HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations

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Hypoxia occurs frequently in human cancers and induces adaptive changes in cell metabolism that include a switch from oxidative phosphorylation to glycolysis, increased glycogen synthesis, and a switch from glucose to glutamine as the major substrate for fatty acid synthesis. This broad metabolic reprogramming is coordinated at the transcriptional level by HIF-1, which functions as a master regulator to balance oxygen supply and demand. HIF-1 is also activated in cancer cells by tumor suppressor (e.g., VHL) loss of function and oncogene gain of function (leading to PI3K/AKT/mTOR activity) and mediates metabolic alterations that drive cancer progression and resistance to therapy. Inhibitors of HIF-1 or metabolic enzymes may impair the metabolic flexibility of cancer cells and make them more sensitive to anticancer drugs.

Introduction

All human cells require a constant supply of O2 to carry out oxidative phosphorylation in the mitochondria for ATP generation. Under hypoxic conditions when O2 availability is reduced, cells generally respond in three ways: (a) cell proliferation is inhibited to prevent any further increase in the number of O2-consuming cells; (b) the rate of oxidative phosphorylation is decreased and the rate of glycolysis is increased in order to decrease O2 consumption per cell; and (c) the production of angiogenic factors is increased in order to increase O2 delivery. Mutations in cancer cells dysregulate cell growth and metabolism, but the mechanisms and consequences of this dysregulation vary widely from one cancer to another and even one from cancer cell to another. In some cancer cells, O2 still alters the rate of cell proliferation, whereas others continue to divide even under severely hypoxic conditions; some cancers are well vascularized and perfused, whereas most cancers contain steep O2 gradients that reflect the distance to the nearest blood vessel, the number of intervening cells and their metabolic activity, and the rate at which blood is flowing through the vessel. The metabolism of individual cancer cells reflects the presence of particular genetic alterations, which may alter metabolism in an O2-independent manner, as well as the spatial and temporal heterogeneity of O2 availability within the tumor microenvironment. This Review summarizes the role of HIF-1 in the regulation of cancer cell metabolism, focusing primarily on the use of glucose as a metabolic substrate.

HIF-1 mediates adaptive responses to reduced O2 availability

HIF-1 is a heterodimer, consisting of an O2-regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit (1, 2), that binds to the consensus sequence 5’-RGCGTG-3’ that is present within or near HIF-1-regulated genes (3). HIF-1α protein stability is negatively regulated by O2-dependent prolyl hydroxylases (Figure 1), which enables binding of the von Hippel-Lindau tumor suppressor protein (VHL), the recognition subunit of an E3 ubiquitin ligase that ubiquitylates HIF-1α, thereby targeting it for proteasomal degradation (4). HIF-1α stability is also modulated according to cellular metabolic status because, in addition to O2, the TCA cycle intermediate α-ketoglutarate is also a reaction substrate for prolyl hydroxylases. The hydroxylases insert one oxygen atom into a proline residue (either Pro-403 or Pro-564 in human HIF-1α), and the other oxygen atom is inserted into α-ketoglutarate, splitting it into succinate and CO2.

Database searches using the HIF-1α sequence identified HIF-2α, which is also O2-regulated, dimerizes with HIF-1β, and activates gene transcription (5, 6). HIF-1α homologs have been identified in all metazoan species analyzed and are expressed in all cell types, whereas HIF-2α homologs are only found in vertebrates and are expressed in a restricted number of cell types (7, 8), although many cancer cells express both HIF-1α and HIF-2α (9, 10). Because the battery of genes that is activated by HIF-1 and HIF-2 in response to hypoxia is unique within each cell, the number of HIF target genes, which currently exceeds 1,000, continues to increase as new cell types are analyzed by ChIP techniques such as ChIP-chip (11, 12) and ChIP-seq (13).

Many cancers contain areas of intratumoral hypoxia, and primary tumors with low oxygenation (PO2 < 10 mmHg) are associated with an increased risk of metastasis and patient mortality (14). Increased HIF-1α levels are also associated with increased risk of mortality in many human cancers, including those of the bladder, brain, breast, colon, esophagus, head/neck/oropharynx, liver, lung, pancreas, skin, stomach, and uterus, as well as in acute lymphocytic and myeloid leukemias (15).

