# Oxygen availability and metabolic reprogramming in cancer

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**Hong Xie**[‡§1] **and M. Celeste Simon**[‡¶2]

*From the* [‡]*Abramson Family Cancer Research Institute and Departments of* [§]*Cancer Biology and* [¶]*Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104*

Edited by Ruma Banerjee

**Hypoxia and dysregulated metabolism are defining features of solid tumors. How cancer cells adapt to low $O_2$ has been illuminated by numerous studies, with "reprogrammed" metabolism being one of the most important mechanisms. This metabolic reprogramming not only promotes cancer cell plasticity, but also provides novel insights for treatment strategies. As the most studied $O_2$ "sensor," hypoxia-inducible factor (HIF) is regarded as an important regulator of hypoxia-induced transcriptional responses. This minireview will summarize our current understanding of hypoxia-induced changes in cancer cell metabolism, with an initial focus on HIF-mediated effects, and will highlight how these metabolic alterations affect malignant phenotypes.**

Molecular oxygen ($O_2$) is a key nutrient required for aerobic metabolism to maintain intracellular bioenergetics and as a substrate in numerous organic and inorganic reactions. Hypoxia, defined by a deficiency in the amount of tissue $O_2$ levels, occurs in a variety of physiological as well as pathological conditions. Significant research interest in variable $O_2$ availability in cancers can be traced to the early 20th century, when Otto Warburg found that unlike most normal tissues, cancer cells preferentially "ferment" glucose to pyruvate and then lactate even in the presence of sufficient $O_2$ to support mitochondrial metabolism (the "Warburg effect"). Although the underlying mechanisms of this observation were not clear and numerous subsequent studies demonstrated certain limitations in this theory (especially the hypothesis that cancer cells develop a defect in mitochondria that leads to impaired aerobic respiration), it opened a new territory of investigating how cancer cells rewire metabolism to adapt to changes in $O_2$ levels. When facing hypoxia, cells modulate a number of conserved molecular responses, including those regulated by hypoxia-inducible factors (HIFs),[3] endoplasmic reticulum (ER) stress responses, mechanistic target of rapamycin signaling, autophagy, and others. These processes promote altered metabolism to match $O_2$ supply. In this minireview, we will discuss hypoxia responses according to HIF-dependent and -independent pathways and how they impact progression of solid tumors.

## HIF-dependent metabolic reprogramming

### *The HIF hydroxylase system*

The HIF system itself has been reviewed extensively elsewhere (1, 2). Initially identified as a transcriptional regulator bound to a hypoxia-response element (HRE) of the erythropoietin (*EPO*) gene to promote EPO production, it readily became apparent that this pathway operated much more widely, and it is currently recognized as a key modulator of the transcriptional response to hypoxic stress. Briefly, HIFs are heterodimeric transcription factors consisting of $\alpha$ and $\beta$ subunits. Three HIF$\alpha$ isoforms exist in the mammalian genome (1$\alpha$, 2$\alpha$, and 3$\alpha$), of which HIF1$\alpha$ and HIF2$\alpha$ are the best characterized. HIF$\alpha$ subunits heterodimerize with stable HIF1$\beta$ (or ARNT), recognize, and then bind to HREs ((G/A)CGTG) throughout the genome to regulate downstream gene expression. Hypoxia-inducible behavior is conferred by the HIF$\alpha$ subunits, the protein abundance and transcriptional activity of which are regulated by $O_2$-dependent prolyl and asparaginyl hydroxylation. In well-oxygenated environments, HIF$\alpha$ subunits are hydroxylated by prolyl hydroxylases (PHDs) and factor-inhibiting HIF (FIH), and in turn they are targeted for proteosomal degradation by an E3 ubiquitin ligase, the von Hippel-Lindau protein (pVHL) complex. In hypoxic conditions, PHD and FIH activities are diminished, and HIF$\alpha$ proteins are stabilized, which consequently induces transcription of thousands of genes supplying adaptive functions, such as vascular endothelial growth factor (*VEGF*), carbonic anhydrase IX (*CAIX*), *EPO,* and others.

### *HIF regulation of glucose metabolism*

*Glycolysis*—Because $O_2$ serves as an electron acceptor in oxidative phosphorylation, a central adaptation to hypoxia is a shift toward non-oxidative forms of carbon metabolism and ATP production, such as anaerobic glycolysis (Fig. 1*A*). This process is altered in cancer or other rapidly-dividing cells because of the high demand for glycolytic intermediates for

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[2] Supported by National Institutes of Health NCI Grants P01 CA104838 and R01 158301. To whom correspondence should be addressed. Tel.: 215-746-5532; Fax: 215-746-5511; E-mail: celeste2@mail.med.upenn.edu.

