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MINI REVIEW

HIF-1 mediates the Warburg effect in clear cell renal carcinoma

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Abstract Hypoxia-inducible factor 1 (HIF-1) is a transcription factor that functions as a master regulator of oxygen homeostasis in all metazoan species. O_2-dependent hydroxylation of two proline residues in the HIF-1α subunit is necessary for the binding of the von Hippel–Lindau (VHL) protein, which is a component of a ubiquitin protein ligase that ubiquitinates HIF-1α, leading to its degradation by the proteasome. In the majority of cases of the clear cell type of renal carcinoma, both *VHL* genes are inactivated by mutation or epigenetic silencing, leading to dysregulated HIF-1 transcriptional activity. VHL loss-of-function leads, under aerobic conditions, to a HIF-1-dependent reprogramming of glucose and energy metabolism that includes increased glucose uptake, glycolysis, and lactate production accompanied by a reciprocal decrease in respiration. These findings delineate for the first time the molecular mechanisms underlying the Warburg effect in a human cancer.

Keywords Glucose transport · Glycolysis · Hypoxia · von Hippel–Lindau

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Introduction

In the presence of O_2, cells convert glucose to pyruvate, which is transported into the mitochondria, converted to acetyl coenzyme A (acetyl CoA) and metabolized via the tricaryboxylic acid (TCA) cycle, yielding reducing equivalents that are used to generate ATP through the process of oxidative phosphorylation (Lehninger 1982). Under hypoxic conditions, pyruvate is instead converted to lactate, resulting in a net synthesis of 2 mol of ATP rather than the 38 mol that are produced by the oxidative metabolism of glucose. Hypoxic cells compensate for the inefficiency of glycolytic metabolism by greatly increasing their uptake of glucose, thus increasing flux through the pathway.

Cancer cells, especially in metastatic disease, are characterized by increased glucose uptake, increased lactate production, and decreased respiration, even under aerobic conditions, which is known as the Warburg effect. The increased glucose uptake that is required to maintain ATP production under conditions of reduced respiration is such a universal feature of metastatic cancer cells that it is used to detect them clinically by positron emission tomography (PET scan) following the administration of [^{18}F]-deoxyglucose (reviewed in Gatenby and Gillies 2004). Increased aerobic glycolysis was also observed in cells infected with Rous sarcoma virus, in which it represented one of the earliest detectable signs of cellular transformation (Steck et al. 1968; Singh et al. 1974; Carroll et al. 1978). However, the underlying molecular mechanisms and adaptive significance of this universal feature of advanced cancer cells have not been fully elucidated in the eight decades since Warburg's pioneering studies were performed.

Regulation of oxygen homeostasis by HIF-1

HIF-1 was identified as a DNA-binding activity, which was induced in nuclear extracts of cells that had been subjected to hypoxia, and which activated transcription of the human *EPO* gene, which encodes erythropoietin, the hormone that controls red blood cell production and thus, blood oxygen-carrying capacity (Semenza and Wang 1992). The binding of HIF-1 to an 18-bp oligonucleotide corresponding to the HIF-1 site in the *EPO* gene was used to purify the protein by DNA affinity chromatography from 100 liters of HeLa cells grown in suspension culture (Wang and Semenza 1995).

The protein purification and subsequent isolation of cDNA clones revealed that HIF-1 was a heterodimer composed of HIF-1α and HIF-1β subunits that belonged to the basic helix–loop–helix family of transcription factors (Wang et al. 1995). HIF-1α protein expression was induced by hypoxia (Wang et al. 1995), with levels increasing exponentially as cellular O_2 concentration was decreased from 20 to 0.5%, which correspond to PO_2 values of 140 to 3.5 mmHg, respectively (Jiang et al. 1996). HIF-1α levels rapidly decayed following reoxygenation with a half-life of less than 5 min in post-hypoxic cultured cells (Wang et al. 1995; Jewell et al. 2001) and less than 1 min in isolated ventilated lung preparations subjected to hypoxia and reoxygenation (Yu et al. 1998). No protein has been shown to have a shorter half-life. Database searches identified HIF-2α, a protein with structural similarity to HIF-1α that is regulated by O_2 and dimerizes with HIF-1β to regulate a set of target genes that overlaps with those regulated by HIF-1α: HIF-1β heterodimers (Tian et al. 1997; Wiesener et al. 1998; Elvidge et al. 2006).

The molecular basis for the precise regulation of HIF-1α levels was demonstrated to involve the ubiquitination and proteasomal degradation of HIF-1α (Salceda and Caro 1997; Huang et al. 1998; Kallio et al. 1999). The von Hippel–Lindau tumor suppressor protein (VHL) is required for this process, as clear cell renal carcinoma cells lacking functional VHL constitutively express HIF-1α and HIF-1 target genes under non-hypoxic conditions (Maxwell et al. 1999; Cockman et al. 2000). VHL forms a complex with elongin B, elongin C, cullin 2, and RBX1 to form an E3 ubiquitin–protein ligase capable of functioning with E1 ubiquitin-activating and E2 ubiquitin-conjugating enzymes to mediate the ubiquitination of HIF-1α (Kamura et al. 2000; Fig. 1).

