Regulation of Metabolism by Hypoxia-Inducible Factor 1

G.L. Semenza

Vascular Program, Institute for Cell Engineering, Departments of Pediatrics, Medicine, Oncology, Radiation Oncology, and Biological Chemistry, and McKusick-Nathans Institute of Genetic Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Correspondence: gsemenza@jhmi.edu

The maintenance of oxygen homeostasis is critical for survival, and the master regulator of this process in metazoan species is hypoxia-inducible factor 1 (HIF-1), which controls both O_2 delivery and utilization. Under conditions of reduced O_2 availability, HIF-1 activates the transcription of genes, whose protein products mediate a switch from oxidative to glycolytic metabolism. HIF-1 is activated in cancer cells as a result of intratumoral hypoxia and/or genetic alterations. In cancer cells, metabolism is reprogrammed to favor glycolysis even under aerobic conditions. Pyruvate kinase M2 (PKM2) has been implicated in cancer growth and metabolism, although the mechanism by which it exerts these effects is unclear. Recent studies indicate that PKM2 interacts with HIF-1α physically and functionally to stimulate the binding of HIF-1 at target genes, the recruitment of coactivators, histone acetylation, and gene transcription. Interaction with HIF-1α is facilitated by hydroxylation of PKM2 at proline-403 and -408 by PHD3. Knockdown of PHD3 decreases glucose transporter 1, lactate dehydrogenase A, and pyruvate dehydrogenase kinase 1 expression; decreases glucose uptake and lactate production; and increases O_2 consumption. The effect of PKM2/PHD3 is not limited to genes encoding metabolic enzymes because *VEGF* is similarly regulated. These results provide a mechanism by which PKM2 promotes metabolic reprogramming and suggest that it plays a broader role in cancer progression than has previously been appreciated.

Life on Earth is based on a biological cycle in which plants and blue-green algae capture solar energy and use it to drive the synthesis of glucose from carbon dioxide and water, thereby storing chemical energy in the form of carbon bonds. During this process of photosynthesis, O_2 is formed as a side product. Animals consume the plants and catabolize glucose via glycolysis and the mitochondrial tricarboxylic (TCA) cycle. This oxidative process generates reducing equivalents in the form of NADH and $FADH_2$, which are delivered to Complex I (NADH:ubiquinone oxidoreductase) and Complex II (succinate:ubiquinone oxidoreductase) of the electron transport chain, respectively, and then transferred to Complex III (ubiquinol:cytochrome *c* oxidoreductase) and, finally, to Complex IV (cytochrome *c* oxidase), where the electrons react with O_2 to form H_2O. The energy derived from electron transfer is used to generate a proton gradient, which in turn is used to drive the synthesis of ATP by Complex V (ATP synthase).

Electron transfer at each step of the respiratory chain is not completely efficient, resulting in the escape of electrons that reduce O_2 to superoxide anion, which is converted to hydrogen peroxide by the action of manganese superoxide dismutase. These reactive oxygen species (ROS) have the potential to damage lipids, proteins, and nucleic acids, leading to cellular dysfunction or death. Electron transport chain activity appears to be optimized for physiological PO_2 and deviations from normal PO_2 (in either direction) are associated with increased ROS production. The utilization of O_2 for energy metabolism is a two-edged sword: the complete oxidation of glucose to CO_2 and H_2O greatly increases the yield of ATP as compared with the incomplete oxidation of glucose to lactate; however, the transfer of electrons poses an inherent risk of ROS production. As a result, the cellular O_2 concentration must be very tightly regulated through homeostatic mechanisms that control O_2 delivery and O_2 utilization.

HYPOXIA-INDUCIBLE FACTOR 1: MASTER REGULATOR OF O_2 HOMEOSTASIS

All metazoan species express hypoxia-inducible factor 1 (HIF-1), a transcription factor that mediates adaptive responses to reduced O_2 availability (Semenza and Wang 1992). HIF-1 is a heterodimeric protein that is composed of an O_2–regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit (Wang and Semenza 1995). Isolation of cDNA sequences (Wang et al. 1995) revealed that both subunits are members of a subfamily of basic helix–loop–helix (HLH) factors containing a PAS domain (first identified in the Per, Arnt, and Sim proteins) that dimerize via HLH and PAS domains and bind to DNA via basic domains located in the amino terminal half of each subunit (Jiang et al. 1996a). The carboxyl terminal half of HIF-1α contains a transactivation domain (Jiang et al. 1997b; Pugh et al. 1997) that interacts with the coactivator p300 (Arany et al. 1996; Kallio et al. 1998). To activate target gene transcription, HIF-1 binds to hypoxia response elements (HREs) that contain the core HIF-1 binding site sequence 5′-RCGTG-3′ (Semenza et al. 1996).

 doi: 10.1101/sqb.2011.76.010678

The levels of HIF-1α protein increase exponentially as O_2 concentration declines over the entire physiological range of PO_2 (Jiang et al. 1996b). Changes in HIF-1α protein levels are the result of O_2-dependent ubiquitination and proteasomal degradation of HIF-1α (Salceda and Caro 1997). In the presence of O_2, HIF-1α is hydroxylated on two proline residues by a family of prolyl-4-hydroxylase domain (PHD) proteins (Bruick and McKnight 2001; Epstein et al. 2001). These dioxygenases utilize O_2 and the TCA cycle intermediate α-ketoglutarate as substrates, with one oxygen atom inserted into the prolyl residue and the other oxygen atom used to split α-ketoglutarate into succinate and CO_2. Prolyl hydroxylation increases the binding to HIF-1α of the von Hippel–Lindau protein (VHL), which is the substrate recognition subunit of an E3 ubiquitin–protein ligase that targets HIF-1α for degradation (Kaelin and Ratcliffe 2008).