[3] The abbreviations used are: HIF, hypoxia-inducible factor; ER, endoplasmic reticulum; HRE, hypoxia-response element; 2HG, 2-hydroxyglutarate; ROS, reactive oxygen species; MDH, malate dehydrogenase; LDH, lactate dehydrogenase; ccRCC, clear cell renal cell carcinoma; TCA, tricarboxylic acid; FIH, factor inhibiting HIF; $\alpha$KGDH, $\alpha$-ketoglutarate dehydrogenase; $\alpha$-KG, $\alpha$-ketoglutarate; PHD, prolyl hydroxylase; ETC, electron transport chain; LD, lipid droplet; FASN, fatty-acid synthase ACC, acetyl-CoA carboxylase; PDH, pyruvate dehydrogenase; EPO, erythropoietin; VHL, von Hippel-Lindau; miRNA, microRNA; SDHD, subunit D of succinate dehydrogenase.

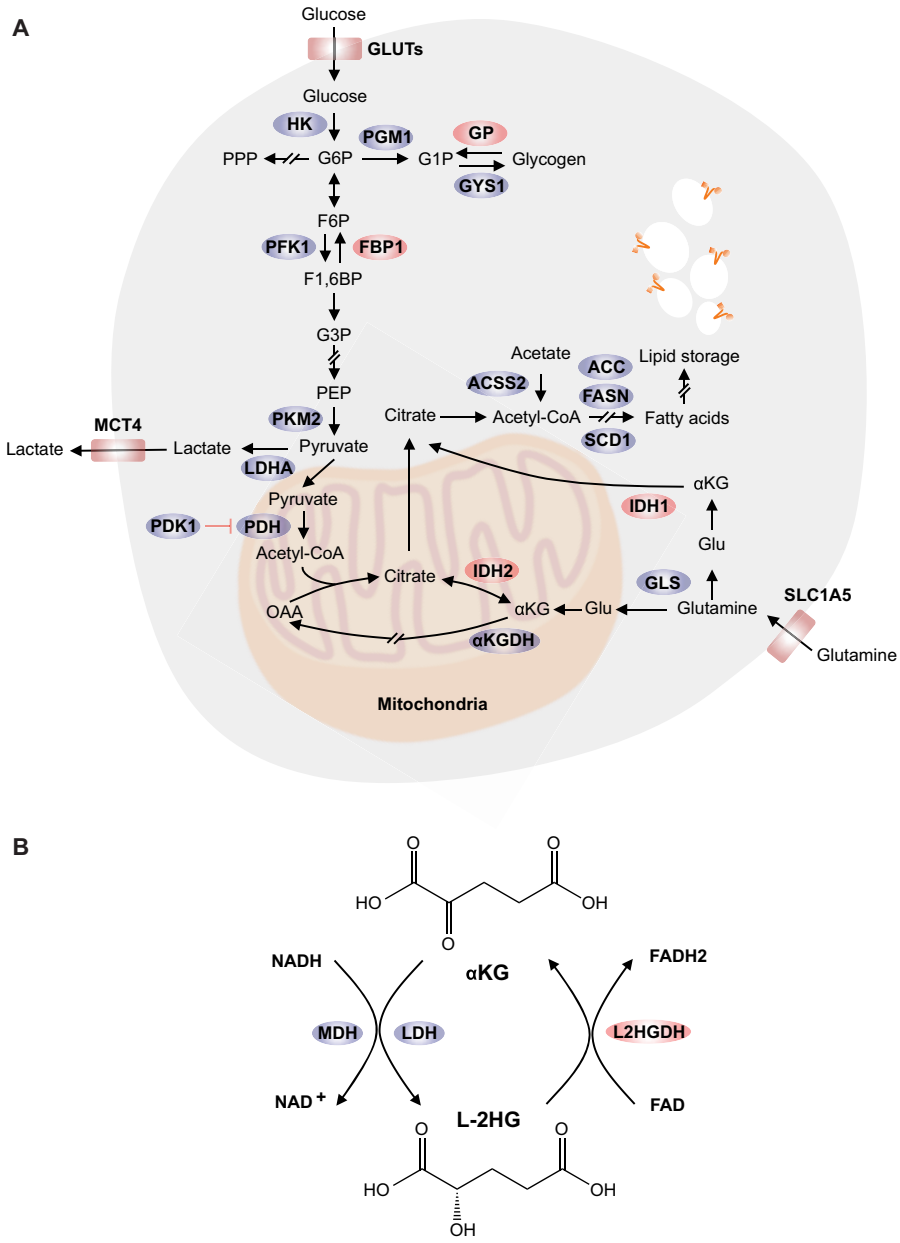


**Figure 1. Metabolic reprogramming in cancer cells under hypoxia.** *A,* low $O_2$ levels promote glucose uptake, glycolysis, lactate secretion, glutamine import, and glutaminolysis. Glucose can be stored as glycogen macromolecules, which are essential to maintain ATP levels and redox balance in hypoxic conditions. In addition, glycolytic enzymes FBP1 and PKM2 regulate HIF$\alpha$ activity through direct physical interaction. Moreover, cells exhibit increased rates of reductive carboxylation upon $O_2$ deficiency. For lipid metabolism, hypoxic cells generate citrate from glutamine, and acetyl-CoA from acetate to compensate for decreased supply of these molecules from the TCA cycle. SCD1 activity is affected directly by changes in $O_2$ availability. However, hypoxia results in increased lipid uptake, which counteracts the effects of SCD1 inhibition. The following genes are regulated by HIF: *GLUT*s (including *GLUT1* and *GLUT3*), *HK*, *PGM1*, *GYS1*, *PFK1*, *FBP1*, *PKM2*, *LDHA*, *PDK1*, *PDH*, and *MCT4*. Also ACSS2, ACC, FASN, SCD1, IDH, and $\alpha$KGDH protein expression/activity is directly or indirectly regulated by low $O_2$ levels. *One-headed arrows* indicate single-direction reactions, and *two-headed arrows* represent those that are reversible. In addition, *arrows with