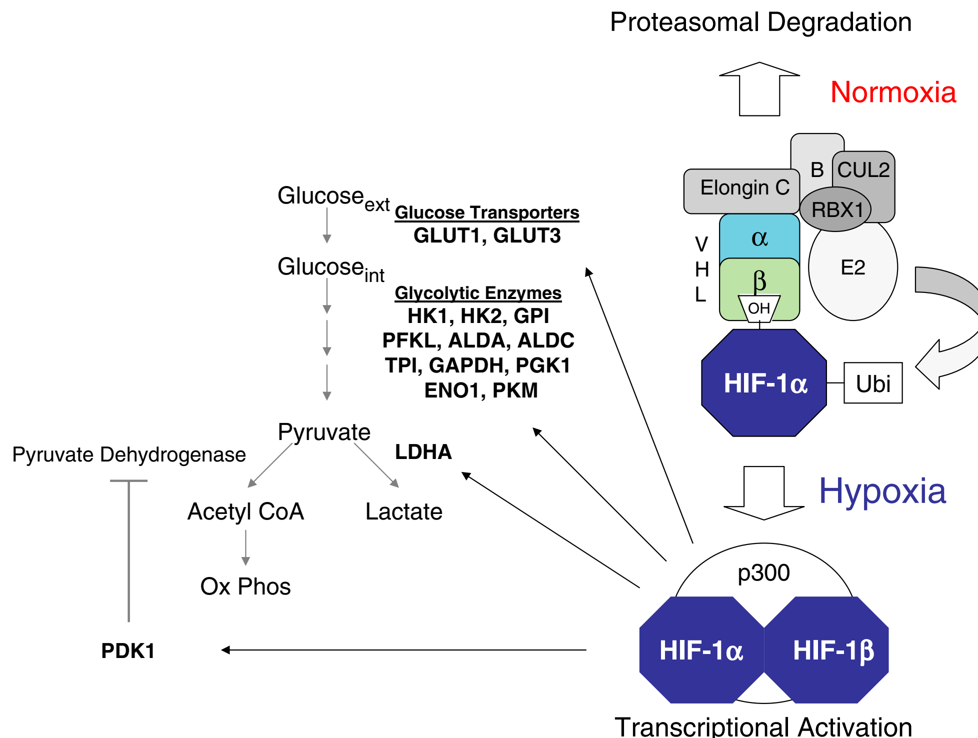

Fig. 1 Regulation of glucose metabolism by HIF-1. Under normoxic conditions, HIF-1α (or HIF-2α) is hydroxylated by PHD2, bound by VHL, ubiquitinated by an E3 ligase complex containing elongin B, elongin C, cullin 2, and Rbx1, and degraded by the proteasome. VHL loss-of-function (in clear cell renal carcinoma) or hypoxic conditions leads to the accumulation of non-hydroxylated, non-ubiquitinated HIF-1α (or HIF-2α), which dimerizes with HIF-1β, recruits the coactivator p300 (or CBP) and activates the transcription of genes encoding glucose transporter (GLUT) 1 and 3, hexokinase (HK) 1 and 2, glucosephosphate isomerase (GPI), phosphofructokinase (PFK) L, aldolase (ALD) A and C, triosephosphate isomerase (TPI), glyceraldephosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK) 1, enolase (ENO) 1, pyruvate kinase (PK) M, lactate dehydrogenase (LDH) A, and pyruvate dehydrogenase kinase (PDK) 1

Hydroxylation of proline residue 402 and/or 564 in human HIF-1α is required for the binding of VHL (Epstein et al. 2001; Ivan et al. 2001; Jaakkola et al. 2001; Yu et al. 2001). Three prolyl hydroxylases were identified in mammalian cells and shown to utilize O_2 as a substrate to generate 4-hydroxyproline at residue 402 and/or 564 of HIF-1α (Bruick and McKnight 2001; Epstein et al. 2001; Ivan et al. 2002). Interestingly, the hydroxylation reaction utilizes the TCA cycle intermediate α-ketoglutarate as a co-substrate and generates succinate and CO_2 as side products. Under hypoxic conditions, the rate of hydroxylation declines, either as a result of substrate (O_2) limitation or inhibition of hydroxylase activity as a result of a physiological increase in mitochondrial generation of reactive oxygen species (ROS) that may oxidize the Fe (II) ion in the catalytic site (Guzy et al. 2005). Decreased hydroxylation under hypoxic conditions is the basis for reduced binding of VHL, leading to increased steady-state levels of HIF-1α. Thus, changes in oxygenation are directly transduced to the nucleus as changes in HIF-1α levels and HIF-1 transcriptional activity.

