REVIEW

Complex role of HIF in cancer: the known, the unknown, and the unexpected

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Abstract: Tumor hypoxia has long been recognized as a driving force of malignant progression and therapeutic resistance. The discovery of hypoxia-inducible transcription factors (HIFs) has greatly advanced our understanding of how cancer cells cope with hypoxic stress by maintaining bioenergetics through the stimulation of glycolysis. Until recently, however, it remained perplexing why proliferative cancer cells opt for aerobic glycolysis, an energy-inefficient process of glucose metabolism. Furthermore, the role of HIF in cancer has also become complex. In this review, we highlight recent groundbreaking findings in cancer metabolism, put forward plausible explanations to the complex role of HIF, and underscore remaining issues in cancer biology.

Keywords: cancer biology, hypoxia, metabolism, oncogenic signaling

Introduction

Cellular adaptation to O_2 deprivation – hypoxia – is a physiological response during the course of normal development and aging in metazoans, and is critical for continued growth and survival of the organism. Central to this adaptive response is hypoxia-inducible factor 1 (HIF-1), a transcription factor that orchestrates global responses to hypoxia in gene expression.[1] HIF-1 is a heterodimer composed of HIF-1α (gene symbol *HIF1A*) and aryl hydrocarbon receptor nuclear translocator (gene symbol *ARNT*, and also known as HIF-1β).[2] Both subunits possess a basic helix-loop-helix domain capable of dimerization and binding to the promoters of hundreds of target genes through the recognition of hypoxia-responsive elements that contain the core sequence RCGTG.[3–7]

As the regulatory subunit of HIF-1, the O_2-labile HIF-1α stabilizes in response to hypoxia, leading to heterodimerization, DNA binding, and recruitment of the transcription co-activators p300 and CBP.[8–10] HIF-1α uses a hydrophobic interface including Cys800 to interact with p300 and CBP.[11–14] Interestingly, in normoxia, HIF-1 target genes are transcriptionally active but paused for RNA polymerase II elongation; hypoxia-activated HIF-1α employs CDK8-Mediator and the super elongation complex to alleviate the pausing for elongation.[15] Numerous HIF-1 target genes are activated by this canonical mechanism of transcription, such as those involved in angiogenesis, metabolic reprogramming, cell survival and proliferation, and migration and metastasis (Figure 1).[16–18] Also contributing to these cancer biological processes is HIF-2α (gene symbol *EPAS1*),[19,20] a paralog of HIF-1α, which shares similar biochemical properties, regulatory mechanisms, and target genes, even though distinct, and even opposing, functions of HIF-2α have begun to be appreciated.[21]

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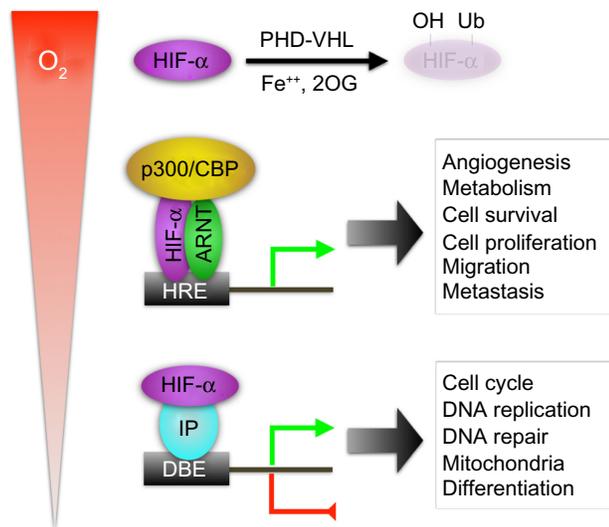Email eric.huang@hsc.utah.edu

Figure 1 Hypoxia-inducible factor alpha (HIF-α) regulation and mechanisms of action. **Notes:** In normoxia, HIF-1α and HIF-2α (designated as HIF-α) are subjected to prolyl hydroxylation by PHD in the presence of ferrous iron (Fe^{++}) and 2-oxoglutarate (2-OG). The E3 ubiquitin ligase VHL recognizes hydroxylated HIF-α (—OH) for polyubiquitination (—Ub) and proteasome degradation (shaded HIF-α). Under low oxygen conditions, HIF-α is stabilized, leading to dimerization with ARNT, recruitment of p300/CBP, and binding to the hypoxia responsive elements (HRE) in the target gene promoter. This mechanism of action is responsible for upregulating numerous target genes in many cancer-related processes as listed. Under severe hypoxia, HIF-α can also act through crosstalk with a number of other interacting proteins (IPs) that occupy DNA-binding elements (DBE), resulting in either gene upregulation or downregulation. This noncanonical mechanism seems responsible for some subtle cellular changes in cancer. **Abbreviations:** PHD, prolyl hydroxylase domain proteins; ARNT, aryl hydrocarbon receptor nuclear translocator; VHL, von Hippel-Lindau protein.