slashes* indicate multistep reactions. *B,* enzymatic and cofactor requirements for L-2HG metabolism. The following abbreviations and names are used in figure: *GLUT*, glucose transporter; *HK,* hexokinase; *PPP*, pentose phosphate pathway; *PGM1*, phosphoglucomutase 1; *GYS1*, glycogen synthase 1; *GP*, glycogen phosphorylase; *G6P*, glucose 6-phosphate; *G1P*, glucose 1-phosphate; *F6P*, fructose 6-phosphate; *F1,6BP*, fructose-1,6-bisphosphatase; *FBP1*, fructose-1,6-bisphosphatase 1; *G3P*, glyceraldehyde 3-phosphate; *PEP*, phosphoenolpyruvate; *PKM2*, pyruvate kinase M2; *LDHA*, lactate dehydrogenase A; *MCT4*, monocarboxylate transporter 4; *PDH*, pyruvate dehydrogenase; *PDK1*, pyruvate dehydrogenase kinase 1; *OAA*, oxaloacetate; *IDH*, isocitrate dehydrogenase; *$\alpha$KG*, $\alpha$-ketoglutarate; *$\alpha$KGDH*, $\alpha$-ketoglutarate dehydrogenase; *Glu*, glutamate; *GLS*, glutaminase; *SLC1A5*, solute carrier family 1 (neutral amino acid transporter), member 5; *ACSS2*, acyl-CoA synthetase short-chain family member 2; *ACC*, acetyl-CoA carboxylase; *FASN*, fatty-acid synthase; *SCD1*, stearoyl-CoA desaturase 1; *MDH*, malate dehydrogenase; *L2HGDH*, L-2-hydroxyglutarate dehydrogenase.

macromolecular synthesis. HIF1$\alpha$ contributes to this shift by promoting the expression of genes at almost every step in central carbon metabolism, such as hexokinases (*HK1* and *HK2*), phosphofructokinase 1 (*PFK1*), and phosphoglycerate kinase 1 (*PGK1*) (3). Glucose transporters GLUT1 and GLUT3 required for initial glucose internalization are also transcriptionally regulated by HIF1$\alpha$ (4, 5), which guarantees adequate glucose uptake and rapid energy production that compensates for its low efficiency. In addition, an end product of glycolysis, *i.e.* lactate, is efficiently removed from the cell through the action

of HIF1$\alpha$-inducible plasma membrane monocarboxylate transporter 4 (MCT4) (6). Taken together, HIF impacts both the glycolytic pathway itself and ancillary processes that support it.

