXL4), which are required for breast cancer invasion and metastasis [42–46].

Intravasation, Circulation, and Extravasation

Intravasation involves cancer cells entering lymphatic or blood vessels. VEGF-A induces blood vessel permeability that enhances intravasation, which is also stimulated by SDF-1 → CXCR4 signaling [47]. Lymphatic intravasation is facilitated by absence of a basal lamina and tight junctions between lymphatic endothelial cells (LECs). Several

cytokines exhibit both lymphangiogenic and angiogenic properties; for example, PDGF-B and PDGFR- β are expressed by cancer cells and ECs/LECs, respectively, in a HIF-dependent manner [35,48]. Moreover, LECs attract cancer cells into lymphatics by expressing SDF-1 and CCL-21, which are ligands for CXCR4 and CCR7, respectively [42]. Anoikis is an apoptotic program induced upon detachment from ECM that cancer cells bypass due to HIF-1-dependent suppression of $\alpha 5$ integrin signaling [49].

Cancer cells arrest in the circulation at metastatic sites by adherence to the luminal surface of ECs, a phenomenon known as margination, which was initially described for blood leukocytes. HIFs increase expression of L1-cell adhesion molecule, which is a transmembrane protein that mediates cancer cell-EC interaction through homophilic binding as well as heterophilic interaction with integrins, neuropilin-1, and CD24 [34]. Cancer cell margination is followed by extravasation. Trans-endothelial passage is mediated by HIF-dependent expression of angiopoietin-like 4 (ANGPTL4), which inhibits EC-EC interactions [34].

Niche Formation and Metastatic Colonization

Restoration of an epithelial phenotype (*i.e.* mesenchymal-epithelial transition) enables cancer cells to proliferate at secondary sites [42]. Few models provide a mechanism for reversal of EMT, but reversal of hypoxia-induced EMT is easily understood: once cancer cells enter the well-oxygenated vasculature, the duration of the mesenchymal phenotype that was induced in the hypoxic tumor microenvironment will be limited by the half-life of hypoxia-induced proteins. Thus, circulating cancer cells have a limited

2 window of opportunity during which they must reach metastatic sites. Many circulating
3 cancer cells that are identified by expression of epithelial cell adhesion molecules may
4 have failed to reach a metastatic site in time. For those that are successful,
5 mesenchymal-epithelial transition will occur followed by initiation of the proliferative
6 program required to form a clinically relevant secondary tumor.

7
8 Studies in mouse models suggest that before cancer cells arrive at the metastatic site,
9 establishment of a pre-metastatic niche is required, in which tumor-secreted factors
10 (TSFs) induce mobilization and recruitment of bone marrow-derived cells to sites of
11 subsequent metastasis, where they modify ECM to generate a microenvironment that
12 stimulates cancer cell extravasation and colonization [50]. HIFs regulate hypoxia-
13 induced expression of multiple TSFs by breast cancer cells, including LOX, LOXL2, and
14 LOXL4 [36,42,46,51]. These enzymes are released into the bloodstream and
15 accumulate at pre-metastatic sites, where they remodel collagen, which is essential for
16 recruitment of CD11b⁺ myeloid cells [46,50,51], which produce MMP-2 that digests
17 collagen to enhance cancer cell invasion. Hypoxia-induced LOX expression also
18 facilitates pre-metastatic niche formation in liver, bone, and brain [51,52]. Taken
19 together, these results demonstrate that HIFs function as master regulators of
20 metastasis, by transactivating genes encoding proteins that are required at every step
21 of the metastatic process ([Box 1](#)).

22 23 **Cancer Stem Cells**

Cancer stem cells (CSCs; also known as tumor initiating cells) are a small subset of cancer cells that are capable of unlimited self-renewal. Division of CSCs can generate both daughter CSCs and differentiated cancer cells, which can undergo a limited number of subsequent cell divisions. Thus, it is CSCs that enable unlimited proliferation and in order for a cancer cell to generate a secondary (recurrent or metastatic) tumor, it must be a CSC [53]. Several lines of evidence indicate that HIF-1 is required for CSC maintenance in multiple cancer types [54]. Exposure of cancer cell lines to hypoxia was shown to induce a HIF-1-dependent expression of *KLF4*, *MYC*, *OCT4*, *SOX2* and *NANOG*, which are known to promote pluripotency and to repress differentiation [55].