HIF-1α is sensitive to O_2, owing to the presence of an O_2-dependent degradation domain that mediates ubiquitin-proteasomal proteolysis.[22] The von Hippel-Lindau (VHL) protein is a part of the E3 ubiquitin ligase,[23,24] which recognizes two highly conserved proline residues (Pro402 and Pro564 in HIF-1α, Pro405 and Pro531 in HIF-2α) for polyubiquitination; however, hydroxyl modification of these proline residues is a prerequisite for VHL recognition.[25–27] Prolyl hydroxylation is catalyzed by three prolyl hydroxylase domain-containing (PHD) proteins of the Fe(II)- and 2-oxoglutarate (2OG)-dependent dioxygenase family: PHD1, PHD2, and PHD3 (gene symbols *EGLN2*, *EGLN1*, and *EGLN3*, respectively), which use molecular O_2 for sensing and signaling.[28–34]

Likewise, the factor inhibiting HIF-1 (FIH1, gene symbol *HIF1AN*)[35] is another type of hydroxylase that modifies Asn803 in the HIF-1α transactivation domain to block p300 interaction, thereby providing an additional mechanism for modulating HIF-1α activity.[36,37] Although FIH1 is a dioxygenase that uses molecular O_2 for substrate modification, its in vivo role for HIF-1α regulation requires further investigation because mice with a *Hif1an*-null mutation demonstrated no alterations of Hif-1 function but

exhibited a hypermetabolic state with hyperventilation and decreased body mass, which was regulated systemically by the nervous system.[38]

Besides hydroxylation, numerous modulatory mechanisms have been identified, adding the complexity of HIF regulation. These include oncogenes and tumor-suppressor genes, acetylation and SUMOylation, reactive oxygen species and nitric oxide, and microRNAs. These regulatory mechanisms have been extensively reviewed.[39]

In addition, both HIF-1α and HIF-2α employ crosstalk mechanisms to regulate cell proliferation, DNA repair, mitochondrial biology, and cell stemness.[40,41] These noncanonical mechanisms of HIF-1α and HIF-2α actions seem independent of ARNT and direct DNA binding; rather, they require protein–protein interactions (Figure 1). HIF-1α and HIF-2α regulate cell-cycle genes in an opposing fashion; HIF-1α inhibits cell-cycle progression by suppressing c-Myc activity, whereas HIF-2α does the reverse.[42–44] By similar mechanisms, HIF-1α represses, whereas HIF-2α stimulates, DNA repair genes.[45–47] HIF-1α engages in the Notch signaling pathway to block cell differentiation, and the β-catenin signaling pathway to regulate embryonic and adult cell proliferation, albeit in an opposite way.[48–51] Finally, HIF-1α also inhibits DNA replication through direct interaction with the adenosine triphosphatase Cdc6.[52] Further studies are required to elucidate detailed mechanisms of noncanonical actions of HIF-1α and HIF-2α.

HIF-1α, HIF-2α, and cancer

Solid cancers frequently harbor hypoxic regions where HIF-1α and HIF-2α become overexpressed and activated.[53] Furthermore, oncogenic signaling and growth factor stimulation lead to the activation of the phosphotidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK) pathways, thereby stimulating HIF-1α and HIF-2α synthesis.[16,17,54–56] As a result, increased expression of both HIF-1α and HIF-2α has been observed in a wide variety of human cancers and, in general, is associated with poor prognosis.[17,57] Likewise, numerous studies support the notion that HIF-1α and HIF-2α promote tumor angiogenesis and growth.[17,18,57] Furthermore, rare somatic, gain-of-function mutations in *EPAS1* have been identified to be associated with paraganglioma with polycythemia[58] and pheochromocytoma.[59]

It has been recognized, however, that HIF-1α expression is also associated with favorable prognosis, eg, in patients with neuroblastoma and renal cell carcinomas.[21,60] Forced expression of HIF-1α in renal cell carcinoma cell lines retarded tumor growth in xenografts,[61,62] suggesting a tumor-suppressing role

for HIF-1α. Conversely, it is suggested that Hif-1α is not required for renal cyst development in conditional *Vhl*-null mice.[63] Furthermore, rare somatic, loss-of-function *HIF1A* mutations have been identified in the specimens of clear-cell renal carcinoma,[64,65] and common focal, homozygous deletions have been detected in cell lines of VHL-deficient renal cell carcinomas.[66] Despite this cogent evidence, the mechanism by which HIF-1α acts as a tumor suppressor remains unclear, and how cancer cells might escape from HIF-1α suppression needs to be addressed, especially in majority of renal cell carcinomas where HIF-1α is commonly overexpressed. What may explain retarded tumor growth in the xenograft studies, however, is that HIF-1α inhibits cell proliferation.[40] Consistently, renal cell carcinomas with HIF-1α expression are statistically much smaller than those without, and intriguingly seem more metastatic.[47] Therefore, the role of HIF-1α in malignant progression and metastasis cannot be ruled out in VHL-deficient renal cell carcinomas.