In some cancers, such as renal clear cell carcinoma, clinical data indicate that HIF-2α overexpression is associated with disease progression and mortality, whereas HIF-1α expression is silenced, often by gene deletion (16). In contrast, in colon carcinoma, clinical data indicate that HIF-1α overexpression is associated with disease progression and HIF-2α expression is silenced (17). At the molecular level, there is also heterogeneity. In most cell types, genes have been identified that are transcriptionally (a) only by HIF-1α, such as lactate dehydrogenase A (LDHA) and other glycolytic enzyme genes; (b) only by HIF-2α, such as TGFα; or (c) by both HIF-1α and HIF-2α, such as VEGF (18). Yet in MCF-7 breast cancer cells, although binding of both HIF-1α and HIF-2α to HIF target genes was demonstrable by ChIP, only knockdown of HIF-1α resulted in decreased gene expression (19). In contrast, in renal carcinoma cell lines, in

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which HIF-2α but not HIF-1α is expressed, all HIF target genes (including glycolytic enzyme genes) are expressed in a HIF-2α-dependent manner (20). This cell type–specific HIF-α subunit target gene selectivity may be due to the regulated recruitment of as-yet-unidentified coactivators or corepressors.

HIF-1α contributes to the regulation of multiple adaptive responses to hypoxia, including cell proliferation, metabolism, and angiogenesis. The regulation of angiogenesis and metabolism are mediated via the transcriptional activity of the HIF-1α/HIF-1β heterodimer, whereas the regulation of cell proliferation is mediated through both transcriptional and nontranscriptional effects of HIF-1α (Figure 1).

Angiogenesis. HIF-1 activates the transcription of VEGF (21) and many other genes encoding angiogenic cytokines and growth factors in hypoxic cells, which stimulate angiogenesis and vascular remodeling that lead to improved tissue perfusion and increased O₂ delivery in normal tissues (22). However, in many cancers, the vascular response is dysregulated, such that the blood vessels are structurally and functionally abnormal, leading to persistent defects in perfusion and oxygenation (23). Both the abnormal tumor vessels and the resulting intratumoral hypoxia that these vessels perpetuate contribute to metastasis, the process by which most cancers kill their hosts, and HIF-1 activates the transcription of genes that control multiple steps in the metastatic process (24).

Proliferation. HIF-1α mediates G1 cell cycle arrest by transcriptional or nontranscriptional mechanisms in different cell types. Several transcriptional mechanisms have been reported: (a) HIF-1α may displace MYC from the promoter of CDKN1A gene, which encodes the cyclin-dependent kinase inhibitor p21Cip1, thereby de-repressing its transcription (25); (b) HIF-1α may interact with β-catenin and block β-catenin–dependent MYC transcription (26); and (c) HIF-1 may inhibit MYC activity by activating transcription of the MXT2 gene, which encodes a MYC repressor (27). More recently, HIF-1α was shown to bind directly to components of the prereplicative complexes that assemble at origins of replication and inhibit activation of the minichromosome maintenance helicase, thereby directly blocking DNA replication by a mechanism that is independent of transcription (28).

**HIF-1 reprograms the metabolism of hypoxic cells**

Reduced O₂ availability has profound effects on cellular metabolism. Glucose and glutamine are considered the primary metabolic substrates of cancer cells, and their utilization is markedly changed in response to hypoxia, as described below.

*Glucose catabolism and energy metabolism.* Because HIF-1 is present even in simple metazoan species with no specialized systems for O₂ delivery, it is likely that the primordial role of HIF-1 was to regulate O₂ consumption by orchestrating the switch from oxidative to glycolytic metabolism under hypoxic conditions. Analysis of Hif1a–/– mouse embryonic stem cells revealed that expression of genes encoding glucose transporters and virtually all of the glycolytic enzymes were induced by hypoxia and/or regulated by HIF-1 (29). The consequences of HIF-1α deficiency are particularly dramatic in mouse embryonic fibroblasts (MEFs): when cultured in the presence of 1% O₂, most of these cells die within 96 hours, whereas wild-type cells continue to proliferate, albeit at reduced rates compared with the standard tissue culture conditions (30, 31). Conventional wisdom holds that cells switch from oxidative to glycolytic metabolism under hypoxic conditions to maintain ATP production, but 1% O₂ was not limiting for ATP production in Hif1α–/– MEFs, which had higher ATP levels than wild-type cells (31). Rather than dying of ATP depletion, Hif1α–/– MEFs die from excess production of ROS, which results from the failure to switch from oxidative to glycolytic metabolism (30, 31).

