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Review

Regulation of cancer cell metabolism by hypoxia-inducible factor 1

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ABSTRACT

The induction of hypoxia-inducible factor 1 (HIF-1) activity, either as a result of intratumoral hypoxia or loss-of-function mutations in the *VHL* gene, leads to a dramatic reprogramming of cancer cell metabolism involving increased glucose transport into the cell, increased conversion of glucose to pyruvate, and a concomitant decrease in mitochondrial metabolism and mitochondrial mass. Blocking these adaptive metabolic responses to hypoxia leads to cell death due to toxic levels of reactive oxygen species. Targeting HIF-1 or metabolic enzymes encoded by HIF-1 target genes may represent a novel therapeutic approach to cancer.

1. Introduction

For decades, students of biochemistry have learned that in the presence of O_2 cells generate ATP by completely oxidizing glucose to carbon dioxide and water through the activity of glycolytic enzymes, pyruvate dehydrogenase (PDH), the tricarboxylic acid (TCA) cycle enzymes, and the electron transport chain. In contrast, under hypoxic conditions, glucose is converted to lactate, through the activity of glycolytic enzymes and lactate dehydrogenase A (LDHA), which is a much less efficient means of generating ATP. Lactate production has been viewed as a default pathway that is followed when O_2 is not available for respiration. However, lactate production increases several-fold when cells are exposed to 1% O_2 (corresponding to a partial pressure [PO_2] of ~7 mmHg at sea level), which is well above the critical O_2 concentration required for electron transport chain activity in isolated mitochondria (~0.1 μM; PO_2 = 0.05 mmHg). Recent studies have demonstrated that the switch from oxidative to glycolytic metabolism is an active response to hypoxia that is mediated by hypoxia-inducible factor 1 (HIF-1).

2. HIF-1

HIF-1 is a heterodimeric protein, composed of HIF-1α and HIF-1β subunits [1,2], which modulates the regulation of hundreds of genes according to the cellular O_2 concentration [3]. HIF-1α levels increase dramatically as O_2 concentration declines [4]. Under normoxic conditions, HIF-1α is subjected to ubiquitination and proteasomal degradation [5-7] due to the binding of the von Hippel-Lindau tumor suppressor protein [8], which is the substrate recognition subunit of an E3 ubiquitin-protein ligase [9]. VHL binds to HIF-1α only when the latter is hydroxylated on proline residue 402 and/or 564 [10–12]. The hydroxylation reaction is performed by prolyl hydroxylases (PHDs) that utilize O_2 and α-ketoglutarate as substrates and generate carbon dioxide and succinate as byproducts [13]. Under hypoxic conditions, hydroxylation, ubiquitination and degradation are inhibited, leading to the accumulation of HIF-1α (Fig. 1). Under normoxic conditions, asparagine residue 803 is also hydroxylated. This reaction, which is mediated by factor inhibiting HIF-1 (FIH-1), prevents the binding of the co-activators CBP and p300 to HIF-1α [14]. Thus, O_2-dependent hydroxylation regulates both the stability and transcriptional activity of HIF-1. Once activated, HIF-1 mediates a variety of adaptive responses to hypoxia. Two general classes of responses are, first, those that serve to increase O_2 delivery (for example, by stimulating angiogenesis by activation of the gene encoding vascular endothelial growth factor [VEGF]); and, second, those that serve to regulate O_2 utilization. Recent discoveries with regard to the latter responses are described below.

3. Intratumoral PO_2, lactate, and pH

The mean PO_2 in human tumors is significantly reduced compared to surrounding normal tissue and tumors with the greatest reduction in PO_2 are most likely to invade, metastasize, and kill

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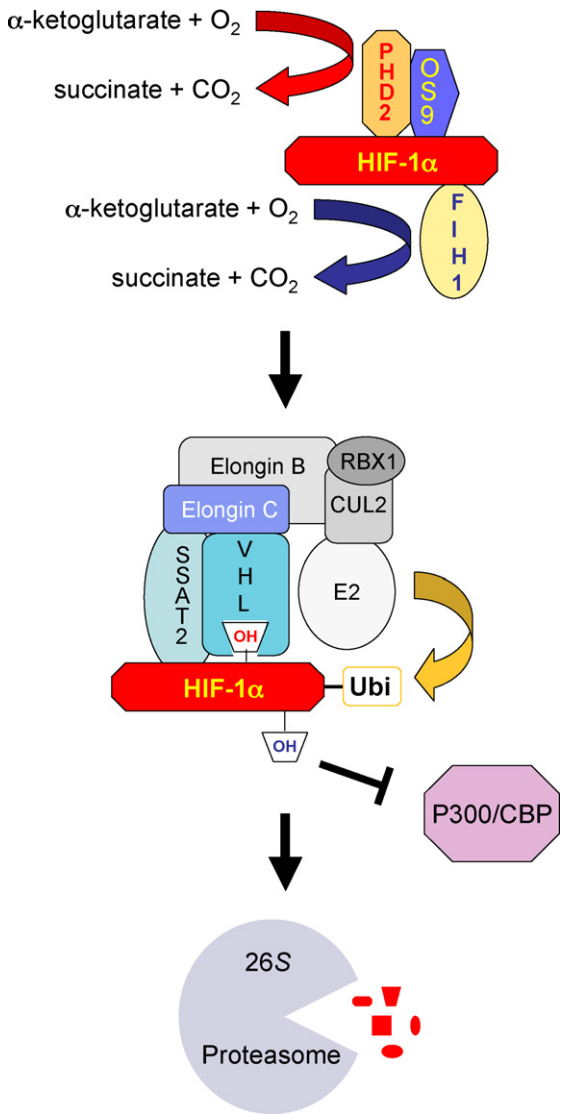