HIF-2α, on the other hand, is believed to be a major contributor to VHL-deficient renal carcinogenesis.[33] However, in other experimental settings, HIF-2α seems to be a tumor suppressor; HIF-2α increases apoptosis in glioma[67] and inhibits oncogenic signaling and activates a tumor-suppressor gene in non-small-cell lung cancer.[68] Clearly, the role of HIF-1α and HIF-2α in cancer biology is complex, and their biological functions are likely context dependent.

Critical role of HIF-1α in metabolic switch from oxidative phosphorylation to anaerobic glycolysis

Among the first recognized biological functions of HIF-1α was metabolic adaptation to decreased O_2 availability, resulting from transcriptional upregulation of multiple genes involved in glucose transport and glycolysis, such as those encoding aldolase A (*ALDOA*), phosphoglycerate kinase 1 (*PGK1*), lactate dehydrogenase A (*LDHA*), and glucose transporters (*SLC2A1* and *SLC2A3*).[1] Genetic deletion of *Hif1a* gene in mouse embryonic cells confirmed the critical role for HIF-1α in glycolysis and lactate production.[69,70]

Under normal O_2 tensions, glycolysis catabolizes glucose to pyruvate, which is converted to acetyl-CoA by pyruvate dehydrogenase for oxidative phosphorylation in the tricarboxylic acid (TCA) or Krebs cycle. Under hypoxia, cells decrease oxidative phosphorylation in the mitochondria and adopt anaerobic glycolysis and conversion of pyruvate to lactate. As a key regulator of this process, HIF-1α actively suppresses

mitochondrial O_2 consumption by transcriptionally upregulating both pyruvate dehydrogenase kinases 1 and 3 (PDK1 and PDK3, respectively),[71–73] which subsequently inactivate pyruvate dehydrogenase to block pyruvate conversion and flux of acetyl-CoA into the TCA cycle. Moreover, HIF-1 has been shown to inhibit oxidative phosphorylation by inhibiting mitochondrial biogenesis.[74]

In addition to the critical role in the metabolic switch from oxidative phosphorylation to anaerobic fermentation – the Pasteur effect, HIF also stimulates energy storage for hypoxic cell survival through the induction of glycogen and lipid synthesis.[75] Primarily, HIF-1α promotes glycogen accumulation through transcriptional activation of several genes involved in glycogen biosynthesis, such as *GYS1*, *PPP1R3C*, and *PGM1*.[76–78] HIF-1α also upregulates expression of the peroxisome proliferator-activated receptor γ (gene symbol *PPARG*), thereby increasing fatty acid uptake and triglyceride biosynthesis in cardiac hypertrophy.[79] Additional HIF-1α target genes responsible for lipid accumulation include *HILPDA* and *LPIN1*.[80,81] Interestingly, conditional deletion of murine *Vhl* in hepatocytes indicates a role for Hif-2α, rather than Hif-1α, in lipid synthesis, oxidation, and storage,[82] another example of context dependence for HIF function.

HIF-1α diverts glycolytic metabolites into biosynthesis by blunting pyruvate production

In comparison to oxidative phosphorylation, glycolysis is inefficient in energy generation. Consistent with the role of HIF-1α in energy maintenance and conservation,[83] it stands to reason that cancer cells adopt glycolysis under low O_2 tensions for survival. However, cancer cells generally manifest characteristics of increased proliferation associated with high glucose uptake and lactate production even in the presence of O_2 – aerobic glycolysis or the Warburg effect.[84–86] Until recently, the significance of aerobic glycolysis remained debatable because it was unclear why cancer cells prefer energy-inefficient glycolysis to support proliferation, notwithstanding the recognition of the role of HIF-1α in aerobic glycolysis.[87]

