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Current Opinion in Genetics & Development

HIF-1: upstream and downstream of cancer metabolism

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Hypoxia-inducible factor 1 (HIF-1) plays a key role in the reprogramming of cancer metabolism by activating transcription of genes encoding glucose transporters and glycolytic enzymes, which take up glucose and convert it to lactate; pyruvate dehydrogenase kinase 1, which shunts pyruvate away from the mitochondria; and BNIP3, which triggers selective mitochondrial autophagy. The shift from oxidative to glycolytic metabolism allows maintenance of redox homeostasis and cell survival under conditions of prolonged hypoxia. Many metabolic abnormalities in cancer cells increase HIF-1 activity. As a result, a feed-forward mechanism can be activated that drives HIF-1 activation and may promote tumor progression.

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Current Opinion in Genetics & Development 2010, **20**:51–56

This review comes from a themed issue on
Genetic and cellular mechanisms of oncogenesis
Edited by Tony Hunter and Richard Marais

Available online 26th November 2009

0959-437X/$ – see front matter

DOI 10.1016/j.gde.2009.10.009

Introduction

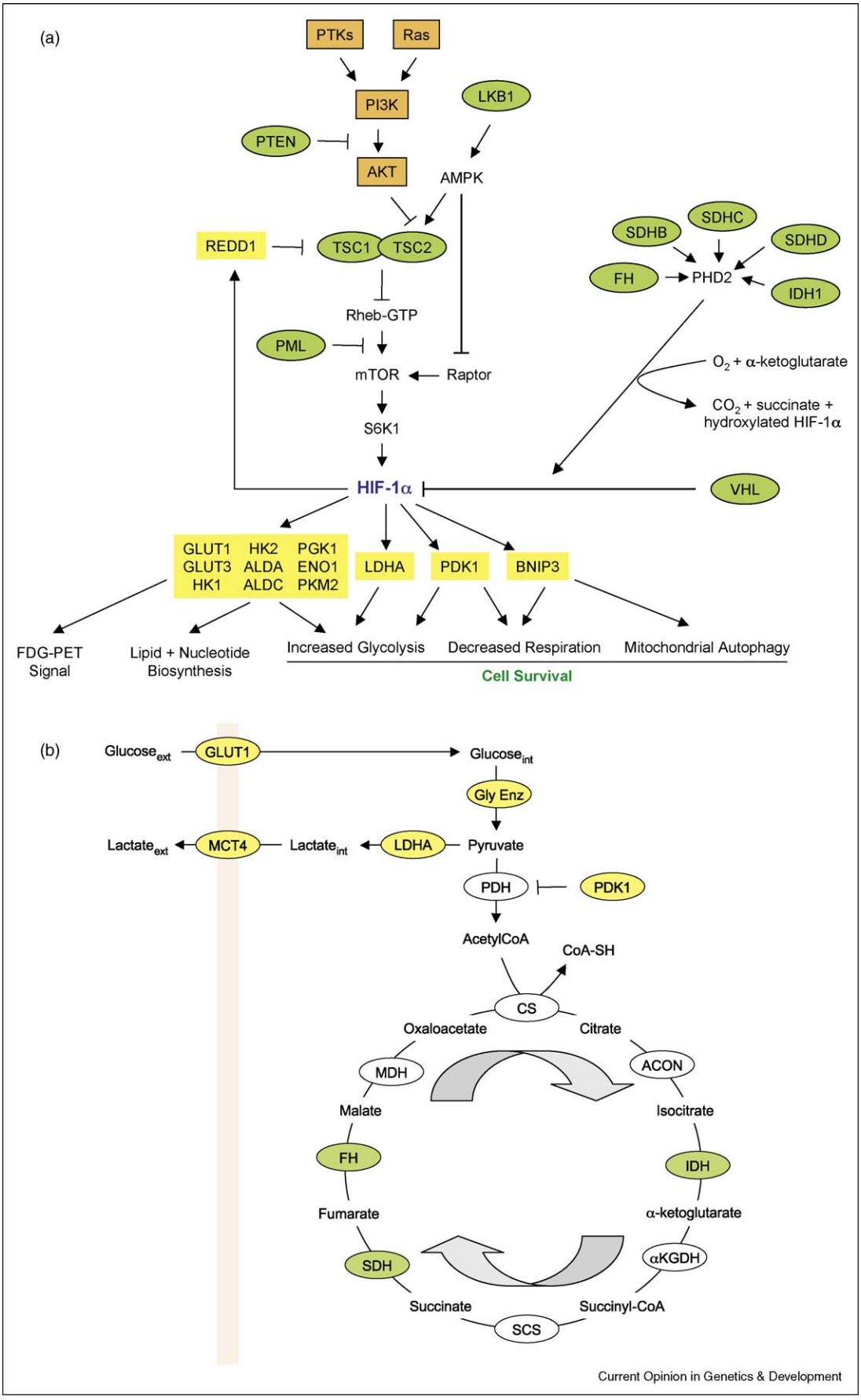
Metastatic cancer is characterized by reprogramming of cellular metabolism leading to increased uptake of glucose for use as both an anabolic and a catabolic substrate. Increased glucose uptake is such a reliable feature that it is utilized clinically to detect metastases by positron emission tomography using ^{18}F-fluorodeoxyglucose (FDG-PET) with a sensitivity of ~90% [1]. As with all aspects of cancer biology, the details of metabolic reprogramming differ widely among individual tumors. However, the role of specific signaling pathways and transcription factors in this process is now understood in considerable detail. This review will focus on the involvement of hypoxia-inducible factor 1 (HIF-1) in both mediating metabolic reprogramming and responding to metabolic alterations. The placement of HIF-1 both upstream and downstream of cancer metabolism results in a feed-forward mechanism that may play a major role in the development of the invasive, metastatic, and lethal cancer phenotype.

