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 divides 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’-RCGTG-3’ that is present within or near HIF-1-regulated genes (3). HIF-1α protein stability is negatively regulated by O2-dependent prolyl hydroxylation (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 activated (a) only by HIF-1α, such as lactate dehydrogenase A (LDHA) and other glycolytic enzyme genes; (b) only by HIF-2α, such as TGFA; 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-undefined 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-1α may inhibit MYC activity by activating transcription of the MXI1 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 Hif1a−/− MEFs, which had higher ATP levels than wild-type cells (31). Rather than dying of ATP depletion, Hif1a−/− 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 (acCoA) 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...
...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), and that increased ROS levels drive HIF-1α that increased ROS levels drive HIF-1α that increased ROS levels drive HIF-1α that increased ROS levels drive HIF-1α that increased ROS levels drive HIF-1α that increased ROS levels drive HIF-1α that increased ROS levels drive HIF-1α that increased ROS levels drive HIF-1α that increased ROS levels drive HIF-1α that increased ROS levels drive HIF-1α that increased ROS levels drive HIF-1α that increased ROS levels drive HIF-1α.
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Figure 4 Glycogen synthesis and glycogenolysis. PGM1 shunts glucose-6-phosphate away from glycolysis for use in glycogen synthesis, which requires the activity of three enzymes: UGP2, GYS1, and GBE1. Glucose-1-phosphate is released from glycogen (glycogenolysis) by PYGL. GYS1 and PYGL are positively and negatively regulated by protein phosphatase 1 regulatory subunit 3C (PPP1R3C), respectively (77–79). Activation of tyrosine kinases (e.g., EGFR and HER2 in prostate and breast cancer, respectively) also increases HIF-1 activity (15). For example, PTEN or p53 loss is dependent manner (76).

Cancer-associated mutations alter metabolism in an HIF-dependent manner

One of the consequences of many key genetic alterations in cancer cells is an increase in HIF activity (15). For example, PTEN or p53 loss of function leads to increased HIF-1α synthesis and stability, respectively (77–79). Activation of tyrosine kinases (e.g., EGFR and HER2 in prostate and breast cancer, respectively) also increases HIF-1α synthesis 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 transactivation domain function (81). Studies of the Rous sarcoma virus (RSV) four decades ago revealed that one of the earliest changes in the phenotype of RSV-infected cells was an increased rate of glycolysis that was dependent upon the activity of the V-SRC oncoprotein (82–84), and V-SRC was the first oncogene shown to induce HIF-1 transcriptional activity, leading to increased expression of genes encoding glycolytic enzymes under nonhypoxic conditions (85).

The genetic alteration that most dramatically increases HIF activity is VHL loss of function, which is the hallmark of the clear cell type of renal cell carcinoma (86). In the absence of VHL, HIF-1α is not subjected to O2-dependent ubiquitination and degradation (87), and increased HIF transcriptional activity is the earliest detectable sign of VHL loss of function in renal tubular cells (88). Loss of VHL resulted in an HIF-dependent, but O2-independent, switch from oxidative to glycolytic metabolism in RCC4 and RCC10 human renal carcinoma cells that dramatically reduced O2 consumption and respiration (89). VHL loss of function also led to HIF-dependent inhibition of PPARγ coactivator 1β (PGC-1β) expression and decreased mitochondrial biogenesis. All of these changes were reversed by forced expression of VHL or short-hairpin RNAs targeting HIF-1α and HIF-2α (89). These findings provided a definitive molecular basis for the observation first made by Otto Warburg that cancer cells manifest increased rates of lactate production under aerobic conditions (90). The transcription of genes encoding glycolytic enzymes is regulated in an HIF-1α-dependent, but an HIF-2α-independent, manner in virtually all cell types except VHL-null renal carcinoma cells, in which HIF-2α acquires this function by unknown mechanisms.

In addition to reprogramming glucose metabolism, VHL loss of function in renal carcinoma cells also leads to the switch from oxidative glucose metabolism to reductive glutamine metabolism as a source of acCoA for fatty acid synthesis that is described above as a response to hypoxia (Figure 2), but in VHL-null renal carcinoma cells this switch occurs in an O2-independent manner (55).

Whereas mutations in VHL directly block the ubiquitination of HIF-α subunits, mutations in the genes encoding succinate dehydrogenase (SDH) and fumarate hydratase (FH) block ubiquitination indirectly by increasing the concentration of TCA cycle intermediates that inhibit prolyl hydroxylase activity (91–93). In either case, these mutations increase HIF activity under nonhypoxic conditions, and this state is sometimes referred to as “pseudo-hypoxia,” a term that should be discouraged because there are many cellular responses to hypoxia that are not regulated by VHL, PHD, or HIF activity. In contrast to the effect of loss-of-function mutations in genes encoding SDH or FH, oncogenic missense mutations in the IDH1 or IDH2 gene encoding isocitrate dehydrogenase alter the catalytic activity of the enzyme such that it con-

suggestions that other factors may determine the relative flux through these two pathways in hypoxic cancer cells. Notably, depletion of glucosephosphate isomerase (GPI) or phosphoglycerate mutase 1 (PGAM1) (Figure 3) by RNA interference also induces senescence (60), indicating the critical role of glucose metabolism in maintaining proliferation. PGAM1 activity is required to maintain a proper balance between glycolysis and biosynthesis (via the PPP) for proliferation (61).

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 β-catenin (73–75). HIF-1 activates transcription of PKM2, the protein product of which interacts with HIF-1α to stimulate coactivator recruitment, chromatin binding, and transcriptional activation (74). This feed-forward loop increases expression of HIF-1 target genes encoding proteins that mediate the metabolic reprogramming of cancer cells (e.g., LDHA and PDK1), angiogenesis (e.g., VEGF), and other aspects of cancer progression. Finally, PKM2 functions as a protein kinase that phosphorylates STAT3, thereby increasing its transcriptional activity (76).

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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 TKT2, 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 TKT2 expression provides a measure of metabolic flexibility by allowing return of intermediates to the glycolytic pathway. Treatment with oxithiamine, an inhibitor of TKT/TKT2, 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). Clinical implications of these findings include the possibility that PDK activity by 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.

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