Hypoxic MEFs reprogram glucose metabolism through transactivation of the pyruvate dehydrogenase (PDH) kinase, isozyme 1 (Pdk1) and the BCL2/adenovirus E1B 19-kDa interacting protein 3 (Bnip3) genes by HIF-1 (30–32). PDK1 phosphorylates and inactivates PDH, thereby inhibiting the conversion of pyruvate to acetyl-CoA for entry into the TCA cycle (Figure 2A). HIF-1 also activates expression of Ldha, which converts pyruvate to lactate, the terminal glycolytic product (3, 29). Bnip3 encodes a protein that promotes mitochondrial-selective autophagy as a means to reduce oxidative metabolism (31). The increased activities of BNIP3, PDK1, and LDHA dramatically alter the fate of pyruvate in hypoxic cells. Expression of the BNIP3 and PDK1 homologs BNIP3L and PDK3, respectively, is also induced by hypoxia in a HIF-dependent manner and contributes to mitochondrial autophagy and metabolic reprogramming in cancer cells (33–35).
Forced expression of PDK1 or BNIP3 was sufficient to rescue Hif1a−/− MEFs from ROS-mediated cell death under conditions of prolonged hypoxia (30, 31). Whereas ROS is associated with increased O2 levels, recent studies have demonstrated that decreased O2 concentrations also trigger increased mitochondrial ROS production (36). These findings represent a paradigm shift in our understanding of oxygen homeostasis, in which cellular energy metabolism is modulated primarily to maintain redox homeostasis. This conclusion was supported by the finding that HIF-1 also modulates respiratory chain function in cancer cells by orchestrating a subunit switch (from COX4I1 to COX4I2) in cytochrome c oxidase that may increase the efficiency of electron transfer to O2 and allow continued respiration without increased ROS levels when O2 availability is modestly reduced (37).

The role of HIF-1 in mediating survival under hypoxic conditions is both cell type/tissue specific and context specific. For example, in mice exposed to 10% O2, Cox4i2 mRNA expression was increased in liver and lung, but not in brain, heart, or kidney (37). In an IL-3–responsive cell line, HIF-1α was required for survival under hypoxic conditions in the presence, but not absence, of IL-3, which was attributed to a requirement for HIF-dependent glycolysis in IL-3–stimulated cells (38).

HIFs also transactivate genes encoding microRNAs (miRs), which are small RNAs that bind to mRNAs in a sequence-specific manner to either inhibit their translation or induce their degradation (39). miR-210 expression is induced in response to hypoxia in many cell types (40). Among the targets of miR-210 that are downregulated in hypoxic cells are mRNAs encoded by the ISCU gene, which encodes an iron-sulfur cluster assembly enzyme that is required for the activity of mitochondrial electron transport complex I (41, 42). This represents another molecular strategy by which hypoxic cells decrease oxidative metabolism.

ROS levels vary widely in cancer cells, with high ROS levels often associated with cell proliferation (43), whereas low ROS levels
have been associated with resistance to chemotherapy (44) and the stem cell phenotype (45, 46). Thus, it is not clear to what extent decreased ROS production is utilized for cancer cell survival. Data from PC3 prostate cancer cells and P493 lymphoid cells indicate that increased ROS levels drive HIF-1α expression in these cells and that antioxidants such as ascorbate or N-acetylcysteine reduce HIF-1α levels and tumor growth (47). Prolyl hydroxylases contain their catalytic centers Fe(II), which is oxidized to Fe(III) during the reaction, inactivating the enzyme until it is reduced to Fe(II), which is facilitated by antioxidants, thereby promoting hydroxylation-dependent degradation of HIF-1α.

**pH regulation.** Cancer cells decrease extracellular pH by activation of a battery of HIF-1 target genes encoding plasma membrane proteins (48). SLC16A3 encodes monocarboxylate transporter 4 (MCT4), which allows efflux of lactate out of cancer cells (49). SLC9A1 encodes sodium-hydrogen exchanger 1 (NHE1), which actively pumps H+ ions into the extracellular space (50). CAR9 encodes carbamic anhydrase 9 (CA9), which converts CO2 + H2O to H+ + HCO3- and, acting in concert with HCO3- transporters, converts to net efflux of H+ from cancer cells (51). The consequences of MCT4, NHE1, and CA9 activity are intracellular alkalization, which promotes proliferation, and extracellular acidification, which promotes invasion (52).