Interestingly, although it is well-known that HIF activates genes encoding glucose metabolic enzymes, recent evidence indicates that some enzymes in turn affect HIF activity through direct physical interaction. One important example is pyruvate kinase, which catalyzes the transfer of a phosphate group from phosphoenolpyruvate to adenosine diphosphate (ADP), yielding one molecule each of pyruvate and ATP. As the last step in glycolysis, pyruvate kinase is a key enzyme that determines glycolytic flux. HIF1$\alpha$ induces transcription of the *PKM* gene (7), which encodes PKM1 and PKM2 isoforms. It has been extensively reported that cancer cells replace PKM1 with the less active PKM2, thereby reducing pyruvate production and redirecting glycolytic carbon to biosynthetic pathways to support cell growth (8). Importantly, Luo *et al.* (7) showed that PKM2, but not PKM1, interacts directly with HIF1$\alpha$ subunits and promotes transactivation of HIF target genes by enhancing HIF1$\alpha$ binding and p300 recruitment to HREs, at least partially explaining the function of PKM2 in cancer cells. This observation has more recently been extended to macrophages (9): lipopolysaccharide, which initiates immune responses found in endotoxemia, induces *PKM2* expression, enhancing the "Warburg effect" and shifting glucose metabolism away from oxidative phosphorylation toward a glycolytic program. This metabolic adaptation helps macrophages meet the increased demand for biosynthetic precursors required for mounting an immune response. Similarly, PKM2 interacts with HIF1$\alpha$ in activated macrophages to promote IL-1$\beta$ production (9).

Another important regulatory enzyme is the gluconeogenic fructose-1,6-bisphosphatase 1 (FBP1). Li *et al.* (10) found FBP1 is uniformly depleted in over 600 clear cell renal cell carcinoma (ccRCC) tumors examined, in which pVHL is lost and HIF activity is constitutively maintained at high levels. Importantly, FBP1 opposes tumor growth not only though its enzymatic function by antagonizing glycolytic flux and inhibiting the "Warburg effect," but also by inhibiting nuclear HIF function via direct interaction with the HIF$\alpha$ "inhibitory domain" (10). These two examples demonstrate complex regulation between HIF and its transcriptional targets and provide potential alternative therapeutic strategies (apart from targeting HIF directly) in tumors addicted to HIF signaling. Finally, whether and how other metabolic enzymes affect HIF activity is under active investigation.

*Glycogen metabolism*—Intracellular glucose is stored in the form of glycogen, a macromolecule essential for energy supply and glucose homeostasis (Fig. 1*A*) (11). Glycogen accumulation has been described in various cancer cells, although its abundance varies greatly across tumor types (12). Along with lipid deposition, glycogen accumulation contributes to the "clear cell" phenotype in a subset of breast, kidney, and ovary cancers (13). As with glycolysis, genes involved in glycogen biosynthesis have been identified as HIF targets in both normal and cancer cells, including phosphoglucomutase 1 (*PGM1*), protein phosphatase 1 regulatory subunit 3C (*PPP1R3C*), glycogen synthase 1 (*GYS1*), UTP–glucose-1-phosphate uridylyltransferase (*UGP2*), and 1,4-$\alpha$-glucan-branching enzyme (*GBE1*) (14–17). The induction of these proteins correlates with significant increase in glycogen buildup in cells exposed to hypoxia. In addition, glycogenolysis allows these "hypoxia-preconditioned" cells to confront and survive glucose deprivation.

Several studies investigated the role of glycogen metabolism in the cancer setting. In a glioblastoma model, hypoxia induces *GYS1* expression and subsequent glycogen accumulation (17). Interestingly, however, the glycogen degradation enzyme glycogen phosphorylase (PYGL) is also regulated by hypoxia. The authors demonstrated that GYS1 increases rapidly in hypoxia, and then declines, whereas PYGL climbs more slowly and reaches maximal levels at a later time point. This expression pattern is consistent with cellular glycogen levels, which are initially elevated and then gradually decline. In addition, PYGL knockdown leads to a p53-dependent induction of senescence and impaired tumorigenesis (17). Mechanistically, PYGL depletion is associated with glycogen deposition as well as increased reactive oxygen species (ROS), potentially due to a reduction in glucose carbon shuttling to the pentose phosphate pathway and impaired NADPH production. Furthermore, Lee *et al.* (18) show that the glycogen phosphorylase inhibitor CP-320626 reduces proliferation and increases apoptosis in pancreatic tumor cells, probably by limiting glucose oxidation, as well as *de novo* nucleic acid and fatty acid synthesis. These two studies provide a rationale to target glycogen degradation pathways in cancer cells, although more complete underlying mechanisms need to be defined. Finally, HIF-dependent regulation of glycogen synthesis has also been described in human ccRCC lines RCC4 and 786-O, which are characterized by pVHL loss and constitutive HIF activation (15). However, it remains to be determined whether and how this observation plays a role in ccRCC tumorigenesis.