Angiogenesis inhibitors reduce tumor perfusion, which leads to intratumoral hypoxia and induction of HIF-1, which increases the percentage of breast CSCs (BCSCs) [56]. Knockdown or knockout of HIF-1 α reduced the percentage of BCSCs in autochthonous and orthotopic mouse models of breast cancer [56–58]. HIF-1 induces the BCSC phenotype through modulation of the Hippo pathway [58], which is known to endow breast cancer cells with self-renewal capacity [59]. Under hypoxia, HIF-1 triggers expression of the transcriptional co-activator TAZ, an effector of the Hippo pathway, through direct binding to the *WWTR1* gene as well as induction of SIAH1-dependent TAZ translocation to the nucleus [58], where it enhances transcription of genes crucial for BCSCs [59]. Direct protein-protein interaction of HIF-1 α and TAZ leads to establishment of bidirectional crosstalk that amplifies transcription of downstream target genes [60,61]. HIF-1 α also interacts with YAP, which is a paralog of TAZ [62]. In yet another mechanism by which hypoxia induces the BCSC phenotype, HIFs increase

NANOG mRNA stability by transactivating *ALKBH5*, which encodes an N⁶-methyladenosine demethylase [63].

A major factor limiting chemotherapy efficacy is CSC enrichment following treatment. HIF-1-dependent BCSC enrichment was observed after treatment of tumor-bearing mice with cytotoxic chemotherapy [64]. In triple negative breast cancers, HIF-1 contributes to BCSC enrichment by enhancing expression of interleukin-6 (IL-6), IL-8 and multidrug resistance-1 (MDR-1), with the latter mediating cellular efflux of chemotherapy, thereby increasing cell survival [64]. Co-administration of HIF inhibitors, such as digoxin or acriflavine, inhibited paclitaxel-or gemcitabine-dependent induction of IL-6, IL-8 and MDR-1 expression, and combination therapy with digoxin and gemcitabine led to tumor eradication [64]. Induction of HIF-1 by chemotherapy also increases expression of *SLC7A11* and *GCLM*, encoding subunits of the cystine-glutamate antiporter and glutamate-cysteine ligase, which are involved in glutathione biosynthesis [65], and *PHGDH*, which is required for NADPH generation [33]. Glutathione-dependent chelation of copper inhibited MEK-ERK activity, resulting in FoxO3 nuclear translocation and transcriptional activation of *NANOG* [65]. Finally, in a subset of breast cancers, HIF-1 also activates transcription of *CD47*, which promotes the BCSC phenotype and protects cancer cells from phagocytosis by macrophages [66]. Taken together, these results indicate that HIFs function as master regulators of the CSC phenotype by transactivating genes that promote CSC specification or maintenance (Figure 4).

Immune Evasion

Hypoxic cancer cells evade innate immunity through HIF-dependent expression of the “don’t eat me” signal, CD47 [66]. HIFs also mediate suppression of the adaptive immune system. One mechanism is by stimulating recruitment and differentiation of monocytic myeloid-derived suppressor cells (MDSCs) and regulatory T cells [67–70]. Expression of HIF-1 α in macrophages is required for their ability to suppress T cell function [71]. T cell-mediated anti-tumor immunity is also suppressed through production of extracellular adenosine, which is generated from ATP by CD39 and CD73, which are both products of HIF target genes [72]. Another mechanism by which cancer cells block anti-tumor immunity is through expression of programmed death ligand PD-L1, which binds to the cell surface checkpoint receptor PD-1 on effector T cells to block their activation [73]. Hypoxia induces HIF-dependent PD-L1 expression by cancer cells [74] and by MDSCs, which is required for inhibition of T cell function [75]. Thus, hypoxia induces both recruitment of MDSCs and activation of their immunosuppressive properties. Tumor-derived VEGF may inhibit the function of dendritic cells and stimulate them to produce PD-L1 [76]. HIF-1-dependent metabolic reprogramming also contributes to immune evasion, as T cells subjected to glucose deprivation (due to increased glucose uptake by hypoxic cancer cells) have diminished anti-tumor effector functions [77,78]. Immune checkpoint blockade, such as anti-PD-1 therapy, has led to dramatic responses in some cancer patients, but overall response rates are less than 30% [73], which likely reflects the fact that multiple mechanisms of immune evasion are active, such that a single targeted therapy is insufficient to restore anti-tumor immunity.

2 **Concluding Remarks**

3 The hypoxic tumor microenvironment drives cancer progression in large part by
4 inducing HIF activity. Hypoxic cancer cells are resistant to radiation therapy [79] and
5 chemotherapy [80]. Anti-angiogenic agents increase intratumoral hypoxia, which inhibits
6 tumor growth, but the resulting induction of HIF activity stimulates invasive, metastatic,
7 and CSC phenotypes as described above [63,81]. Cytotoxic chemotherapy also induces
8 HIF-dependent CSC enrichment in an O₂-independent manner [64,65]. The counter-
9 therapeutic enrichment of CSCs induced by chemotherapy or angiogenesis inhibitors is
10 blocked by concomitant administration of a HIF inhibitor [64,65,82,83]. HIF inhibitors
11 also improve the response to gemcitabine by promoting immunogenic cell death [84].
12 Thus, preclinical data suggest that addition of a HIF inhibitor to current standard of care
13 may improve patient survival, but it is not known whether patients who are most likely to
14 respond can be identified by immunohistochemistry, transcriptome profiling, tumor
15 subtype, or some other method of stratification (Outstanding Questions Box).