HIF-1α transactivation domain function is also negatively regulated by O_2 (Jiang et al. 1997b; Pugh et al. 1997), through the activity of factor inhibiting HIF-1 (FIH-1) (Mahon et al. 2001), which uses O_2 and α-ketoglutarate to hydroxylate an asparaginyl residue in the transactivation domain, thereby blocking the binding of p300 (Lando et al. 2002a,b). Thus, both the half-life and transcriptional activity of HIF-1α are regulated by O_2-dependent hydroxylation reactions that provide a direct mechanism to transduce changes in O_2 availability to the nucleus as changes in transcription mediated by HIF-1, which is now known to regulate the expression of hundreds of genes (Manalo et al. 2005; Mole et al. 2009; Xia et al. 2009; Schödel et al. 2011).

HIF-1 was discovered based on its regulation of the human *EPO* gene, which encodes erythropoietin, the hormone that controls red cell production and, thus, blood O_2-carrying capacity (Semenza and Wang, 1992). HIF-1 was subsequently shown to regulate the expression of *VEGF*, which encodes vascular endothelial growth factor, the principal protein for stimulating the formation of new blood vessels (Forsythe et al. 1996). EPO and VEGF illustrate the role of HIF-1 in increasing O_2 delivery in response to systemic or local hypoxia, respectively. Mouse embryos lacking HIF-1α die at midgestation with defects in the heart and vasculature (Iyer et al. 1998; Ryan et al. 1998; Compernolle et al. 2003) and reduced blood cell production (Yoon et al. 2006), indicating that all three components of the circulatory system are dependent upon HIF-1 for normal development.

HIF-1 also increases the ability to survive O_2 deprivation by switching cells from oxidative to glycolytic metabolism by coordinately regulating the genes encoding glycolytic enzymes (Semenza et al. 1996; Iyer et al. 1998; Seagroves et al. 2001) and pyruvate dehydrogenase (PDH) kinase 1 (PDK1) (Kim et al. 2006; Papandreou et al. 2006). The key decision point for glucose metabolism is the fate of pyruvate, which is a substrate for either PDH, which converts it to acetyl coenzyme A for entry into the mitochondrial TCA cycle, or lactate dehydrogenase A (LDHA), which converts it to lactate for export from the cell. Under hypoxic conditions, HIF-1 activates expression of both LDHA and PDK1, thereby shunting pyruvate away from mitochondrial oxidation. HIF-1 also activates the expression of BNIP3 and BNIP3L, two proteins that trigger mitochondrial-selective autophagy, which is a strategy that eliminates oxidative metabolism of both glucose and fatty acids by eliminating the mitochondria in which these reactions occur (Zhang et al. 2008; Bellot et al. 2009). HIF-1 also activates transcription of a microRNA, miR-210, which inhibits the expression of a protein required for formation of iron-sulfur clusters that are components of the TCA cycle enzyme aconitase and electron transport chain Complex I (Chan et al. 2009; Chen et al. 2010; Favaro et al. 2010).

According to conventional wisdom, the driving force behind the switch from oxidative to glycolytic metabolism is the maintenance of ATP production. However, mouse embryo fibroblasts (MEFs) that are homozygous for a null (knockout) allele at the locus encoding HIF-1α die when exposed to 0.5%–1% O_2 for >48 h, despite the fact that these cells have higher ATP levels than their wild-type counterparts (Zhang et al. 2008). In the absence of HIF-1 activity, the MEFs do not die from ATP depletion but instead are killed by toxic levels of ROS because of the failure to switch from oxidative to glycolytic metabolism (Kim et al. 2006; Zhang et al. 2008).

HIF-1 is expressed in *Caenorhabditis elegans* (Epstein et al. 2001), a nematode that consists of more than 1000 cells and has no specialized systems for O_2 delivery. However, these worms must adapt their metabolism when they burrow into the soil for food. Thus, a major (and probably primordial) function of HIF-1 is to balance the risks and benefits of O_2 utilization over the entire physiological range of PO_2. This point is underscored by the finding that HIF-1 also regulates respiration by directing a subunit switch in cytochrome *c* oxidase that appears to increase the efficiency of electron transfer at Complex IV under hypoxic conditions (Fukuda et al. 2007). Remarkably, a homologous subunit switch in Complex IV occurs when *Saccharomyces cerevisiae* is subjected to hypoxia (Burke and Poyton 1998), although by a different mechanism because HIF-1 is only present in the metazoan lineage, which suggests that metabolic adaptation to hypoxia is critical for survival of all eukaryotic cells.