### *HIF regulation of glutamine metabolism*

Glutamine is the most abundant amino acid in the circulation and is an essential precursor for the synthesis of proteins, fatty acids, nucleotides, and many other important molecules (19). Glutamine metabolism has been reviewed extensively (20) and is briefly outlined here. Upon cellular entry via transporters such as SLC1A5, glutamine is converted by glutaminases to generate glutamate. Glutamate plays a variety of important roles in eukaryotic cells (Fig. 1*A*). For example, it is required for glutathione synthesis, the major cellular antioxidant, and is also the source of amino groups for other non-essential amino acids such as proline, alanine, aspartate, serine, and glycine. In addition, glutamate can be converted to $\alpha$-ketoglutarate ($\alpha$-KG), which is either oxidized by $\alpha$-ketoglutarate dehydrogenase ($\alpha$KGDH) to succinate and enters a "forward" tricarboxylic acid (TCA) cycle to generate ATP or reductively carboxylated by isocitrate dehydrogenase (cytosolic IDH1 or mitochondrial IDH2) to produce isocitrate and citrate by a "reverse" TCA cycle.

Under hypoxia, Wise *et al.* (21) demonstrated that glutamine becomes a major source of citrate through reductive carboxylation via wild-type IDH2 activity in the glioblastoma cell line SF188. Consequently, hypoxic cells are unable to proliferate when they are either glutamine-starved or rendered IDH2-de-

ficient. This metabolic reprogramming might be partly through HIF, as constitutive HIF activation recapitulates the preferential reductive metabolic pathway even in normoxic conditions (21). In addition, IDH1 is also essential for HIF-mediated production of citrate at 1% $O_2$, and citrate produced via IDH1-dependent reductive carboxylation is utilized for *de novo* lipogenesis, required for maintaining cell growth in hypoxic conditions (22–24). This shift appears to be due to proteolysis of the E1 subunit of the αKGDH complex, named "oxoglutarate dehydrogenase 2" (OGDH2) (25). Sun and Denko (25) found that the E3 ubiquitin ligase SIAH2 destabilized OGHD2 in hypoxia in an HIF-dependent manner, which results in diminished αKGDH activity and decreased glutamine oxidation. In addition, a ubiquitination-resistant OGDH2 336KA mutant impedes tumor growth *in vivo*, accompanied by a reduction in glutamine incorporation into lipids (25), highlighting this pathway as an essential mechanism to rewire glutamine fate in HIF-stabilized tumor cells. Finally, in *VHL*$^{-/-}$ ccRCC cells, glutamine is also utilized to generate aspartate for *de novo* pyrimidine biosynthesis via reductive carboxylation and glutathione for redox balance (26). When glutaminase is inhibited, nucleoside depletion and increased ROS levels lead to DNA replication stress, which sensitizes cells to poly(ADP-ribose) polymerase inhibition *in vitro* and *in vivo*. Taken together, these studies exemplify alternative fates of glutamine in an HIF-dependent manner to support cancer cell proliferation and viability under hypoxic stress or as a consequence of genetic mutations.

### *HIF regulation of lipid synthesis*

Lipid synthesis provides essential building blocks and signaling molecules for tumor growth. Thus, despite a high-energy cost, *de novo* lipogenesis is clearly increased in most cancer cells (27–29). In this process, acetyl-CoA and NADPH together generate free fatty acid chains through stepwise enzymatic reactions via acetyl-CoA carboxylase (ACC) and fatty-acid synthase (FASN) (Fig. 1*A*). These fatty acids are then: 1) used to generate phospholipids, the basic units of lipid bilayers making up cell and organelle membranes; 2) incorporated into neutral lipids, such as triglycerides and cholesterol esters, for storage; 3) utilized to produce essential signaling molecules, such as sphingosine 1-phosphate, lysophosphatidic acid, diglyceride, and inositol 1,4,5-trisphosphate, which control inflammation, cell migration, and survival in cancer; and 4) employed to modify proteins, among others.

In hypoxic conditions, glucose-derived pyruvate entry into the TCA cycle is inhibited, as well as subsequent citrate and acetyl-CoA production. Therefore, cells must shift to alternative carbon sources to generate acetyl-CoA for fatty acid synthesis. These sources include citrate synthesis from reductive carboxylation of glutamate (reviewed above) and acetyl-CoA production through metabolizing acetate via cytoplasmic acetyl-CoA synthetase (ACSS2) (30). Indeed, based on $^{13}$C-tracing and mass spectrometry, cells cultured in $O_2$-replete conditions produce more than 90% acetyl-CoA from glucose and glutamine-derived carbon. However, at 1% $O_2$, acetate instead appears to be the primary hypoxia-induced contributor to acetyl-CoA in multiple cell lines (31). In addition, hypoxia enhances *ACSS2* expression, increasing the use of acetate to sustain lipid biomass production and imparting a competitive growth advantage under microenvironmental stress (32). Exactly how *ACSS2* is up-regulated in hypoxia remains unclear, although it has been suggested that sterol regulatory element-binding protein 2 (SREBP2) mainly controls *ACSS2* expression, whereas HIF signaling enhances the up-regulation of *ACSS2* by SREBP2 (32).