A reinterpretation of the Warburg effect is that aerobic glycolysis is not merely employed for bioenergetics but, more importantly, for biosynthesis of macromolecules (nucleotides, amino acids, and lipids) necessary for cell proliferation.[85,86,88] This view has integrated oncogenic signaling (PI3K-AKT-mTOR and c-Myc) with regulation of metabolic pathways, biosynthesis, and cell proliferation. Recent studies have

pointed out the M2 splice isoform of pyruvate kinase (PKM2, a splice variant of the *PKM* gene) as a metabolic switch for aerobic glycolysis and tumorigenesis.[89,90] In normal adult tissues, the splice variant PKM1 catalyzes the final step of glycolysis by transferring the phosphate from phospho-enolpyruvate to adenosine diphosphate, thereby yielding pyruvate and adenosine triphosphate, whereas PKM2 is expressed primarily in lung tissues and proliferating cells including stem cells and cancer cells.[91] The transcription factor c-Myc promotes PKM2 splicing by inducing the expression of three heterogeneous nuclear ribonucleoproteins (hnRNPs).[92] It has been shown that oncogenic signaling converges on the activation of mTOR to increase HIF-1α levels for *PKM* transcription and concomitantly c-Myc levels for PKM2 splicing (Figure 2).[90]

In addition to being a glycolytic enzyme, PKM2 can function as a protein kinase for gene transcription in the nucleus.[93–95] Activation of epithelial growth factor receptor induces extracellular signal-regulated kinase 2 (gene symbol *MAPK1*)-mediated phosphorylation and translocation of PKM2 into the nucleus. Nuclear PKM2 interacts with β-catenin to activate target genes such as *CCND1* and *MYC*

through phosphorylation of histone H3, which leads to the dissociation of HDAC3 and acetylation of histone H3. Accordingly, nuclear PKM2 promotes cell-cycle progression and cell proliferation by stimulating cyclin D1 and c-Myc expression. c-Myc further enhances PKM2 expression in a positive-feedback loop and also induces glycolytic gene expression.[93–95] PKM2 can also phosphorylate signal transducer and activator of transcription 3 (gene symbol *STAT3*) to drive gene expression.[96] In addition, PKM2 is a transcriptional coactivator of HIF-1α transcriptional activity.[97] PKM2 can be hydroxylated by PHD3, and hydroxylated PKM2 enhances HIF-1α activity through direct interaction. Taken together, PKM2 stimulation of c-Myc and HIF-1α further promotes glycolysis.

So, is PKM2 essential to tumor growth and lactate production? On the contrary, mice with a conditional allele that abolishes Pkm2 but not Pkm1 expression show accelerated mammary tumor formation and heterogeneous Pkm1 expression.[98] Pkm1 was detected in non-proliferating cells but not in those in proliferation, which suggests that active pyruvate kinase is required for non-proliferating, but not necessarily for proliferating, tumor cells. Interestingly, PKM2
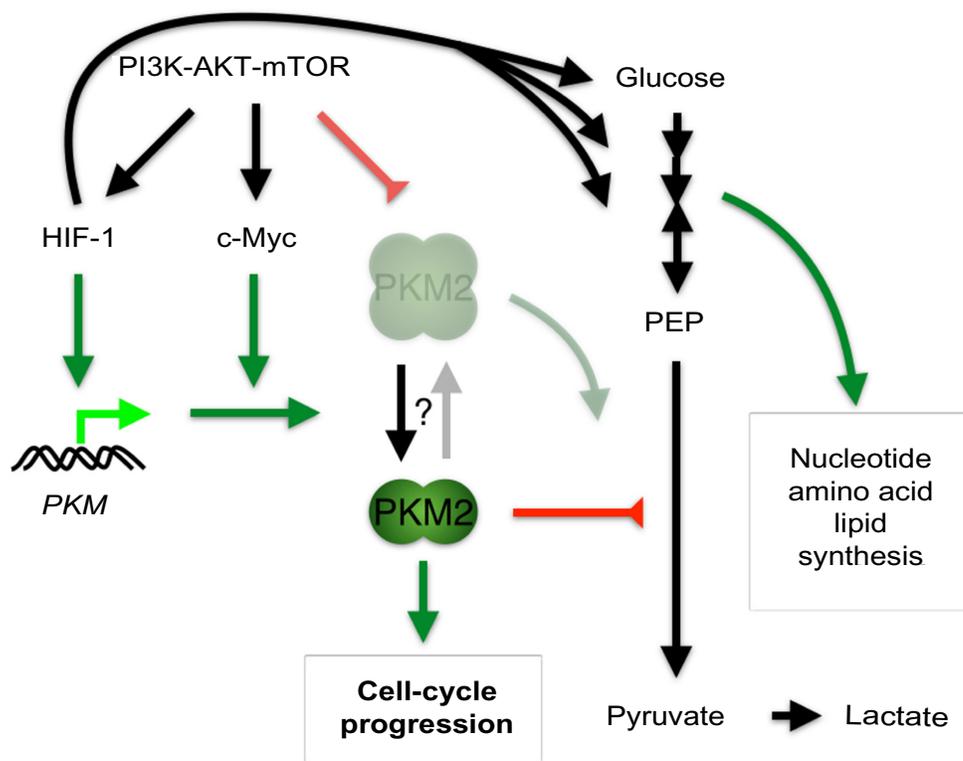