Fig. 1. Regulation of HIF-1. Under normoxic conditions, HIF-1α is hydroxylated by PHD2. The protein OS-9 binds to both HIF-1α and PHD2, thereby promoting the hydroxylation reaction [45], in which O_2 and α-ketoglutarate are consumed, with CO_2 and succinate generated as byproducts. Hydroxylated HIF-1α is bound by VHL, which recruits an ubiquitin-protein ligase complex consisting of Elongin C, Elongin B, Cullin 2 (CUL2), Ring Box Protein 1 (RBX1), and an E2 ubiquitin conjugating enzyme (E2). SSAT2 promotes ubiquitination by binding to HIF-1α, VHL, and Elongin C [46]. Ubiquitinated HIF-1α is subjected to degradation by the 26*S* proteasome.

the patient [15]. Many carcinomas also manifest an increased concentration of lactate, which is also associated with increased risk of metastasis [16]. The increased lactate production is associated with increased expression of LDH-A [17] and the monocarboxylate transporter MCT4, which transports lactate out of cancer cells [18]. Cancer cells also overexpress the sodium–hydrogen exchanger NHE1 and carbonic anhydrase 9, which function to maintain an alkaline intracellular pH and an acidic extracellular pH [19]. Expression of the *LDHA*, *MCT4*, *NHE1*, and *CA9* genes is induced by hypoxia through the activity of HIF-1 [20]. Thus, along with its control of genes encoding glucose transporters and glycolytic enzymes [21], HIF-1 coordinately regulates all of the proteins required for glucose uptake and its conversion to lactate (Fig. 2). The induction of CA9, MCT4, and NHE1 allows cancer cells to maintain an alkaline intracellular pH and an acidic extracellular pH, which are critical for cell proliferation and invasion, respectively.

4. Effects of HIF-1α deficiency on cell metabolism

When HIF-1α-null mouse embryo fibroblasts (MEFs) are subjected to hypoxia for 3 days, the cells die due to increased production of reactive oxygen species (ROS) [22,23]. In contrast, ROS levels decrease in wild type (WT) cells in response to chronic hypoxia. This adaptive response is mediated by HIF-1 through the transactivation of genes encoding pyruvate dehydrogenase kinase 1 (PDK1) and BNIP3. In hypoxic cells, PDK1 phosphorylates and inactivates PDH, thereby blocking the conversion of pyruvate to acetyl coenzyme A (CoA), which is required for entry into the TCA cycle [22,24]. In concert with LDH-A, PDK1 leads to the preferential conversion of pyruvate to lactate rather than acetyl CoA. Overexpression of PDK1 in HIF-1α-null MEFs is sufficient to reduce ROS levels and prevent cell death [22].

A second key response to hypoxia that is mediated by HIF-1 is the induction of BNIP3, which is a member of the BCL2 family of mitochondrial proteins. Expression of BNIP3 triggers selective mitochondrial autophagy in WT MEFs, but not in HIF-1α-null MEFs, which results in a two- to fourfold decrease in mitochondrial mass and O_2 consumption within 48 h [23]. Experimental knockdown of BNIP3 or a key component of the autophagy machinery, such as Atg5 or Beclin1, phenocopies the effect of HIF-1α deficiency, whereas forced overexpression of BNIP3 in HIF-1α-null MEFs reduces ROS levels and cell death, similar to the effect of PDK1 overexpression.

These data suggest that there is an optimal PO_2 for mitochondrial respiration, that increased or decreased PO_2 is associated with increased ROS production, and that a major role of HIF-1 is in balancing energy and redox homeostasis. This hypothesis is supported by the finding that HIF-1 also regulates the composition of cytochrome *c* oxidase (COX; electron transport complex IV) by activating transcription of the *COX4I2* gene, which encodes COX4-2, a regulatory subunit that allows the enzyme to function optimally under hypoxic conditions, and of the *LON* gene, which encodes a mitochondrial protease that is required for degradation of the COX4-1 subunit under hypoxic conditions [25]. Thus, in response to modest reduction of PO_2, this subunit switch may provide a mechanism that allows continued ATP production via oxidative phosphorylation without increased ROS production.

Previous studies have demonstrated that acute hypoxia results in increased ROS levels, which are required for the inhibition of PHD activity and stabilization of HIF-1α in hypoxic cells [26]. These responses are lost in cells lacking cytochrome *c* [27]. Thus, hypoxia leads to increased mitochondrial ROS, which induce HIF-1, which then mediates adaptive responses to reduce ROS levels through the modulation of mitochondrial oxidative metabolism.

5. The molecular basis of the Warburg effect in renal clear-cell carcinoma

Although many cancer cells utilize the physiological responses to hypoxia described above, in some cancers, genetic alterations can result in a fixed and O_2-independent reprogramming of metabolism. Warburg noted increased production of lactate in the tissue culture media of liver tumor explants as compared to normal liver explants cultured under aerobic conditions [28]. In renal cell carcinoma lines in which VHL is inactivated by mutation, HIF-1α and HIF-2α are constitutively expressed and mediate glycolytic metabolism. Reintroduction of WT VHL into the cell results in loss of HIF-1α and HIF-2α expression under aerobic conditions and a dramatic increase in mitochondrial mass and O_2 consumption [29]. In VHL-deficient renal carcinoma cells HIF-1 blocks the biogenesis of mitochondria through inhibition of MYC, which would otherwise activate transcription of the gene encoding PGC-1β, a transcription factor that controls mitochondrial biogenesis. Loss of