HIF-1 regulates the expression of hundreds of genes in human cells (Manalo et al. 2005; Elvidge et al. 2006) and is essential for embryonic development in mice (Iyer et al. 1998; Ryan et al. 1998). Many of these genes contribute to two essential functions of HIF-1. First, HIF-1 promotes the delivery of oxygen to cells through its control of erythropoiesis and angiogenesis (reviewed in Hirota and Semenza 2006). Second, HIF-1 promotes cell survival under hypoxic conditions by reprogramming cellular glucose and energy metabolism. The remainder of this review will focus on recent discoveries regarding this latter program of gene expression.

Regulation of glucose transport, glycolysis, and lactate production by HIF-1

Hypoxia response elements containing HIF-1 binding sites were identified in genes encoding several glucose transporters and glycolytic enzymes (Semenza et al. 1994, 1996; Ebert et al. 1995; Firth et al. 1995; Fig. 1). Analysis of mRNA expression in mouse embryonic stem cells that were either wild type or homozygous for a knockout allele at the *Hif1a* locus encoding the HIF-1α subunit revealed that expression of the genes encoding glucose transporters 1 and 3 and the glycolytic enzymes hexokinase 1 and 2, glucose phosphate isomerase, phosphofructokinase L, aldolase A and C, triosephosphate isomerase, phosphoglycerate kinase 1, enolase 1, pyruvate kinase M, and lactate dehydrogenase A (LDH-A) were all regulated by HIF-1 (Fig. 1), representing the most extensive example of coordinate metabolic control at the transcriptional level that has been described in any metazoan species (Iyer et al. 1998). These studies provided a molecular basis for the observed stimulation of glucose transport and glycolysis that are necessary to maintain ATP production in hypoxic cells (Seagroves et al. 2001). In human VHL-deficient renal cell carcinoma, upregulation of GLUT1 protein expression has been demonstrated at the earliest stages of tumor formation (Mandriota et al. 2002).

Regulation of pyruvate metabolism by HIF-1

The results described above suggest that the upregulation of LDH-A results in increased conversion of pyruvate to lactate at the expense of mitochondrial utilization of pyruvate as a substrate for pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl CoA. However, recent studies have demonstrated that HIF-1 plays a direct role in actively shunting pyruvate away from the mitochondria through its regulation of the *PDK1* gene encoding PDH kinase 1 in multiple cell types including VHL-deficient renal carcinoma cells (Kim et al. 2006; Papandreou et al. 2006; Fig. 1). Phosphorylation of the catalytic subunit of PDH by PDK1 inactivates the enzyme. In mouse embryo fibroblasts cultured from HIF-1α-null embryos, prolonged hypoxic incubation induces ROS production leading to cell death that can be rescued by forced expression of PDK1 (Kim et al. 2006). HIF-1α-null mouse embryo fibroblasts also manifest increased cell death (relative to wild type cells) when incubated under hypoxic conditions in the presence of the hypoxic cytotoxin tirapazamine (Papandreou et al. 2006). These results provided the first evidence that HIF-1 actively inhibits the oxidative metabolism of glucose under hypoxic conditions.

Regulation of mitochondrial biogenesis by HIF-1

Reduced levels of mitochondrial DNA and respiratory chain proteins as well as increased levels of glycolytic enzymes have been reported in renal cell carcinoma (Simonnet et al. 2002; Unwin et al. 2003; Meierhofer et al. 2004; Craven et al. 2006). Forced expression of wild-type VHL protein in cell lines derived from VHL-deficient renal carcinoma leads to increased mitochondrial electron transport complex activity and increased levels of mitochondrial DNA and respiratory chain proteins (Hervouet et al. 2005), but the molecular pathways leading to these alterations have not been delineated (Hervouet and Godinot 2006). We have recently demonstrated that introduction of a VHL expression vector into the clear cell renal carcinoma cell lines results in a dramatic increase in mitochondrial mass, mitochondrial DNA, and O_2 consumption (Zhang et al. 2007). Similar results were obtained when the cells were

transfected with an expression vector encoding a dominant negative form of HIF-1α (Jiang et al. 1996) that can dimerize with HIF-1β but cannot bind to DNA or activate transcription (Zhang et al. 2007).

Perspective

The recent studies reviewed above have demonstrated that in VHL-deficient clear cell renal carcinoma, HIF-1 mediates increased glucose uptake, increased lactate production, and decreased respiration, thus delineating for the first time the molecular mechanisms underlying the switch from oxidative to glycolytic metabolism in human cancer. A large body of data suggests that HIF-1 may also contribute to the Warburg effect in other human cancers (Semenza 2003). High LDH levels and low mitochondrial respiratory chain content are each associated with poor prognosis in advanced renal cell carcinoma (Simonnet et al. 2002; Motzer et al. 2004). Additional studies are required to investigate whether the increased dependence of these cancer cells on glycolytic metabolism can be exploited therapeutically.

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