Figure 2 Role of hypoxia-inducible factor 1 (HIF-1) in regulating bioenergetics and biosynthesis through the induction of pyruvate kinase M2 (PKM2) in proliferating cancer cells.
Notes: Increased HIF-1 expression through oncogenic signaling not only promotes glucose uptake and glycolysis, but also cooperates with c-Myc to increase PKM2 expression through transcriptional upregulation of *PKM* and PKM2 splicing, respectively. PKM2 is present mainly in the nucleus as a dimer and acts as a protein kinase to drive gene transcription for cell-cycle progression. Its low activity as pyruvate kinase blunts the conversion of phosphoenolpyruvate (PEP) to pyruvate, thereby diverting upstream glycolytic metabolites into the biosynthesis pathways. Although PKM2 can also exist as a tetramer for glycolysis, its glycolytic activity is inhibited by oncogenic signaling.

found in tumor cells is usually dimeric with low enzymatic activity for glycolysis, whereas the tetrameric form in normal cells has a high activity.[91] Furthermore, oncogenic signaling inhibits the tetrameric form of PKM2 through direct and selective binding to tyrosine-phosphorylated peptides, thereby enabling anabolism from glucose metabolites for tumor growth.[99]

Thus, HIF-1α-mediated PKM2 expression provides a regulated, bifunctional protein that can act on the one hand as a tetrameric glycolytic enzyme for bioenergetics and lactate production but on the other as a dimeric transcriptional coactivator/protein kinase for cell proliferation. Most importantly, loss of glycolytic activity resulting from either a dimer or a tyrosine-phosphorylated peptides-bound tetramer diverts glycolytic metabolites into the anabolic pathways (Figure 2).

Hereditary mutations in fumarate hydratase and succinate dehydrogenase

Neither HIF-1α nor HIF-2α is known to transcriptionally regulate genes in the TCA cycle; rather, it is the intermediate metabolites including fumarate and succinate that have been shown to increase the activities of these transcription factors.[100] Whereas germline mutation in the *FH* gene (encoding fumarate hydratase) predisposes individuals to hereditary leiomyomatosis and renal cell carcinoma, hereditary mutations in the genes encoding four subunits of succinate dehydrogenase (*SDHA*, *SDHB*, *SDHC*, and *SDHD*) and one cofactor (*SDHAF2*) are linked to familial pheochromocytomas and paragangliomas.[100–102] These tumors are characterized by the induction of pseudo-hypoxia, ie, increased HIF activities and target gene expression in normoxia. At the molecular level, inactivation of fumarate hydratase and succinate dehydrogenase leads to the accumulation of fumarate and succinate, respectively,[103] resulting in allosteric inhibition of prolyl hydroxylase PHDs and induction of HIF-1α and HIF-2α.[104–106] Targeted inactivation of mouse *Fh1* confirmed the resultant activation of Hif-1α and Hif-2α and development of proliferative renal cysts.[107] *FH*-deficient renal cancer cells also exhibit aerobic glycolysis and increased expression of HIF-1α.[108] Additionally, accumulated fumarate produces succinated glutathione, resulting in increased mitochondrial reactive oxygen species and HIF-1α activation.[109] In fact, it has been shown that fumarate can modify numerous proteins including mitochondrial aconitase 2 for the inhibition of aconitase activity in *Fh1*-null mouse embryonic fibroblasts,[110] even

though reduced cytosolic, but not mitochondrial, aconitase activity is observed in *FH*-deficient renal cancer cells associated with iron deficiency.[108]

Despite these findings, whether activation of HIF pathway has a causal effect on renal cyst formation was questioned.[111] Indeed, in mouse genetic studies, *Fh1*$^{-/-}$-associated renal cyst formation was independent of Hif-1α and Hif-2α, and was further exacerbated by inactivation of Hif-1α but not Hif-2α.[112] In fact, *FH*-deficient cysts and tumors were associated with upregulation of the KEAP1-NRF2 antioxidant pathway (Figure 3A), resulting from derepression of nuclear factor erythroid 2-like 2 (NRF2, gene symbol *NFE2L2*) upon fumarate inhibition of the negative regulator kelch-like ECH-associated protein 1 (gene symbol *KEAP1*) by succination. Consistently, upregulation of antioxidant response genes through the KEAP1-NRF2 axis is a distinct feature of type 2 papillary renal cell carcinoma caused by *FH* mutation, in contrast to those of clear cell carcinomas arising from *SDH* or *VHL* mutation.[113] Furthermore, NRF2 apparently decreases HIF-1α levels by reducing reactive oxygen species in *FH*-deficient cells.[109] The NRF2-mediated antioxidant and detoxification program is involved in oncogene-induced tumorigenesis.[114] Intriguingly, NRF2 has also been shown to promote biosynthesis by redirecting glucose and glutamine into the anabolic pathways in the presence of activated PI3K-AKT signaling.[115] Despite the lack of involvement of Hif-1α in *Fh1*$^{-/-}$-associated renal cyst development, upregulation of HIF-1α remains essential to glycolytic metabolism and oncogenic growth of *FH*-deficient renal cancer,[108] consistent with the aforementioned role of HIF-1α in glycolysis and anabolism.