O_2 concentrations are significantly reduced in many human cancers compared with the surrounding normal tissue. The median PO_2 in breast cancers is ~10 mmHg, as compared with ~65 mmHg in normal breast tissue [2]. Reduced O_2 availability induces HIF-1, which regulates the transcription of hundreds of genes [3•,4•] that encode proteins involved in every aspect of cancer biology, including: cell immortalization and stem cell maintenance; genetic instability; glucose and energy metabolism; vascularization; autocrine growth factor signaling; invasion and metastasis; immune evasion; and resistance to chemotherapy and radiation therapy [5].

HIF-1 is a transcription factor that consists of an O_2-regulated HIF-1α and a constitutively expressed HIF-1β subunit [6]. In well-oxygenated cells, HIF-1α is hydroxylated on proline residue 402 (Pro-402) and/or Pro-564 by prolyl hydroxylase domain protein 2 (PHD2), which uses O_2 and α-ketoglutarate as substrates in a reaction that generates CO_2 and succinate as byproducts [7]. Prolyl-hydroxylated HIF-1α is bound by the von Hippel–Lindau tumor suppressor protein (VHL), which recruits an E3-ubiquitin ligase that targets HIF-1α for proteasomal degradation (Figure 1a). Asparagine 803 in the transactivation domain is hydroxylated in well-oxygenated cells by factor inhibiting HIF-1 (FIH-1), which blocks the binding of the coactivators p300 and CBP [7]. Under hypoxic conditions, the prolyl and asparaginyl hydroxylation reactions are inhibited by substrate (O_2) deprivation and/or the mitochondrial generation of reactive oxygen species (ROS), which may oxidize Fe(II) present in the catalytic center of the hydroxylases [8].

The finding that acute changes in PO_2 increase mitochondrial ROS production suggests that cellular respiration is optimized at physiological PO_2 to limit ROS generation and that any deviation in PO_2 – up or down – results in increased ROS generation. If hypoxia persists, induction of HIF-1 leads to adaptive mechanisms to reduce ROS and re-establish homeostasis, as described below. Prolyl and asparaginyl hydroxylation provide a molecular mechanism by which changes in cellular oxygenation can be transduced to the nucleus as changes in HIF-1 activity. This review will focus on recent advances in our understanding of the role of HIF-1 in

Figure 1



controlling glucose and energy metabolism, but it should be appreciated that any increase in HIF-1 activity that leads to changes in cell metabolism will also affect many other crucial aspects of cancer biology [5] that will not be addressed here.