It is important to note that the cancer microenvironment is metabolically heterogeneous: cells adjacent to functional blood vessels are well oxygenated and more likely to utilize oxidative metabolism, whereas cells far from blood vessels are poorly oxygenated and dependent upon glycolysis. In hypoxic cells, HIF-1 mediates increased expression of glucose transporters, glycolytic enzymes including LDHA, and MCT4. These changes in gene expression lead to increased glucose uptake, conversion to lactate, and extrusion of lactate from the cell (Figure 3). In contrast, oxygenated cells express MCT1 and LDHB, which mediate lactate uptake and conversion to pyruvate for oxidative metabolism. Disruption of this metabolic symbiosis by treatment of tumor-bearing mice with α-cyano-4-hydroxycinnamate inhibited MCT1 activity such that aerobic cells were forced to take up glucose rather than lactate, leading to glucose depletion and the death of hypoxic cancer cells (53).

**Fatty acid synthesis.** The increased expression of BNIP3, LDHA, and PDK1 shunts pyruvate away from the mitochondria and reduces flux through the TCA pathway, the generation of NADH and FADH2 that feed the electron transport chain, and thus ROS generation under hypoxic conditions. However, reduced acCoA levels present a dilemma for those cells that continue to proliferate under hypoxic conditions with respect to fatty acid synthesis, which is normally dependent on glucose-derived acCoA that is converted to citrate, shuttled to the cytoplasm, and converted to acCoA by ATP citrate lyase (Figure 2A). Instead, hypoxic cancer cells utilize glutamine to generate α-ketoglutarate, which is converted to citrate by isocitrate dehydrogenase and aconitase (54, 55). The switch from oxidative glucose metabolism to reductive glutamine metabolism as the source of acCoA for lipid synthesis under hypoxic conditions (Figure 2B) is HIF dependent and has been attributed to shunting of glucose-derived pyruvate away from the mitochondria by PDK1 (54, 55).

**Glycogen synthesis.** Conversion of glucose to glycogen provides a means to store metabolic substrate in reserve. Hypoxia induces the expression of genes encoding the enzymes required to convert glucose to glycogen, including hexokinase (HK1 or HK2), phosphoglucomutase 1 (PGM1), UDP-glucose pyrophosphorylase (UGP2), glycogen synthase (GYS1), glycogen branching enzyme (GBE1), as well as the gene encoding PPP1R3C, which activates GYS1 and inhibits liver-type glycogen phosphorylase (PYGL), the enzyme that breaks down glycogen (Figure 4). HIF-1 transactivates these genes as determined by microarray and ChIP assays (11, 12, 29, 56–59). PYGL is expressed at high levels in many cancer cells, and knockdown of PYGL expression in U87 glioma cells increased glycogen accumulation but also induced senescence, which was associated with increased ROS levels (58). The inability to liberate glucose-1-phosphate from glycogen in PYGL-knockdown U87 cells may reduce flux through the pentose phosphate pathway (PPP), which generates the NADPH required for ROS scavenging and lipid synthesis as well as nucleotides required for DNA synthesis.

HIF-1 controls the expression of genes encoding the enzymes required for the conversion of glucose either to glycogen (as described above) or to lactate (via the glycolytic pathway), which
serves as a result of PI3K and AKT signaling that stimulates mTOR activity, which stimulates translation of HIF1A mRNA into protein by phosphorylation of eIF4E binding protein (78, 80). mTOR has also been reported to stimulate HIF-1α stabilization and transcriptional activity, which stimulates translation of HIF1A mRNA into protein by phosphorylation of eIF4E binding protein (78, 80).

Multifunctional role of pyruvate kinase M2. HIF-1 activates the expression of pyruvate kinase M2 (PKM2), which encodes pyruvate kinase isozymes PKM1 and PKM2 by alternative splicing that includes sequences encoded by exon 9 or exon 10, respectively, which differ at 23 of 56 amino acids. In cancer cells, the expression of splicing factors favors the generation of PKM2 mRNA (62–65). PKM2 promotes tumor growth (66) by a complex and growing list of activities. First and foremost, PKM2 functions as a pyruvate kinase in glycolysis (Figure 3) but, in contrast to PKM1, its activity is regulated by allosteric interactions with phosphopeptides (67) and intermediary metabolites (68, 69) as well as by phosphorylation, acetylation, and oxidation (70–72). Second, PKM2 functions as a transcriptional coactivator for OCT4, HIF-1α, and HIF-2α, and α1-4-glucosidase alter the catalytic activity of the enzyme such that it con-
verts α-ketoglutarate into D-2-hydroxyglutarate, which stimulates prolyl hydroxylase activity and results in decreased HIF-α protein levels (94), which contrasts with earlier reports of increased HIF-α levels in IDH-mutant cells (95). The effects of D-2-hydroxyglutarate as an oncometabolite may be due in part to its inhibition of the S′-methylcytosine hydroxylase TET2 (96).