ACTIVATION OF HIF-1 IN CANCER

HIF-1α protein levels are increased in the majority of human cancers and their metastases (Zhong et al. 1999), and high levels of HIF-1α in the primary tumor biopsy are associated with increased risk of mortality in many cancer types (Semenza 2010). There are three general mechanisms by which HIF-1α levels are increased in human cancer. First, intratumoral hypoxia is common and often severe. In breast cancer, the mean PO_2 is 10 mmHg, compared with 65 mmHg in normal breast tissue, and patients with intratumoral PO_2<10 mmHg are at increased risk of mortality, independent of tumor size, stage, histology, grade, or lymph node status (Vaupel

et al. 2004). Second, other alterations of the tumor microenvironment also increase HIF-1α levels, such as the presence of nitric oxide (Quintero et al. 2006) or ROS (Gao et al. 2007).

Third, cancer-specific genetic alterations lead to dysregulation of HIF-1α levels in an O_2-independent manner. The most dramatic effect is observed with loss of function of the VHL tumor suppressor protein in the clear cell type of renal cell carcinoma and in cerebellar hemangioblastoma (Maxwell et al. 1999; Kaelin 2007). Increased HIF-1α levels are also associated with loss of function of p53 (Ravi et al. 2000) and PTEN (Zhong et al. 2000; Zundel et al. 2000), which are the two most commonly mutated tumor suppressors. Other tumor suppressors whose inactivation is associated with increased HIF-1 activity include $p14^{ARF}$ (Fatyol and Szalay 2001), TSC2 (Brugarolas et al. 2003), PML (Bernardi et al. 2006), and LKB1 (Shackelford et al. 2009). The *SDHB*, *SDHC*, *SDHD*, and *FH* genes encode the TCA cycle enzymes succinate dehydrogenase and fumarate hydratase, which also function as tumor suppressors that, when inactivated, result in increased HIF-1α levels (Isaacs et al. 2005; Selak et al. 2005). Activated oncogenes such as $HER2^{neu}$ in breast cancer (Laughner et al. 2001), EGFR in prostate (Zhong et al. 2000) and lung (Phillips et al. 2005) cancer, and BCR-ABL in chronic myelogenous leukemia (Mayerhofer et al. 2002) increase HIF-1α levels through the phosphatidylinositol-3-kinase/AKT/mTOR signaling pathway. HIF-1 activity is also increased by transforming proteins of many oncogenic viruses, including Rous sarcoma virus (Jiang et al. 1997a), Kaposi sarcoma herpesvirus (Sodhi et al. 2000), hepatitis B virus (Yoo et al. 2004), Epstein–Barr virus (Wakisaka et al. 2004), and human papillomavirus (Nakamura et al. 2009).

Taken together, the data suggest that HIF-1 activation represents a final common pathway in most advanced human cancers. The selection for cells with HIF-1 activation reflects the many HIF-1 target genes that play important roles in critical aspects of cancer biology, including angiogenesis (Liao and Johnson 2007); epithelial–mesenchymal transition, invasion, and metastasis (Chan and Giaccia 2007; Sullivan and Graham 2007); genetic instability (Bindra et al. 2007); immortalization and stem cell maintenance (Barnhart and Simon 2007); immune evasion (Lukashev et al. 2007); metabolism (Kim et al. 2007a); pH regulation (Swietach et al. 2007); and resistance to radiation therapy (Moeller et al. 2007).

REPROGRAMMING OF METABOLISM IN CANCER CELLS BY HIF-1

Compared with normal cells, cancer cells have long been known to exhibit dramatically increased glucose uptake and lactate production, even under conditions in which O_2 is not limiting for respiration (Warburg et al. 1927). Infection of normal cells with Rous sarcoma virus was shown to increase aerobic glycolysis and this effect was temporally and genetically associated with V-SRC-mediated transformation (Steck et al. 1968; Singh et al. 1974; Carroll et al. 1978), which provided the first link between an oncogene and metabolism. Oncogenic forms of SRC increase HIF-1α protein levels and HIF-1-dependent activation of glycolytic gene expression, providing the first molecular mechanism by which an oncogene promotes metabolic reprogramming (Jiang et al. 1997a; Karni et al. 2002). Oncogenic forms of MYC cooperate with HIF-1 to activate glycolytic gene transcription (Kim et al. 2007b). In renal carcinoma cells, loss of VHL tumor suppressor function leads to a switch from oxidative to glycolytic metabolism that can be dramatically reversed by expression of wild-type VHL or inhibition of HIF-1 activity (Zhang et al. 2007).

The mechanisms and consequences of alterations of metabolism in cancer cells are the focus of considerable research effort (McKnight 2010; Dang et al. 2011; Frezza et al. 2011; Maddocks and Vousden 2011; Semenza, 2011; Shanware et al. 2011; Yecies and Manning 2011). One area of particular recent interest is the role of the pyruvate kinase M2 (PKM2) isoform in cancer metabolism. PKM2 is normally expressed in the embryo, whereas PKM1 is expressed in adult cells. These two isoforms arise from alternative splicing of the primary RNA transcript: PKM1 mRNA contains sequences encoded by exon 9, whereas the PKM2 mRNA contains sequences encoded by exon 10 (Noguchi et al. 1986; Mazurek et al. 2005). Many cancer cells re-express PKM2 (Mazurek et al. 2005) as a result of MYC-dependent expression of heterogeneous nuclear ribonucleoprotein 1, A1, and A2, which bind to RNA sequences encoded by exon 9 and promote the splicing out of the PKM1-specific sequences (Clower et al. 2010; David et al. 2010). PKM2, but not PKM1, is subject to allosteric regulation that is modulated by tyrosine kinase signaling (Christofk et al. 2008b; Hitosugi et al. 2009).