HIF-1 target genes involved in glucose and energy metabolism

HIF-1 activates the transcription of *SLC2A1* and *SLC2A3*, which encode the glucose transporters GLUT1 and GLUT3, respectively, as well as *HK1* and *HK2*, which encode hexokinase, the first enzyme of the Embden–Meyerhoff (glycolytic) pathway [9]. Once taken up by GLUT and phosphorylated by HK, FDG cannot be metabolized further; thus, FDG-PET signal is determined by FDG delivery to tissue (i.e. perfusion) and GLUT/HK expression/activity. Unlike FDG, glucose is further metabolized to pyruvate by the action of the glycolytic enzymes, which are all encoded by HIF-1 target genes (Figure 1a). Glycolytic intermediates are also utilized for nucleotide and lipid synthesis [10]. Lactate dehydrogenase A (LDHA), which converts pyruvate to lactate, and monocarboxylate transporter 4 (MCT4), which transports lactate out of the cell (Figure 1b), are also regulated by HIF-1 [9,11]. Remarkably, lactate produced by hypoxic cancer cells can be taken up by non-hypoxic cells and used as a respiratory substrate [12••].

Pyruvate represents a crucial metabolic control point, as it can be converted to acetyl coenzyme A (AcCoA) by pyruvate dehydrogenase (PDH) for entry into the tricarboxylic acid (TCA) cycle or it can be converted to lactate by LDHA (Figure 1b). Pyruvate dehydrogenase kinase (PDK), which phosphorylates and inactivates the catalytic domain of PDH, is encoded by four genes and *PDK1* is activated by HIF-1 [13,14]. (Further studies are required to determine whether *PDK2*, *PDK3*, or *PDK4* is regulated by HIF-1.) As a result of PDK1 activation, pyruvate is actively shunted away from the mitochondria, which reduces flux through the TCA cycle, thereby reducing delivery of NADH and $FADH_2$ to the electron transport chain. This is a crucial adaptive response to hypoxia, because in HIF-1α-null mouse embryo fibroblasts (MEFs), PDK1 expression is not induced by hypoxia and the cells die as a result of excess ROS production, which can be ameliorated by forced expression of PDK1 [13]. MYC, which is activated in ~40% of human cancers, cooperates with HIF-1 to activate transcription of *PDK1*, thereby amplifying the hypoxic response [15]. Pharmacological inhibition of HIF-1 or PDK1 activity increases O_2 consumption by cancer cells and increases the efficacy of a hypoxia-specific cytotoxin [16].

HIF-1 also activates transcription of the gene encoding the BH3 domain protein BNIP3 (Figure 1a), which induces selective mitochondrial autophagy by competing with Beclin 1 for binding to Bcl2, thereby freeing Beclin 1 to trigger autophagy [17••]. BNIP3-induced autophagy was originally associated with hypoxic cell death [18], but studies of HIF-1α-null MEFs have revealed that mitochondrial autophagy is an adaptive response that maintains cell viability under conditions of prolonged hypoxia [17••]. MEFs exposed to 1% O_2 reduce their mitochondrial mass by >50% within 48 h. Hypoxic HIF-1α-null MEFs, which died as a result of excess ROS production, were rescued by treatment with superoxide scavenger or by forced expression of BNIP3 or PDK1 [17••]. Hypoxia induced mitochondrial autophagy in wild type but not in HIF-1α-null MEFs and knockdown of BNIP3, Beclin 1, or Atg5 expression also resulted in ROS-induced cell death, demonstrating that autophagy triggered by HIF-1-dependent BNIP3 expression is required for cell survival under conditions of prolonged hypoxia [13,17••].

HIF-1α-null MEFs exposed to 1% O_2 for 48 h have higher ATP levels than do wild type MEFs cultured at 20% O_2, indicating that O_2 is not limiting for ATP production under these conditions [17••]. Maintenance of respiration in hypoxic HIF-1α-null MEFs is associated with the production of toxic levels of ROS. HIF-1 appears to play a crucial homeostatic role in managing O_2 consumption to balance ATP and ROS production. This is illustrated by the recent finding that HIF-1 orchestrates a subunit switch in cytochrome *c* oxidase (complex IV) that optimizes the efficiency of respiration in response to changes in the cellular O_2 concentration [20].