Although mutations in *SDHD* is linked to hereditary paraganglioma,[116] heterozygous deletion of *Sdhd* in mice develops no tumor but subtle glomus cell hypertrophy and hyperplasia.[117] Succinate accumulation has been shown to specifically inhibit PHD3 apoptotic activity, thereby blocking the apoptosis of sympathetic neuronal precursor cells during development and contributing to the pathogenesis of familial pheochromocytoma (Figure 3A).[118] Interestingly, mice with germline deletion of *Egln3* also exhibited reduced apoptosis and increased cell numbers in the superior cervical ganglia, adrenal medulla, and carotid body.[119] Furthermore, a combined heterozygous deletion of *Epas1* rendered sympathetic neurons more sensitive to induced apoptosis, indicating an anti-apoptotic role of HIF-2α in this setting. Conversely, as mentioned above, rare somatic, gain-of-function mutations in *EPAS1*,[58,59] as well as germline mutations in *FH*[120] have been associated with malignant paragangliomas and
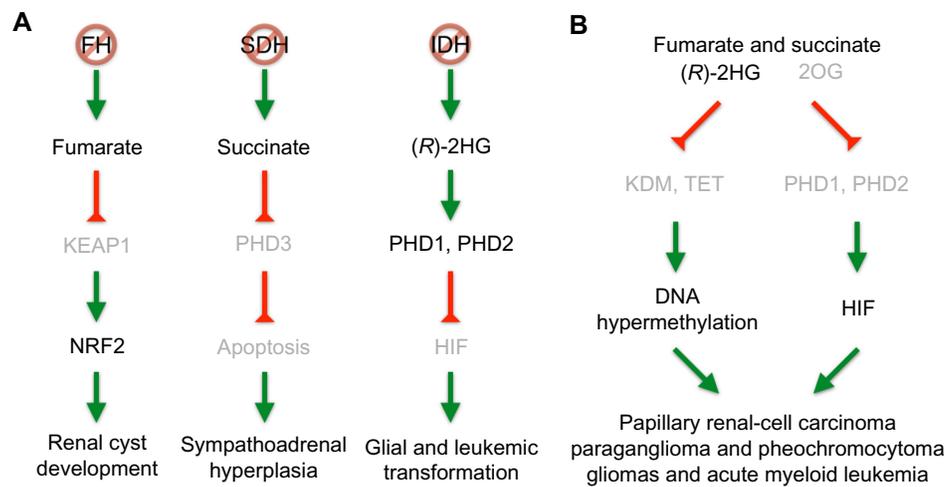
Figure 3 Pathways of FH, SDH, and IDH mutations leading to tumorigenesis.

Notes: (**A**) FH, SDH, IDH mutations result in the accumulation of fumarate, succinate, and (*R*)-2HG, respectively. During incipient tumor development, accumulation of fumarate inhibits KEAP1, thereby activating the NRF2 antioxidant response and resulting in renal cyst development. Similarly, succinate has been shown to inhibit PHD3-mediated apoptosis of neuronal cells during embryonic development, resulting in hyperplasia in the sympathoadrenal tissues. (*R*)-2HG stimulates PHD activity as a cosubstrate and reduces HIF signaling for glial and leukemic transformation. (**B**) During tumor progression, increased levels of fumarate, succinate, and (*R*)-2HG all share a common pathway by inhibiting the JmjC domain-containing histone demethylases (KDM) and the TET family of DNA hydroxylases. This leads to genome-wide DNA hypermethylation and tumorigenesis. Furthermore, increased levels of fumarate and succinate, as well as decreased levels of 2OG inhibit the HIF prolyl hydroxylases to activate the HIF signaling. Inhibited steps are shaded.