16 One group of patients who might benefit from a HIF inhibitor are those with the
17 clear cell type of renal cell carcinoma (RCC), in which the hallmark genetic or epigenetic
18 alteration is VHL loss of function, leading to dysregulated HIF activity [85]. In a phase
19 I/IIa trial, 22 patients with metastatic RCC refractory to multiple prior therapies were
20 treated with CRLX101, a camptothecin-containing nanoparticle that inhibits HIF activity,
21 and bevacizumab, an anti-VEGF-A monoclonal antibody. Mean progression-free
22 survival was 10 months and partial responses were observed in 5 patients [86]. Based
23 on these encouraging results, an expanded phase II trial (NCT02187302ⁱ) was

2 conducted comparing standard of care with or without CRLX101. However, the trial
3 missed its primary endpoint, which was an increase in progression free survival [87].

4 Additionally, cardiac glycosides such as digoxin potently inhibit HIF activity by
5 interfering with the accumulation of HIF-1 α protein, thereby blocking the growth and
6 metastasis of human prostate and breast cancer cells in xenograft and orthotopic
7 transplantation models [34-36;88]. However, a major obstacle to the effective use of
8 digoxin as an anticancer agent in humans is dose-limiting cardiac toxicity due to
9 inhibition of the sodium-potassium ATPase, which is not observed in mice.

10 Perhaps the most promising and selective class of HIF inhibitors comprises
11 compounds that bind directly to the HIF-1 α and/or HIF-2 α subunit and block their
12 dimerization with HIF-1 β . The first drug in this class that was shown to have an anti-
13 cancer effect *in vivo* is acriflavine [11]. More recently, compounds that bind selectively
14 to the HIF-2 α subunit to block dimerization and inhibit the growth of tumor xenografts
15 have been reported [89,90]. Variable sensitivity to the HIF-2 α inhibitor PT2399 was
16 observed: analysis of 18 RCC cell lines revealed that the growth of 10 lines was
17 inhibited *in vivo* [89]. In resistant cell lines, the drug disrupted subunit heterodimerization
18 but had no effect on gene expression [89]. HIF-1 α expression or p53 pathway status
19 may play a role in determining sensitivity to PT2399 [90]. Gene expression profiling
20 revealed a signature of sensitive tumors that may allow for the selection of patients who
21 are most likely to respond to the therapy [90].

22 It is likely that over the next several years, additional HIF inhibitors and their
23 effects in additional cancer subtypes will be evaluated in trials combining these novel

agents with angiogenesis inhibitors, chemotherapy, immune checkpoint inhibitors, and other targeted therapies. As with all cancer therapies, the challenge will be to identify what combination of drugs in which patients with what type of cancer will result in a significant therapeutic benefit without adverse side effects.

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Resources

<https://clinicaltrials.gov/ct2/show/NCT02187302?term=NCT02187302&rank=1>

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Figure Legends

Figure 1. Intratumoral hypoxia induces HIF-dependent cancer progression. (A) Immunohistochemistry was performed to detect hypoxia-inducible factor (HIF) 1α in a human glioblastoma biopsy section. The section can be divided into three zones: Zone I, which is defined by proximity to a blood vessel (red arrows); Zone II, which is defined by expression of HIF- 1α ; and Zone III, which is defined by the presence of necrosis. (B) Hypoxic cells in Zone II express high levels of HIFs, which activate target genes that impact on every critical aspect of cancer progression. Abbreviations: ECM, extracellular matrix; EMT, epithelial-mesenchymal transition.

Figure 2. O_2 -dependent hydroxylation regulates HIF- α degradation and transactivation. Under normoxic conditions, hydroxylation (OH) of an asparagine (N) residue in the transactivation domain (TAD) blocks interaction with the coactivator P300, and hydroxylation of proline (P) residues in the O_2 -dependent degradation domain (ODD) enables interaction with the von Hippel-Lindau (VHL) tumor suppressor protein, which targets HIF- α subunits for ubiquitylation and proteasomal degradation. Under hypoxic conditions, hydroxylation is inhibited, leading to the HIF- α stabilization and coactivator recruitment; dimerization of HIF- α subunits with HIF- 1β is mediated by the basic-helix-loop-helix Per-Arnt-Sim homology (bHLH-PAS) domain.