(Figure 1 Legend) HIF-1 and metabolism. (**a**) Regulation of HIF-1α protein synthesis and stability and HIF-1-dependent metabolic reprogramming. The rate of translation of HIF-1α mRNA into protein in cancer cells is dependent upon the activity of the mammalian target of rapamycin (mTOR), which is determined by the activity of upstream tumor suppressor proteins (green ovals) and oncoproteins (orange rectangles). Arrows indicate stimulation, blocked lines indicate inhibition. HIF-1α protein stability is regulated by O_2- and α-ketoglutarate-dependent prolyl hydroxylation catalyzed by PHD2. Hydroxylation is required for the binding of the von Hippel–Lindau protein (VHL), which recruits a ubiquitin ligase that targets HIF-1α for proteasomal degradation. Loss of function for any of the tumor suppressor genes encoding fumarate hydratase (FH), isocitrate dehydrogenase (IDH), or succinate dehydrogenase (SDH) inhibits PHD2 activity. HIF-1α dimerizes with HIF-1β (not shown) and activates transcription of target genes encoding proteins (yellow rectangles) that play key roles in the metabolic reprogramming of cancer cells. Abbreviations: PTKs, protein tyrosine kinases; PI3K, phosphatidylinositol-3-kinase; S6K, ribosomal protein S6 kinase; ALD, aldolase; PGK, phosphoglycerate kinase; ENO, enolase; and PKM, pyruvate kinase M. Other HIF-1-regulated glycolytic enzymes that are not shown: glucosephosphate isomerase, phosphofructokinase L, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, and phosphoglyceromutase. (**b**) Oxidative and glycolytic metabolism of glucose. HIF-1-regulated genes (yellow ovals) play key roles in converting extracellular glucose to extracellular lactate and blocking entry of pyruvate into the tricarboxylic acid (TCA) cycle. Loss of function for each of the TCA cycle enzymes shown in green is associated with tumor formation and HIF-1α stabilization owing to PHD2 inhibition by accumulation of the enzyme substrate. Arrows indicate the direction of the TCA cycle. CS, citrate synthase; ACON, aconitase; αKGDH, α-ketoglutarate dehydrogenase; SCS, succinyl-CoA synthetase; and MDH, malate dehydrogenase.

Hypoxia also induces mitochondrial autophagy in many human cancer cell lines through HIF-1-dependent expression of BNIP3 and a related BH3 domain protein, BNIP3L [19••]. Autocrine signaling through the platelet-derived growth factor receptor in cancer cells increases HIF-1 activity and thereby increases autophagy and cell survival under hypoxic conditions [21]. Autophagy may also occur in a HIF-1-independent manner in response to other physiological stimuli that are associated with hypoxic conditions, such as a decrease in the cellular ATP:AMP ratio, which activates AMP kinase signaling [22].

In clear cell renal carcinoma, VHL loss of function (LoF) results in constitutive HIF-1 activation, which is associated with impaired mitochondrial biogenesis that results from HIF-1-dependent expression of MXI1, which blocks MYC-dependent expression of PGC-1β, a coactivator that is required for mitochondrial biogenesis [23]. Inhibition of wild type MYC activity in renal cell carcinoma contrasts with the synergistic effect of HIF-1 and oncogenic MYC in activating PDK1 transcription [24].

Genetic and metabolic activators of HIF-1

Hypoxia plays a crucial role in cancer progression [2,5] but not all cancer cells are hypoxic and a growing number of O_2-independent mechanisms have been identified by which HIF-1 is induced [5]. Several mechanisms that are particularly relevant to cancer metabolism are described below.

Activation of mTOR

LoF for any of five different tumor suppressors – LKB1 [25••], PML [26], PTEN [27,28], and TSC1/TSC2 [29] induces HIF-1α expression by disregulation of the mammalian target of rapamycin (mTOR), which can also be activated by gain of function affecting protein tyrosine kinases (e.g. epidermal growth factor receptor [27], $HER2^{neu}$ [30]), Ras, and/or the downstream phosphotidylinositol-3-kinase/AKT pathway (Figure 1a). mTOR is a serine–threonine protein kinase that phosphorylates ribosomal protein S6 kinase and eIF-4E binding protein 1, which increases the rate of translation of HIF-1α mRNA into protein [30]. LKB1, which is mutated in the Peutz–Jeghers intestinal hamartoma syndrome, is the upstream activator of AMP kinase, which activates TSC2 and inactivates Raptor, thereby inhibiting mTOR activity by two mechanisms (Figure 1a). Analysis of LKB1-deficient mice revealed a dramatic upregulation of HIF-1, GLUT1, and HK2 in gastrointestinal polyps, which were strongly positive by FDG-PET imaging; all of these findings were abolished after treatment with rapamycin [25••]. Expression of constitutively active AKT in prostatic epithelium also induced neoplasia and the HIF-1 metabolic transcriptome in a mTOR-dependent manner [31].