Knockdown of PKM2 in cancer cells and replacement with PKM1 expression resulted in decreased lactate production and increased O_2 consumption as well as decreased tumor xenograft growth, indicating that PKM2 plays important roles in tumor growth and metabolic reprogramming (Christofk et al. 2008a). However, the mechanism by which PKM2 altered the balance between oxidative and glycolytic metabolism was unclear because PKM2 catalyzes the conversion of pho*enol*pyruvate to pyruvate—upstream of the decision point, which is the conversion of pyruvate to either acetyl CoA or lactate that is regulated by HIF-1 as described above.

We performed a proteomics-based screen to identify proteins that interact with the carboxyl terminus (amino acid residues 531–826) of HIF-1α and identified PKM2, which was subsequently shown to co-immunoprecipitate with HIF-1α in nuclear extracts of hypoxic HeLa cells (Luo et al. 2011). HIF-1α bound to the PKM2-specific domain encoded by exon 10 but not to the PKM1-specific domain encoded by exon 9. We hypothesized that PKM2 contributes to metabolic reprogramming of cancer cells by stimulating HIF-1 activity. To test this hypothesis, we co-transfected HeLa

cells with an HIF-1-dependent luciferase reporter plasmid and an expression vector encoding PKM1 or PKM2. PKM2 vector increased hypoxia-induced luciferase activity, whereas PKM1 did not. PKM2 did not affect the levels of HIF-1α or HIF-1β protein. However, PKM2 stimulated luciferase activity in cells co-transfected with an expression vector encoding the DNA binding domain from GAL4 fused to the HIF-1α transactivation domain and a luciferase reporter plasmid containing five GAL4 binding sites (Luo et al. 2011).

The amino acid sequence encoded by exon 10 was remarkable for the presence of the hexapeptide ^{398}LRRLAP^{403}, which fits the consensus for HIF-1α prolyl hydroxylation sites in all vertebrate species (LXXLAP). Mass spectrometry revealed that both P^{403} and P^{408} were hydroxylated. Mutation of both prolines (P403A/P408A) resulted in decreased physical and functional interaction of PKM2 with HIF-1α (Luo et al. 2011). Similar effects were observed when cells were treated with the α-ketoglutarate analog dimethyloxalylglycine (DMOG), which inhibits hydroxylase activity. The prolyl hydroxylase PHD3 was found to bind to PKM2 and stimulate its hydroxylation and interaction with HIF-1α. Surprisingly, PKM2 was hydroxylated at similar levels in HeLa cells incubated at 20% or 1% O_2, despite the known loss of hydroxylase activity at lower O_2 concentrations (Epstein et al. 2001; Kaelin and Ratcliffe 2008). Analysis of PHD3 protein levels revealed a marked increase under hypoxic conditions, consistent with previous studies, which reported that PHD3 is encoded by an HIF-1 target gene (Aprelikova et al. 2004; Marxsen et al. 2004; Pescador et al. 2005). Thus, the increased expression of PHD3 compensates for the loss of enzymatic activity under conditions of chronic hypoxia to maintain PKM2 hydroxylation, as has previously been reported for HIF-1α hydroxylation (Stiehl et al. 2006).

Chromatin immunoprecipitation (ChIP) assays revealed that knockdown of PKM2 reduced the hypoxia-induced occupancy by HIF-1α, HIF-1β, and p300 of HREs in the *LDHA* and *PDK1* genes in HeLa cells (Luo et al. 2011). Recruitment of both PKM2 and PHD3 to these HREs in hypoxic cells was also demonstrated. Knockdown of PKM2 reduced the levels of histone H3 acetylated at lysine 9 (H3K9ac) at HREs as determined by ChIP, whereas there was no effect on total H3 binding to the HRE or on total cellular levels of H3 or H3K9ac. These data indicate that PKM2 specifically enhances the formation of an active HIF-1 complex at HREs and promotes H3K9 acetylation, which is an epigenetic modification that is associated with transcriptional activation. PKM2 binding to HIF-1α may provide a mechanism to overcome the repressive effect of asparaginyl hydroxylation by FIH-1, thereby increasing transactivation function under aerobic conditions in cancer cells in which HIF-1α protein expression has been dysregulated by genetic alterations as described above.

Knockdown of PKM2 in HeLa cells significantly reduced the levels of GLUT1, LDHA, and PDK1 mRNA and protein under hypoxic conditions (Luo et al. 2011). Knockdown of PHD3 in RCC4 renal carcinoma cells, in which HIF-1α is constitutively expressed because of VHL loss of function, significantly reduced GLUT1, LDHA, and PDK1 mRNA and protein levels, leading to significantly decreased glucose uptake and lactate production and increased O_2 consumption under non-hypoxic conditions.