Figure 3. Intratumoral hypoxia induces HIF-1-dependent metabolic reprogramming. HIF-1 induces multiple changes in gene expression that mediate a switch from oxidative to glycolytic metabolism in order to reduce mitochondrial generation of ROS. Abbreviations: LDH, lactate dehydrogenase; PDK, pyruvate dehydrogenase kinase; BNIP, BCL2/adenovirus E1B 19-kDa interacting protein; COX, cytochrome c oxidase; miR, microRNA; NDUFA4L2, NADH dehydrogenase (ubiquinone) 1 α subcomplex subunit 4-like 2; SIAH, seven in absentia homolog; PDH, pyruvate dehydrogenase; ISCU, iron-sulfur cluster scaffold homolog; ETC, electron transport chain; α -KGDH, α -ketoglutarate dehydrogenase; PGC, PPAR gamma coactivator; MCAD and LCAD, medium- and long-chain acyl-CoA dehydrogenase.

Figure 4. HIFs are master regulators of the breast cancer stem cell (BCSC) phenotype. Exposure of breast cancer cells to hypoxia or cytotoxic chemotherapy induces HIF-dependent changes in gene expression that promote specification and maintenance of the BCSC phenotype. Blue rectangles indicate HIF target genes. Red ovals denote proteins whose activity is inhibited under hypoxic conditions. Abbreviations: LATS, large tumor suppressor; WWTR, WW domain-containing transcription regulator; IL, interleukin; MDR, multi-drug resistance; SLC, solute carrier family; GCLM, glutamate-cysteine ligase modifier subunit; ALKBH, AlkB homolog; GSH, glutathione; MEK, MAP kinase/ERK kinase; ERK, extracellular signal-regulated protein kinase; FOX, forkhead box.

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2 Table 1. HIF target genes that promote cancer progression.

3	<i>ALKBH5</i>	CSC maintenance
4	<i>ANGPT2</i>	Angiogenesis
5	<i>ANGPTL4</i>	Extravasation
6	<i>AXL</i>	Motility, Invasion
7	<i>BNIP3</i>	Metabolism
8	<i>CA9</i>	Metabolism
9	<i>CD39</i>	Immune evasion
10	<i>CD47</i>	CSC maintenance, Immune evasion
11	<i>CD73</i>	Immune evasion
12	<i>CD274 (PDL1)</i>	Immune evasion
13	<i>COX4I2</i>	Metabolism
14	<i>GCLM</i>	Metabolism, CSC maintenance
15	<i>KITL (SCF)</i>	Angiogenesis
16	<i>L1CAM</i>	Margination
17	<i>LDHA</i>	Metabolism
18	<i>LOX</i>	Invasion, Metastatic niche formation
19	<i>LOXL2</i>	Invasion, Metastatic niche formation
20	<i>LOXL4</i>	Invasion, Metastatic niche formation
21	<i>MCT4</i>	Metabolism

2	<i>MET</i>	Motility
3	<i>MIR210</i>	Metabolism
4	<i>MMP2</i>	Invasion
5	<i>MMP9</i>	Invasion
6	<i>MMP14</i>	Invasion
7	<i>MXI1</i>	Metabolism
8	<i>NDUFA4L2</i>	Metabolism
9	<i>NHE1</i>	Metabolism
10	<i>P4HA1</i>	ECM remodeling
11	<i>P4HA2</i>	ECM remodeling
12	<i>PDGFB</i>	Angiogenesis, Lymphangiogenesis
13	<i>PKD1</i>	Metabolism
14	<i>PGF</i>	Angiogenesis
15	<i>PKM2</i>	Metabolism
16	<i>PLAUR</i>	Invasion
17	<i>PLOD1</i>	ECM remodeling
18	<i>PLOD2</i>	ECM remodeling
19	<i>RHOA</i>	Motility
20	<i>ROCK1</i>	Motility
21	<i>SDF1</i>	Angiogenesis

2	<i>SHMT2</i>	Metabolism
3	<i>SIAH1</i>	CSC maintenance
4	<i>SIAH2</i>	Metabolism
5	<i>SLC2A1</i>	Metabolism
6	<i>SLC7A11</i>	Metabolism, CSC maintenance
7	<i>SNAI1</i>	EMT
8	<i>SNAI2</i>	EMT
9	<i>TCF3</i>	EMT
10	<i>TWIST</i>	EMT
11	<i>VEGFA</i>	Angiogenesis
12	<i>WWTR1</i>	CSC maintenance
13	<i>ZEB1</i>	EMT
14	<i>ZEB2</i>	EMT
15		