Abbreviations: SDH, succinate dehydrogenase; FH, fumarate hydratase; IDH, isocitrate dehydrogenase; PHD, prolyl hydroxylase domain-containing protein; KEAP1, kelch-like ECH-associated protein 1; (*R*)-2HG, (*R*)-enantiomer of 2-hydroxyglutarate; NRF2, nuclear factor erythroid 2-like 2; 2OG, 2-oxoglutarate; HIF, hypoxia-inducible factor.

pheochromocytomas. Furthermore, hypermethylation has also been identified in tumors with SDH and FH mutations. Methylome analysis of a large cohort of paraganglioma patients has revealed a hypermethylator phenotype similar to that in gliomas.[121] *Sdhb* deletion in mouse chromaffin cells led to DNA hypermethylation, resulting from succinate inhibition of the JmjC domain-containing histone demethylases and DNA hydroxylases (Figure 3B). Finally, DNA hypermethylation has also been identified in tumors with FH mutations.[109,121]

Somatic mutations in isocitrate dehydrogenase

There has been rapid expansion of knowledge in recent years of single somatic mutations in cytosolic isocitrate dehydrogenase 1 (gene symbol *IDH1*), and these mutations were identified initially in >70% WHO grade II–III gliomas and secondary glioblastomas.[122] Tumors lacking mutations in *IDH1* often had single mutations in *IDH2*, a mitochondrial gene in the TCA cycle for interconversion of isocitrate and 2OG. Similar mutations have also been found in <23% acute myeloid leukemia, albeit mainly in *IDH2*.[123–125] Interestingly, all the mutations are heterozygous and affect the same active site of the enzyme at a single arginine residue (Arg132 of IDH1 and Arg172 of IDH2), thereby reducing the intracellular concentration of 2OG,[122] a cosubstrate of the HIF prolyl hydroxylases. Accordingly, it was found that mutant

IDH1 forms a catalytically inactive heterodimer with the wild type, resulting in a decrease of PHD activity and an increase of HIF-1α levels in human gliomas harboring an *IDH1* mutation.[126]

This loss-of-function theory was only part of the story, however, because a wild type allele of *IDH1* or *IDH2* always remains in these tumors, raising the possibility of gain of function as result of mutations. Indeed, both mutant IDH1 and mutant IDH2 acquire the ability to catalyze the nicotinamide adenine dinucleotide phosphate-dependent reduction of 2OG to (*R*)-enantiomer of 2-hydroxyglutarate ((*R*)-2HG).[124,127,128] Accordingly, elevated levels of (*R*)-2HG are detectable in IDH mutant gliomas and acute myeloid leukemias.

So, is (*R*)-2HG an oncometabolite stimulating hypoxic signaling for tumorigenesis? On the contrary, it has been suggested that (*R*)-2HG downregulates HIF-1α and HIF-2α levels by increasing PHD1 and PHD2 activity to promote transformation of immortalized human astrocytes (Figure 3A).[129] This finding was supported by the evidence that (*R*)-2HG can substitute 2OG for PHD enzymatic activity in a cell-free reaction, and this finding is correlated with diminished expression of HIF-1α and HIF-2α in IDH1 mutant cells and reduced HIF target gene expression in IDH mutant gliomas. Furthermore, decreased HIF-1α expression is conducive to transformation by mutant IDH1. Moreover, (*R*)-2HG is sufficient to promote leukemogenesis by inducing cytokine independence and blocking hematopoietic differentiation, whereas the

(*S*)-enantiomer of 2-hydroxyglutarate, despite being a more potent inhibitor of 2OG-dependent dioxygenases,[129–131] fails to do so owing to its inhibition of PHD activity to increase, rather than decrease, HIF-1α levels.[132]

The notion that (*R*)-2HG inhibits HIF signaling seems at odds with the genetic evidence of mouse studies; increased Hif-1α protein levels and target gene transcription were observed in the embryo of brain *Idh1* (R132H) knock-in mice where high levels of (*R*)-2HG were produced,[133] and no alteration of Hif-1α signaling was found in the hematopoietic stem cells and progenitor cells from the knock-in mice of the myeloid lineage.[134] Although the mechanisms by which HIF-1α prevents astrocyte transformation and leukemogenesis remain unclear, a lower level of HIF-1α expression in IDH1 mutant cells might be conducive to tumor initiation because HIF-1α also upregulates histone demethylases,[135,136] which have been shown to be inhibited by (*R*)-2HG for transformation.