Alterations in mitochondrial metabolism

Recent studies have revealed that that tumor suppressor genes mutated in hereditary paraganglioma, hereditary leiomyomatosis/renal carcinoma, and secondary glioblastoma encode enzymes of the mitochondrial TCA cycle: succinate dehydrogenase (SDH), fumarate hydratase (FH), and isocitrate dehydrogenase (IDH), respectively (Figure 1a). SDH and FH LoF lead to increased levels of the metabolic substrate of the enzyme – succinate and fumarate, respectively (Figure 1b), which inhibit PHD2 by competing with α-ketoglutarate for binding to the catalytic center, thereby reducing hydroxylation, ubiquitination, and proteasomal degradation of HIF-1α [32–35]. By contrast, IDH LoF leads to decreased levels of the reaction product, α-ketoglutarate (Figure 1b), which is a necessary substrate for PHD2 [36••].

Mutations in the gene encoding the ND2 subunit of respiratory complex I have been reported to increase ROS levels and thereby increase HIF-1α levels in head and neck squamous cell carcinoma [37]. Administration of antioxidants, such as ascorbate or *N*-acetylcysteine, has been shown to dramatically reduce tumor xenograft growth by inhibiting HIF-1α levels [38]. In ND2-deficient cells, expression of PDK2 was increased and administration of the PDK inhibitor dichloroacetate decreased HIF-1α levels. This result is consistent with previous studies demonstrating that both pyruvate and lactate (whose levels increase in the presence of PDK activity) induce HIF-1α expression [39••]. These results suggest the existence of a feed-forward mechanism, in which induction of HIF-1 leads to PDK activity, elevated pyruvate/lactate, and further increases in HIF-1 activity.

NAD^+ levels

In addition to HIF-1α, the PHD2-VHL pathway also regulates HIF-2α, which dimerizes with HIF-1β and activates target gene expression [40]. Increased levels of both HIF-1α and HIF-2α are observed in many human cancers and associated with increased patient mortality [5]. Studies in mice suggest that HIF-2α may regulate *SOD2* and other genes encoding antioxidant proteins [41]. Thus, whereas HIF-1α inhibits oxidant generation, HIF-2α promotes antioxidant generation. It is therefore of interest that the NAD^+-dependent deacetylase sirtuin 1 (SIRT1) was found to bind to, deacetylate, and increase transcriptional activation by HIF-2α but not HIF-1α [42••]. Another NAD^+-dependent enzyme is poly(ADP-ribose) polymerase 1 (PARP1), which was recently shown to bind to HIF-1α and promote transactivation through a mechanism that required the enzymatic activity of PARP1 [43]. Thus, transactivation mediated by both HIF-1α and HIF-2α can be modulated according to NAD^+ levels. Further studies are required to understand the significance of these novel findings in the context of cancer cell metabolism.

Nitric oxide

Increased expression of nitric oxide (NO) synthase isoforms and increased levels of NO have been shown to increase HIF-1α protein stability in human oral squamous cell carcinoma [44]. In prostate cancer, nuclear co-localization of endothelial NO synthase, estrogen receptor β, HIF-1α, and HIF-2α was associated with aggressive disease and the proteins were found to form chromatin complexes on the promoter of *TERT* gene encoding telomerase [45••]. The *NOS2* gene encoding inducible NO synthase is HIF-1 regulated [5], suggesting another possible feed-forward mechanism.

Acknowledgements

Work in the author's laboratory is supported by grants from the National Cancer Institute, National Heart, Lung, and Blood Institute, National Institute of General Medical Sciences, and the Johns Hopkins Institute for Cell Engineering. G.L.S. is the C. Michael Armstrong Professor at The Johns Hopkins University School of Medicine.

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