Multiple genes involved in lipid metabolism have been reported to be strongly induced under hypoxia. One example is the regulation of *SREBP1* via phosphorylation of AKT followed by HIF1α activation (33). Of note, SREBP1 is a major transcriptional regulator of lipid metabolic genes, such as *FASN*. Moreover, some lipid metabolic genes are direct HIF transcriptional targets. For example, in hypoxic breast cancer MCF-7 cells and glioblastoma U87 cells, induction of fatty acid-binding protein 3 (*FABP3*), *FABP7*, and adipose differentiation-related protein (*ADRP*, also known as perilipin2, *PLIN2*) is an HIF1α-dependent but HIF-2α-independent event. These proteins are essential for lipid droplet (LD) formation, which helps to maintain ROS levels in these cells (34). Furthermore, PLIN2 up-regulation in ccRCC is HIF2α-dependent, which promotes lipid storage and contributes to "clear cell" phenotypes (35). Interestingly, PLIN2-mediated LD formation maintains integrity of the ER, suppressing cytotoxic ER stress responses that otherwise result from elevated protein synthetic activity characteristic of ccRCC cells. Therefore, PLIN2 depletion results in apoptosis and further sensitizes these cells to ER stress, identifying it as a targetable vulnerability created by HIF2α/PLIN2 signaling in this common renal malignancy.

### *HIF and redox stress*

Because of its role as a potent electron acceptor, $O_2$ has the tendency to form highly reactive oxygen species. Mitochondrial $O_2$ metabolism is the dominant source of superoxide production via the mitochondrial electron transport chain (ETC). Several studies found HIFα subunits can be stabilized by mitochondrial ROS (36–39). Reciprocally, suppressing the production of ROS by ETC seems to be a fundamental function of HIF.

Under hypoxic conditions, HIF actively regulates mitochondrial function and subsequent ROS production through multiple mechanisms. First, HIF1α uncouples glycolysis and oxidative mitochondrial metabolism by transcriptionally up-regulating pyruvate dehydrogenase kinase 1 (*PDK1*) (40, 41). PDK1 phosphorylates and inhibits the function of mitochondrial pyruvate dehydrogenase (PDH), which transforms pyruvate into acetyl-CoA, subsequently used in the TCA cycle to carry out cellular respiration (Fig. 1*A*). Enforced PDK1 expression in hypoxic HIF1α-null cells attenuates ROS generation and protects these cells from hypoxia-induced apoptosis (40). At the same time, accumulating pyruvate is metabolized by lactate dehydrogenase A (LDHA) to generate lactate, which is then exported from the cell by MCT4, as discussed previously. Importantly, both *LDHA* and *MCT4* are also HIF1α targets (42). Taken together, HIF1α-dependent transcriptional regulation coordinately impedes the entry of substrates for mitochondrial respiration and toxic ROS production. Second, HIF1α attenuates mitochondrial function by down-regulating several ETC components. For example, components of cytochrome *c*

oxidase (COX; complex IV), the final ETC enzyme, are regulated in an HIF1α-dependent manner (43). HIF1α reciprocally manipulates COX4 subunit expression by activating transcription of the gene encoding COX4-2, while at the same time enhancing COX4-1 protein degradation by increasing the abundance of LON, a mitochondrial protease (43). The replacement of COX4-1 with COX4-2 allows improved adaption to hypoxia, with reduced ROS production. In addition, HIF1α increases mitochondrial NDUFA4L2 (NADH dehydrogenase 1α subcomplex, 4-like 2), which attenuates mitochondrial oxygen consumption through reduced complex I activity, limiting intracellular ROS production under low $O_2$ conditions (44). Furthermore, HIF1α suppresses succinate dehydrogenase subunit B, a component of mitochondrial complex II (45), but whether this contributes to ROS level regulation is unclear. Finally, HIF influences mitochondrial function through effects at the whole-organelle level. In ccRCC cells lacking VHL, HIF1α negatively regulates c-Myc activity and subsequent mitochondrial biogenesis, $O_2$ consumption, and ROS production (46). Moreover, the HIF target BNIP3 contributes to reduced mitochondrial mass through increased mitophagy (47). Taken together, these adaptive pathways underlying HIF activation attenuate ROS production and provide protective mechanisms for cancer cell growth. Whether they are "Achilles heels" and how we can utilize them against cancer are under investigation.