In addition to PHDs, (*R*)-2HG is a potent inhibitor of other 2OG-dependent dioxygenases,[137] including the TET family of 5-methylcytosine hydroxylases, which convert 5-methyl-cytosine to 5-hydroxymethylcytosine for DNA demethylation.[138–140] In fact, *IDH1* somatic mutations in glioblastomas and lower-grade gliomas have a CpG island methylator phenotype, displaying DNA hypermethylation at a large number of loci.[141,142] Similarly, acute myeloid leukemias with *IDH1* or *IDH2* mutations also exhibit genome-wide DNA hypermethylation with a specific signature shared with those harboring mutations in *TET2*,[125] a member of the TET protein family. Consistently, expression of IDH mutants was found to impair TET2 catalytic function, and mutations in *IDH1* and *IDH2* were mutually exclusive with those in *TET2* in a large cohort of acute myeloid leukemias.[125]

Although both forms of 2-HG have been shown to be competitive inhibitors of multiple 2OG-dependent dioxygenases, including the JmjC domain-containing histone demethylases and the TET family of 5-methylcytosine hydroxylases as well as PHDs,[130] the half-maximal inhibitory concentration of (*R*)-2HG for histone demethylases is 200-fold less than for PHD2, indicating more important effects of IDH mutations on chromatin remodeling.[131] In keeping with this, IDH mutation has been shown to inhibit histone demethylation and induce DNA hypermethylation in cell culture and animal model, thereby blocking cell differentiation.[134,143,144] Furthermore, targeted inhibition of mutant IDH2 with the small molecule AGI-6780 induces differentiation of established erythroleukemia cells and primary human acute myeloid leukemia cells in vitro.[145] The small-molecule inhibitor of mutant IDH1 (AGI-5198) also promotes differentiation of glioma cells harboring *IDH1* mutation and inhibits the growth of tumor xenografts.[146] Interestingly, AGI-5198 induces histone demethylation and expression of genes associated with glial differentiation without appreciable changes in genome-wide DNA methylation, suggesting additional mechanisms of IDH1 mutation for glioma growth.

Conclusion

It took nearly two decades to appreciate that HIF-1α stimulation of glycolysis is not merely to maintain bioenergetics for cell survival, but equally importantly is to promote biosynthesis of macromolecules for cell proliferation. HIF-1α does the latter by blunting the glycolytic pathway through the induction of PKM2. PKM2 engages in various mechanisms as a dimer to drive cell proliferation[147] and as a glycolytic enzyme in tetramer is further suppressed by oncogenic signaling. Although how PKM2 oscillates between dimer and tetramer is not well understood, receptor tyrosine kinase-mediated activation of the PI3K-AKT-mTOR pathway seems essential in orchestrating cellular biosynthesis of nucleotides, amino acids, and lipids that involves multiple pathways including HIF-1α and c-Myc signaling, and glutamine-dependent anaplerosis for cell proliferation.[148]

Yet, rapid cell proliferation induces hypoxia, which is known to suppress mTORC1 through multiple mechanisms.[21] In fact, HIF-1α is known to inhibit mTORC1 activity through transcriptional upregulation of *DDIT4* (encoding REDD1), a negative regulator of mTORC1 activity,[149,150] whereas HIF-2α, unrelated to aerobic glycolysis, stimulates mTORC1 activity by transcriptionally upregulating *SLC7A5* (encoding an amino acid transporter).[151] So, how do cancer cells escape from these mechanisms for proliferation? One possible answer to this conundrum is to inactivate HIF-1α, as mentioned above, albeit rarely, which implies the benefit of keeping HIF-1α expression in cancer cells. Alternatively, cancer cells may decrease expression of the stress-sensor protein ATM (ataxia telangiectasia mutated, gene symbol *ATM*), which is required for transcriptional activation of *REDD1* through HIF-1α phosphorylation,[152] or they may attempt to maintain mTORC1 activity by relying on exogenous desaturated lipids from serum for survival.[153] Lastly, they may adopt a "stop-and-go" mechanism by entering cell-cycle arrest while promoting an angiogenic response to alleviate hypoxic stress for continued proliferation.[154]

By altering the intracellular or intercellular context, cancer cells can adopt aberrant ways of maintaining survival and proliferation. Therefore, the selection of NRF2 signaling

for detoxification and anabolism, rather than anti-proliferative HIF-1α signaling, might be beneficial to renal cyst development at the early stage in FH-mutant cells. Similarly, an initial decrease in HIF-1α levels may facilitate DNA hypermethylation in cells harboring FH, SDH, or IDH mutations, because HIF-1α does the opposite by inducing histone demethylases and DNA demethylation, as also observed in metastasis of VHL-deficient renal cancer.[155] It is also noteworthy that none of the FH, SDH, and IDH mouse models has recapitulated a corresponding phenotype of the human diseases, suggesting the requirement of additional genetic/epigenetic alterations for tumorigenesis. Further studies are warranted to elucidate the mechanisms underlying these changes and the logistics of utilizing various signaling pathways to cancer's best advantage. Understanding of these dynamic processes is expected to provide more unexpected answers to the mystery of cancer.

Acknowledgments

Disclosure

The authors have no conflicts of interest in this work.

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