Analysis of PKM1 and PKM2 expression in immortalized mouse embryo fibroblasts revealed that both mRNAs were induced by hypoxia in an HIF-1-dependent manner (Luo et al. 2011). ChIP assays identified HIF-1 binding sites within intron 1 of the human *PKM2* gene and a 55-bp sequence encompassing these sites functioned as an HRE in reporter gene assays. Thus, the genes encoding PKM2 and PHD3 are both direct HIF-1 targets, thereby establishing a feed-forward mechanism for amplifying HIF-1-dependent transcriptional activation in cancer cells. PKM2 coactivator function, by increasing the expression of *LDHA*, *PDK1*, and other HIF-1 target genes that mediate the metabolic switch from oxidative to glycolytic metabolism, provides a mechanism underlying the contribution of PKM2 to metabolic reprogramming of cancer cells. However, the effect of PKM2 coactivator function was not restricted to genes encoding metabolic enzymes, as recruitment of HIF-1α and HIF-1β to the *VEGF* gene was also impaired by PKM2 knockdown. These data imply that PKM2 may play a far broader role in cancer progression than has been appreciated heretofore. Finally, these data suggest that therapeutic strategies based on the inhibition of PKM2 catalytic activity in cancer cells may have limited efficacy, because mutation of a key catalytic residue in PKM2 that eliminated its enzymatic activity as a glycolytic enzyme had no effect on its HIF-1 coactivator function (Luo et al. 2011).

ACKNOWLEDGMENTS

Work in the author's laboratory is supported by grants from the National Institutes of Health (HHSN268201000032C and U54-CA143868), Japanese Science and Technology Agency (ERATO-ICORP, Gas Biology Project), Emerald Foundation, and Johns Hopkins Institute for Cell Engineering. G.L.S. is the C. Michael Armstrong Professor at the Johns Hopkins University School of Medicine.

REFERENCES

Aprelikova O, Chandramouli GV, Wood M, Vasselli JR, Riss J, Maranchie JK, Linehan WM, Barrett JC. 2004. Regulation of HIF prolyl hydroxylases by hypoxia-inducible factors. *J Cell Biochem* **92:** 491–501.

Arany Z, Huang LE, Eckner R, Bhattacharya S, Jiang C, Goldberg MA, Bunn HF, Livingston DM. 1996. An essential role for p300/CBP in the cellular response to hypoxia. *Proc Natl Acad Sci* **93:** 12969–12973.

Barnhart BC, Simon MC. 2007. Metastasis and stem cell pathways. *Cancer Metastasis Rev* **26:** 251–271.

Bellot G, Garcia-Medina R, Gounon P, Chiche J, Roux D, Pouysségur J, Mazure NM. 2009. Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction

of BNIP3 and BNIP3L via their BH3 domains. *Mol Cell Biol* **29:** 2570–2581.

Bernardi R, Guernah I, Jin D, Grisendi S, Alimonti A, Teruya-Feldstein J, Cordon-Cardo C, Simon MC, Rafii S, Pandolfi PP. 2006. PML inhibits HIF-1α translation and neoangiogenesis through repression of mTOR. *Nature* **442:** 779–785.

Bindra RS, Crosby ME, Glazer PM. 2007. Regulation of DNA repair in hypoxic cancer cells. *Cancer Metastasis Rev* **26:** 249–260.

Brugarolas JB, Vazquez F, Reddy A, Sellers WR, Kaelin WG Jr. 2003. TSC2 regulates VEGF through mTOR-dependent and -independent pathways. *Cancer Cell* **4:** 147–158.

Bruick RK, McKnight SL. 2001. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* **294:** 1337–1340.

Burke PV, Poyton RO. 1998. Structure/function of oxygen-regulated isoforms in cytochrome c oxidase. *J Exp Biol* **201:** 1163–1175.

Carroll RC, Ash JF, Vogt PK, Singer SJ. 1978. Reversion of transformed glycolysis to normal by inhibition of protein synthesis in rat kidney cells infected with temperature-sensitive mutant of Rous sarcoma virus. *Proc Natl Acad Sci* **75:** 5015–5019.

Chan DA, Giaccia AJ. 2007. Hypoxia, gene expression, and metastasis. *Cancer Metastasis Rev* **26:** 333–339.

Chan SY, Zhang YY, Hemann C, Mahoney CE, Zweier JL, Loscalzo J. 2009. MicroRNA-210 controls mitochondrial metabolism during hypoxia by repressing the iron-sulfur cluster assembly proteins ISCU1/2. *Cell Metab* **10:** 273–284.

Chen Z, Li Y, Zhang H, Huang P, Luthra R. 2010. Hypoxia-regulated microRNA-210 modulates mitochondrial function and decreases ISCU and COX10 expression. *Oncogene* **29:** 4362–4368.

Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, Fleming MD, Schreiber SL, Cantley LC. 2008a. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumor growth. *Nature* **452:** 230–233.

Christofk HR, Vander Heiden MG, Wu N, Asara JM, Cantley LC. 2008b. Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature* **452:** 181–186.

Clower CV, Chatterjee D, Wang Z, Cantley LC, Vander Heiden MG, Krainer AR. 2010. The alternative splicing repressors hnRNP A1/A2 and PTB influence pyruvate kinase isoform expression and cell metabolism. *Proc Natl Acad Sci* **107:** 1894–1899.

Compernolle V, Brusselmans K, Franco D, Moorman A, Dewerchin M, Collen D, Carmeliet P. 2003. Cardia bifida, defective heart development and abnormal neural crest migration in embryos lacking hypoxia-inducible factor-1α. *Cardiovasc Res* **60:** 569–579.

Dang CV, Hamaker M, Sun P, Le A, Gao P. 2011. Therapeutic targeting of cancer cell metabolism. *J Mol Med* **89:** 205–212.

David CJ, Chen M, Assanah M, Canoll P, Manley JL. 2010. HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. *Nature* **463:** 364–368.

Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, et al. 2001. *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* **107:** 43–54.

Favaro E, Ramachandran A, McCormick R, Gee H, Blancher C, Crosby M, Devlin C, Blick C, Buffa F, Li JL, et al. 2010. MicroRNA-210 regulates mitochondrial free radical response to hypoxia and Krebs cycle in cancer cells by targeting iron sulfur cluster protein ISCU. *PLoS One* **5:** e10345.

Fatyol K, Szalay AA. 2001. The p14ARF tumor suppressor protein facilitates nucleolar sequestration of hypoxia-inducible factor-1α (HIF-1α) and inhibits HIF-1-mediated transcription. *J Biol Chem* **276:** 28421–28429.

Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, Semenza GL. 1996. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol* **16:** 4604–4613.

Frezza C, Pollard PJ, Gottlieb E. 2011. Inborn and acquired metabolic defects in cancer. *J Mol Med* **89:** 213–220.

Fukuda R, Zhang H, Kim JW, Shimoda L, Dang CV, Semenza GL. 2007. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell* **129:** 111–122.

Gao P, Zhang H, Dinavahi R, Li F, Xiang Y, Raman V, Bhujwalla ZM, Felsher DW, Cheng L, Pevsner J, Lee LA, et al. 2007. HIF-dependent antitumorigenic effect of antioxidants in vivo. *Cancer Cell* **12:** 230–238.

Hitosugi T, Kang S, Vander Heiden MG, Chung TW, Elf S, Lythgoe K, Dong S, Lonial S, Wang X, Chen GZ, et al. 2009. Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. *Sci Signal* **2:** ra73.

Isaacs JS, Jung YJ, Mole DR, Lee S, Torres-Cabala C, Chung YL, Merino M, Trepel J, Zbar B, Toro J, et al. 2005. HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: Novel role of fumarate in regulation of HIF stability. *Cancer Cell* **8:** 143–153.

Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY, Semenza GL. 1998. Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1α. *Genes Dev* **12:** 149–162.

Jiang BH, Rue E, Wang GL, Roe R, Semenza GL. 1996a. Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. *J Biol Chem* **271:** 17771–17778.

Jiang BH, Semenza GL, Bauer C, Marti HH. 1996b. Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O_2 tension. *Am J Physiol* **271:** C1172–C1180.

Jiang BH, Agani F, Passaniti A, Semenza GL. 1997a. V-SRC induces expression of hypoxia-inducible factor 1 (HIF-1) and transcription of genes encoding vascular endothelial growth factor and enolase 1: Involvement of HIF-1 in tumor progression. *Cancer Res* **57:** 5328–5335.

Jiang BH, Zheng JZ, Leung SW, Roe R, Semenza GL. 1997b. Transactivation and inhibitory domains of hypoxia-inducible factor 1α. Modulation of transcriptional activity by oxygen tension. *J Biol Chem* **272:** 19253–19260.

Kaelin WG. 2007. Von Hippel–Lindau disease. *Annu Rev Pathol* **2:** 145–173.

Kaelin WG Jr, Ratcliffe PJ. 2008. Oxygen sensing by metazoans: The central role of the HIF hydroxylase pathway. *Mol Cell* **30:** 393–402.

Kallio PJ, Okamoto K, O'Brien S, Carrero P, Makino Y, Tanaka H, Poellinger L. 1998. Signal transduction in hypoxic cells: Inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1α. *EMBO J* **17:** 6573–6586.

Karni R, Dor Y, Keshet E, Meyuhas O, Levitzki A. 2002. Activated pp60c-Src leads to elevated hypoxia-inducible factor (HIF)-1α expression under normoxia. *J Biol Chem* **277:** 42919–42925.

Kim JW, Tchernyshyov I, Semenza GL, Dang CV. 2006. HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* **3:** 177–185.

Kim JW, Gao P, Dang CV. 2007a. Effects of hypoxia on tumor metabolism. *Cancer Metastasis Rev* **26:** 291–298.

Kim JW, Gao P, Liu YC, Semenza GL, Dang CV. 2007b. Hypoxia-inducible factor 1 and dysregulated c-Myc cooperatively induce vascular endothelial growth factor and metabolic switches hexokinase 2 and pyruvate dehydrogenase kinase 1. *Mol Cell Biol* **27:** 7381–7393.

Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK. 2002a. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev* **16:** 1466–1471.

Lando D, Peet DJ, Whelan DA, Gorman JJ, Whitelaw ML. 2002b. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science* **295:** 858–861.

Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. 2001. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1α (HIF-1α) synthesis: Novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Mol Cell Biol* **21:** 3995–4004.

Liao D, Johnson RS. 2007. Hypoxia: A key regulator of angiogenesis in cancer. *Cancer Metastasis Rev* **26:** 281–290.

Lukashev D, Ohta A, Sitkovsky M. 2007. Hypoxia-dependent anti-inflammatory pathways in protection of cancerous tissues. *Cancer Metastasis Rev* **26:** 273–279.

Luo W, Hu H, Chang R, Zhong J, Knabel M, O'Meally R, Cole RN, Pandey A, Semenza GL. 2011. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. *Cell* **145:** 732–744.

Maddocks ODK, Vousden KH. 2011. Metabolic regulation by p53. *J Mol Med* **89:** 237–245.