### *HIF, miR-210, and metabolism*

The microRNAs (miRNAs) are small, non-coding RNA molecules ~22 nucleotides in length that are evolutionarily conserved (48). Targeting most protein-coding transcripts, miRNAs are involved in nearly all physiological and pathological processes, including cancer cell metabolism. In recent years, more than 50 hypoxia-regulated miRNAs have been identified. Among them, *miR-210* exhibits a robust and consistent up-regulation under hypoxia in virtually all cell types through an HIF1α-dependent regulation (49) and influences intracellular metabolism in both normal and malignant cells. For example, first, *miR-210* represses the iron–sulfur cluster assembly proteins (ISCU1 in cytosol and ISCU2 in mitochondria) (50), which promote the assembly of [4Fe-4S] and [2Fe-2S] iron–sulfur clusters, which are incorporated into enzymes responsible for mitochondrial respiration, such as those in ETC complexes I, II, and III (51). Therefore, *miR-210* expression decreases activity of complex I, oxygen consumption, and ATP generation (50). Moreover, an *miR-210* antagonist decreases clonogenic survival in hypoxic MCF7 and HeLa cells (52). In both cases, *miR-210* suppression increases ROS production. Second, the transcript coding for one of ETC components, SDHD, subunit D of succinate dehydrogenase complex, is a *bona fide miR-210* target. *SDHD* knockdown mimics *miR-210* mediated mitochondrial dysfunction. In addition, SDHD targeting also seems to stabilize HIF1α, which creates a positive feedback loop (53). Taken together, *miR-210* serves as an important mediator shifting mitochondrial oxidative phosphorylation to glycolysis downstream of HIF. Whether other miRNAs are involved in more metabolic processes needs to be further investigated.

## HIF-independent regulation of intracellular metabolic reactions

As discussed above, under low $O_2$ tension, HIF coordinately regulates a variety of adaptive pathways to enable cancer cell growth, including but not limited to glucose, glutamine, lipid, and redox metabolism. However, $O_2$ availability also affects cellular responses through HIF-independent mechanisms, the understanding of which will help to more completely describe how cancer cells adapt to hypoxic microenvironment.

### *L-2-Hydroxyglutarate (L-2HG) production and function*

With the discovery of oncogenic mutations in isocitrate dehydrogenase enzymes (IDH1 and IDH2), the enzymatic product D-2HG is broadly appreciated as an oncometabolite that inhibits αKG and a large family of αKG-dependent dioxygenases, including histone demethylases and methylcytosine dioxygenases of the TET family, causing epigenetic dysregulation and a block in cellular differentiation (54). Although intensive efforts have been made to investigate the cellular functions of IDH-mutant derived D-2HG, and a selective, potent inhibitor of mutant IDH2, AG-221, was shown to be effective in IDH2 mutation-positive acute myeloid leukemia (55, 56), its mirror-image enantiomer L-2HG is less understood. Interestingly, recent studies found that hypoxia dramatically induces production of L-2HG in both normal and malignant cells (57, 58).

In different types of cancer cell lines, hypoxia substantially increases 2HG production 5–25-fold, detailed analysis specifies a selective accumulation of the L-enantiomer of 2HG in these cells (57). Surprisingly, this reaction is not through IDH1/2, but LDHA and malate dehydrogenase (MDH1 and MDH2) via their "promiscuous" catalytic activity (57). Further study confirmed these results by showing that purified LDH and MDH enzymes catalyze stereospecific reduction of αKG to L-2HG and that acidic reaction conditions dramatically enhance LDH- and MDH-mediated production of L-2HG *in vitro* and in cells. Mechanistically, hypoxia-induced acidic pH enhances LDHA-mediated reduction of αKG by driving equilibrium toward a protonated form of αKG that binds more stably to the LDHA enzyme (59). In normal cells, including human pulmonary arterial endothelial and smooth muscle cells, hypoxia also directs the production of L-2HG (58), highlighting it as a fundamental mechanism for hypoxia adaptation. Functionally, acid-enhanced production of L-2HG leads to stabilization of HIF1α in normoxic conditions (59). In addition, like D-2HG, L-2HG also inhibits the Jumonji family histone lysine demethylase KDM4C, resulting in aberrant accumulation of trimethylated histone 3 lysine 9 (H3K9me3) (57). Interestingly, a recent report demonstrated that tumor hypoxia reduces TET enzyme activity, which is independent of hypoxia-associated alterations in *TET* expression, proliferation, metabolism, HIF activity, or ROS production, and it is dependent directly on $O_2$ shortage (60). This effect results in hypermethylation at gene promoters (including those suppressing glycolysis), which accounts for up to half of the hypermethylation events in cells. However, whether and how much hypoxia-mediated L-2HG plays a role in this process needs to be investigated in more detail. Furthermore, mitochondrial L-2HG is oxidized and converted back to