Although VHL-deficient renal cell carcinoma provides the most striking example of HIF-dependent metabolic reprogramming, many other genetic alterations appear to reprogram metabolism in a HIF-dependent manner. Chronic myeloid leukemia (CML) is characterized by the Philadelphia chromosome and expression of the transforming BCR-ABL fusion protein, which is targeted therapeutically by the tyrosine kinase inhibitor imatinib, although many patients eventually develop resistance (97). BCR-ABL signaling to mTOR leads to increased HIF-1α protein levels and HIF-1 transcriptional activity in CML cells (98), and HIF-1α is required for the maintenance of CML stem cells (99). When BCR-ABL–transformed cells were cultured in the presence of imatinib, the surviving cells expressed BCR-ABL and HIF-1α at higher levels (100). Increased HIF-1 activity in resistant cells was associated with a shift of glucose flux from the TCA cycle to glycolysis. However, glucose flux also shifted from the oxidative arm of the PPP, which is initiated by glucose-6-phosphate dehydrogenase (G6PD), to the non-oxidative arm due to HIF-1–dependent expression of TKT and TKTL2, which encode transketolase (100). The PPP, a complicated collection of highly interconnected enzymatic reactions, is presented in a highly simplified manner in Figure 3 to illustrate that activation of TKT and TKTL2 expression provides a measure of metabolic flexibility by allowing return of intermediates to the glycolytic pathway. Treatment with oxysteramine, an inhibitor of TKT/TKTL2, led to increased sensitivity to imatinib in vivo (100). As in the cases of PYGL knockdown and MCT1 inhibition described above, these results indicate that pharmacologic strategies designed to limit metabolic flexibility may have anticancer effects.

**Clinical implications**

Translational efforts are underway to devise novel anticancer strategies involving inhibition of the metabolic enzymes that are induced by HIF. An example is the use of dichloroacetate (DCA), which is an inhibitor of PDK activity (101). DCA has been used to treat congenital lactic acidosis in children with mitochondrial disorders (102). When DCA was administered in the drinking water of athymic rats to increase prolyl hydroxylase levels (94), which contrasts with earlier reports of increased HIF-1α levels, and decreased angiogenesis after therapy (105). A clinical trial is underway to study the safety and efficacy of DCA in patients with glioblastoma and other recurrent brain tumors (ClinicalTrials.gov identifier: NCT01111097). An alternative, but not mutually exclusive, strategy is to inhibit HIF activity as a means of altering multiple metabolic pathways as well as blocking the expression of genes encoding proteins that contribute to angiogenesis, invasion, and metastasis (24). Digoxin decreases HIF-1α protein levels and inhibits primary tumor growth, lymphatic metastasis to axillary lymph nodes, and hematogenous metastasis to the lungs in an orthotopic model of breast cancer (106–109), and a study is underway to determine whether digoxin inhibits HIF-1 activity in human breast cancer (ClinicalTrials.gov identifier: NCT01763931). Preclinical data suggest that both metabolic inhibitors such as DCA (110) and HIF inhibitors (111) may improve the currently limited efficacy of angiogenesis inhibitors such as the anti-VEGF antibody bevacizumab.

Several strategies are being employed in an effort to identify subsets of cancers that will respond to inhibition of particular metabolic pathways, including the use of algorithms that infer metabolic phenotypes from RNA/protein expression data (112), metabolomic profiling techniques (113), stable isotope–based metabolic analysis (114, 115), and functional genomic strategies (116). Although the complexity and interconnectedness of metabolic pathways suggest that targeting a single metabolic pathway may have limited anticancer effects, preclinical studies suggest that by limiting metabolic flexibility, inhibitors of metabolic enzymes or master regulators such as HIF-1 may improve the response to cytotoxic chemotherapy and compounds that target signal transduction pathways.

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