Mahon PC, Hirota K, Semenza GL. 2001. FIH-1: A novel protein that interacts with HIF-1α and VHL to mediate repression of HIF-1 transcriptional activity. *Genes Dev* **15:** 2675–2686.

Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, Garcia JG, Semenza GL. 2005. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* **105:** 659–669.

Marxsen JH, Stengel P, Doege K, Heikkinen P, Jokilehto T, Wagner T, Jelkmann W, Jaakkola P, Metzen E. 2004. Hypoxia-inducible factor-1 (HIF-1) promotes its degradation by induction of HIF-alpha-prolyl-4-hydroxylases. *Biochem J* **381:** 761–767.

Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ. 1999. The tumor suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* **399:** 271–275.

Mayerhofer M, Valent P, Sperr WR, Griffin JD, Sillaber C. 2002. BCR/ABL induces expression of vascular endothelial growth factor and its transcriptional activator, hypoxia inducible factor-1alpha, through a pathway involving phosphoinositide 3-kinase and the mammalian target of rapamycin. *Blood* **100:** 3767–3775.

Mazurek S, Boschek CB, Hugo F, Eigenbrodt E. 2005. Pyruvate kinase type M2 and its role in tumor growth and spreading. *Semin Cancer Biol* **15:** 300–308.

McKnight SL. 2010. On getting there from here. *Science* **330:** 1338–1339.

Moeller BJ, Richardson RA, Dewhirst MW. 2007. Hypoxia and radiotherapy: Opportunities for improved outcomes in cancer treatment. *Cancer Metastasis Rev* **26:** 241–248.

Mole DR, Blancher C, Copley RR, Pollard PJ, Gleadle JM, Ragoussis J, Ratcliffe PJ. 2009. Genome-wide association of hypoxia-inducible factor (HIF)-1α and HIF-2α DNA binding with expression profiling of hypoxia-inducible transcripts. *J Biol Chem* **284:** 16767–16775.

Nakamura M, Bodily JM, Beglin M, Kyo S, Inoue M, Laimins LA. 2009. Hypoxia-specific stabilization of HIF-1α by human papillomaviruses. *Virology* **387:** 442–448.

Noguchi T, Inoue H, Tanaka T. 1986. The M1- and M2-type isozymes of rat pyruvate kinase are produced from the same gene by alternative RNA splicing. *J Biol Chem* **261:** 13807–13812.

Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. 2006. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab* **3:** 187–197.

Pescador N, Cuevas Y, Naranjo S, Alcaide M, Villar D, Landázuri MO, Del Peso L. 2005. Identification of a functional hypoxia-responsive element that regulates the expression of the *egl nine* homologue 3 (*egln3*/*phd3*) gene. *Biochem J* **390:** 189–197.

Phillips RJ, Mestas J, Gharaee-Kermani M, Burdick MD, Sica A, Belperio JA, Keane MP, Strieter RM. 2005. Epidermal growth factor and hypoxia-induced expression of CXC chemokine receptor 4 on non-small cell lung cancer cells is regulated by the phosphatidylinositol 3-kinase/PTEN/AKT/mammalian target of rapamycin signaling pathway and activation of hypoxia inducible factor-1α. *J Biol Chem* **280:** 22473–22481.

Pugh CW, O'Rourke JF, Nagao M, Gleadle JM, Ratcliffe PJ. 1997. Activation of hypoxia-inducible factor-1; definition of regulatory domains within the α subunit. *J Biol Chem* **272:** 11205–11214.

Quintero M, Brennan PA, Thomas GJ, Moncada S. 2006. Nitric oxide is a factor in the stabilization of hypoxia-inducible factor-1α in cancer: Role of free radical formation. *Cancer Res* **66:** 770–774.

Ravi R, Mookerjee B, Bhujwalla ZM, Sutter CH, Artemov D, Zeng Q, Dillehay LE, Madan A, Semenza GL, Bedi A. 2000. Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1α. *Genes Dev* **14:** 34–44.

Ryan HE, Lo J, Johnson RS. 1998. HIF-1α is required for solid tumor formation and embryonic vascularization. *EMBO J* **17:** 3005–3015.

Salceda S, Caro J. 1997. Hypoxia-inducible factor 1α (HIF-1α) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. *J Biol Chem* **272:** 22642–22647.

Schödel J, Oikonomopoulos S, Ragoussis J, Pugh CW, Ratcliffe PJ, Mole DR. 2011. High-resolution genome-wide mapping of HIF-binding sites by ChIP-seq. *Blood* **117:** e207–e217.

Seagroves TN, Ryan HE, Lu H, Wouters BG, Knapp M, Thibault P, Laderoute K, Johnson RS. 2001. Transcription factor HIF-1 is a necessary mediator of the Pasteur effect in mammalian cells. *Mol Cell Biol* **21:** 3436–3444.

Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E. 2005. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-α prolyl hydroxylase. *Cancer Cell* **7:** 77–85.

Semenza GL. 2010. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene* **20:** 625–634.

Semenza GL. 2011. A return to cancer metabolism. *J Mol Med* **89:** 203–204.

Semenza GL, Wang GL. 1992. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol* **12:** 5447–5454.

Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Maire P, Giallongo A. 1996. Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J Biol Chem* **271:** 32529–32537.