αKG by L-2HG dehydrogenase (L2HGDH) (61), and αKG and L-2HG form a redox couple linked to NADH/$NAD^+$ and $FADH_2$/FAD (Fig. 1*B*). Therefore, 2HG may provide cells with a reservoir of reducing equivalents, and the αKG-2HG exchange maintains cellular redox balance. Consistent with this idea, a recent study in hematopoietic stem cells reported that loss of the mitochondrial complex III subunit Rieske iron–sulfur protein results in impaired mitochondrial respiration, increased NADH/$NAD^+$ ratio, and increased 2HG levels, accompanied with DNA and histone hypermethylation (62). Because L2HGDH activity is dependent on active complex III (63), loss of complex III activity both increases NADH/$NAD^+$ and impairs L2HGDH activity to favor 2HG production.

How HIF proteins contribute to L-2HG production seems to be context-dependent. In lung fibroblasts, whereas *VHL* knockdown and HIFα stabilization increase total 2HG production in normoxia, HIF1α depletion also elevates L-2HG in hypoxia, indicating HIF1α is sufficient but not necessary for L-2HG accumulation in these cells (58). Consistent with this, in SF188 cancer cells, *HIF1A* knockdown in hypoxia does not decrease but slightly increases L-2HG levels (57). In addition, HIF1α enhances hypoxia-induced L-2HG production in VHL-deficient RCC4 cells, as HIF1α depletion partly reverses hypoxia-mediated L-2HG increments (57). However, in murine $CD8^+$ T lymphocytes, $Hif1\alpha^{-/-}$ abolished 2HG accumulation under hypoxia, and L-2HG constitutes more than 90% of the 2HG pool, suggesting L-2HG production is dependent on HIF1α in this case (64).

### *Alternative effect of hypoxia on lipid metabolism*

$O_2$ deficiency affects lipid metabolism not only through HIF-dependent pathways, as discussed above, but also via direct effects on enzymes involved in this process. Stearoyl-CoA desaturase-1 (SCD1) is a key enzyme catalyzing the rate-limiting step in the formation of monounsaturated fatty acids. This process conducts electron flow from NADPH to the terminal electron acceptor molecular $O_2$, rendering $O_2$ a critical factor in the formation of double bonds in stearoyl-CoA. Indeed, Kamphorst *et al.* (65) found that consistent with impaired SCD1 activity, hypoxic cancer cells display decreased C18:1 production, as well as the C18:1/C18:0 ratios. Furthermore, SCD1 enzymatic activity is required to maintain cell viability in $Tsc2^{-/-}$ mouse embryonic fibroblasts with $O_2$ deprivation (66). This is accompanied by elevated toxic ER stress, which might be caused by an unmet requirement of ER membrane quality in the conditions of increased protein synthesis by dysregulated mTORC1. This mechanism has been confirmed in multiple systems by demonstrating that SCD1 inhibition increases ER stress *in vitro* and *in vivo* (67, 68) and can be employed as a strategy to treat cancers.

## Conclusion

Although our understanding of metabolic alterations in cancer has improved dramatically over recent years, whether and how hypoxia affects these pathways is still not entirely clear. As a typical feature of solid tumors, hypoxia is usually associated with chemoresistance, radioresistance, and poor prognosis. Defining how cancer cells cope with hypoxic stress is therefore critical for identifying potential new therapeutic targets. As discussed above, metabolic rewiring is one of the major hypoxia-induced responses, encompassing HIF-dependent and HIF-independent pathways. These adaptive responses collectively enable cell growth and survival, which at the same time could be an "Achilles heel" of these cells. Indeed, in cell culture and preclinical mouse model systems, targeting these processes results in cell death and tumor regression. However, will metabolic therapy be clinically viable? A therapeutic window may be difficult to achieve, and cancer cells could shift to adaptive pathways during long-term exposure to certain treatments. Therefore, future research needs to focus on how to specifically target cancer cells and improve efficacy, such as combining metabolic intervention with standard chemotherapy, radiotherapy, and immunotherapy.

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