Shackelford DB, Vasquez DS, Corbeil J, Wu S, Leblanc M, Wu CL, Vera DR, Shaw RJ. 2009. mTOR and HIF-1α-mediated tumor metabolism in an LKB1 mouse model of Peutz–Jeghers syndrome. *Proc Natl Acad Sci* **106:** 11137–11142.

Shanware NP, Mullen AR, DeBerardinis RJ, Abraham RT. 2011. Glutamine: Pleiotropic roles in tumor growth and stress resistance. *J Mol Med* **89:** 229–236.

Singh VN, Singh M, August JT, Horecker BL. 1974. Alterations in glucose metabolism in chick-embryo cells transformed by Rous sarcoma virus: Intracellular levels of glycolytic intermediates. *Proc Natl Acad Sci* **71:** 4129–4132.

Sodhi A, Montaner S, Patel V, Zohar M, Bais C, Mesri EA, Gutkind JS. 2000. The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor up-regulates vascular endothelial growth factor expression and secretion through mitogen-activated protein kinase and p38 pathways acting on hypoxia-inducible factor 1α. *Cancer Res* **60:** 4873–4880.

Steck TL, Kaufman S, Bader JP. 1968. Glycolysis in chick embryo cell cultures transformed by Rous sarcoma virus. *Cancer Res* **28:** 1611–1619.

Stiehl DP, Wirthner R, Köditz J, Spielmann P, Camenisch G, Wenger RH. 2006. Increased prolyl 4-hydroxylase domain proteins compensate for decreased oxygen levels. Evidence for an autoregulatory oxygen-sensing system. *J Biol Chem* **281:** 23482–23491.

Sullivan R, Graham CH. 2007. Hypoxia-driven selection of the metastatic phenotype. *Cancer Metastasis Rev* **26:** 319–331.

Swietach P, Vaughan-Jones RD, Harris AL. 2007. Regulation of tumor pH and the role of carbonic anhydrase 9. *Cancer Metastasis Rev* **26:** 299–310.

Vaupel P, Mayer A, Höckel M. 2004. Tumor hypoxia and malignant progression. *Meth Enzymol* **381:** 335–354.

Wakisaka N, Kondo S, Yoshizaki T, Murono S, Furukawa M, Pagano JS. 2004. Epstein–Barr virus latent membrane protein 1 induces synthesis of hypoxia-inducible factor 1α. *Mol Cell Biol* **24:** 5223–5234.

Wang GL, Semenza GL. 1995. Purification and characterization of hypoxia-inducible factor 1. *J Biol Chem* **270:** 1230–1237.

Wang GL, Jiang BH, Rue EA, Semenza GL. 1995. Hypoxia-inducible factor 1 is a basic-helix–loop–helix–PAS heterodimer regulated by cellular O_2 tension. *Proc Natl Acad Sci* **92:** 5510–5514.

Warburg O, Wind F, Negelein E. 1927. The metabolism of tumors in the body. *J Gen Physiol* **8:** 519–530.

Xia X, Lemieux ME, Li W, Carroll JS, Brown M, Liu XS, Kung AL. 2009. Integrative analysis of HIF binding and transactivation reveals its role in maintaining histone methylation homeostasis. *Proc Natl Acad Sci* **106:** 4260–4265.

Yecies JL, Manning BD. 2011. mTOR links oncogenic signaling to tumor cell metabolism. *J Mol Med* **89:** 221–228.

Yoo YG, Cho S, Park S, Lee MO. 2004. The carboxy-terminus of the hepatitis B virus X protein is necessary and sufficient for the activation of hypoxia-inducible factor-1α. *FEBS Lett* **577:** 121–126.

Yoon D, Pastore YD, Divoky V, Liu E, Mlodnicka AE, Rainey K, Ponka P, Semenza GL, Schumacher A, Prchal JT. 2006. Hypoxia-inducible factor-1 deficiency results in dysregulated erythropoiesis signaling and iron homeostasis in mouse development. *J Biol Chem* **281:** 25703–25711.

Zhang H, Gao P, Fukuda R, Kumar G, Krishnamachary B, Zeller KI, Dang CV, Semenza GL. 2007. HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. *Cancer Cell* **11:** 407–420.

Zhang H, Bosch-Marcé M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL. 2008. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *J Biol Chem* **283:** 10892–10903.

Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, Simons JW. 1999. Overexpression of hypoxia-inducible factor 1α in common human cancers and their metastases. *Cancer Res* **59:** 5830–5835.

Zhong H, Chiles K, Feldser D, Laughner E, Hanrahan C, Georgescu MM, Simons JW, Semenza GL. 2000. Modulation of hypoxia-inducible factor 1α expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: Implications for tumor angiogenesis and therapeutics. *Cancer Res* **60:** 1541–1545.

Zundel W, Schindler C, Haas-Kogan D, Koong A, Kaper F, Chen E, Gottschalk AR, Ryan HE, Johnson RS, Jefferson AB, Stokoe D, Giaccia AJ. 2000. Loss of PTEN facilitates HIF-1-mediated gene expression. *Genes Dev* **14:** 391–396.

Cold Spring Harbor Symposia on Quantitative Biology

Regulation of Metabolism by Hypoxia-Inducible Factor 1

G.L. Semenza

Cold Spring Harb Symp Quant Biol 2011 76: 347-353 originally published online July 22, 2011
Access the most recent version at doi:10.1101/sqb.2011.76.010678