Hypoxia-inducible factors and cancer

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Abstract Decreased oxygen availability is a common feature during embryonic development as well of malignant tumours. Hypoxia regulates many transcription factors, and one of the most studied is the hypoxia-inducible factor (HIF). As a consequence of HIF stabilisation, the cell constitutively upregulates the hypoxic programme resulting in the expression of genes responsible for global changes in cell proliferation, angiogenesis, metastasis, invasion, de-differentiation and energy metabolism. Of the three known alpha subunits of HIF transcription factors, HIF-1α and HIF-2α have been the most studied. Their differential expression and function have been widely discussed, however no clear picture has been drawn on how these two transcription factors differently regulate common and unique target genes. Their role as oncogenes has also been suggested in several studies. In this review we provide an overview of the current knowledge on some of the most important aspects of HIFα regulation, its role in tumour angiogenesis and energetic metabolism. We also give an overview of how the modulation of HIF regulating pathways is a potential therapeutic target that may have benefits in the treatment of cancer.

Key words Hypoxia • HIF • Cancer • Angiogenesis • VHL


Introduction

Oxygen is a highly reactive molecule due to its elevated standard reduction potential and the electronegativity of oxygen atoms. During evolution living organisms took advantage of this property to develop a highly efficient oxygen-dependant oxidative metabolism. At the same time cells acquired antioxidant mechanisms to protect their macromolecules from the damaging effects of oxygen. In metazoans, oxygen is required for mitochondrial respiration in aerobic metabolism. Thus, a decrease in oxygen availability severely impairs energy generation through oxidative phosphorylation and forces the cells to depend on the inefficient glycolytic metabolism. However, anaerobic metabolism does not support the energetic needs of most cell types in the long term. Hence, cells respond to decreased oxygen tension by the induction of an adaptive response aimed to restore oxygen supply to the hypoxic region and maintain cell viability in the meantime. This response is mediated through the induction of genes involved in angiogenesis and in the switch to anaerobic metabolism.

It is noteworthy that as most solid tumours develop hypoxia, as a consequence of cellular proliferation, many of these physiological adaptive responses are also commonly found in human cancer. Accordingly, altered glucose metabolism (Warburg effect), resistance to apoptosis and induction of blood vessel growth (angiogenic switch) are all hallmarks of neoplasia. Moreover, these features are critical during tumour progression. Thus, hypoxia is generated during tumour growth and the response to hypoxia results in increased tumour growth in a positive feedback loop.

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The oxygen sensing machinery

As indicated before, most of the responses to hypoxia are mediated through the induction of a specific gene expression programme. In turn, most of these genes are under the control of a family of transcription factors known as hypoxia-inducible factors (HIFs). Other transcription factors, including AP-1 [1] and NFkB [2–4], may be regulated by hypoxia, but their putative role in the response to low oxygen is unclear. Analysis of hypoxia-induced gene expression in animals/cells deficient for HIF factors [5–8] corroborate their central role in this response. In agreement, hypoxia response elements (HRE) have been found on the regulatory regions of many hypoxia-inducible genes [9], including proangiogenic factors, glucose transporters and most of the glycolytic enzymes. HIF transcription factors are heterodimers composed of a constitutively expressed β subunit (HIFβ, also known as aryl receptor nuclear translocator, ARNT) and an oxygen-regulated α subunit (HIFα). Both subunits are basic-helix-loop-helix (bHLH) transcription factors and contain a Pax/Aryl hydrocarbon receptor/Sim (PAS) domain. HIFβ is not regulated by oxygen and interacts with several bHLH factors other than HIFα. To date, three different α subunits have been described: HIF-1α/MOP1, HIF-2α/EPAS (endothelial PAS)/MOP2 and HIF-3α. Most of the studies have focused on the HIF1α and 2α isoforms and comparatively much less is known about HIF-3α. Nonetheless, HIF-3α seems to be regulated by oxygen in a similar fashion to the other two HIFα proteins [10]. HIF-3α’s role in the induction of hypoxia-regulated genes is unclear and also whether it has the same target genes as HIF-1α or -2α. Interestingly, an alternative spliced form of HIF-3α encodes for a HIFα inhibitor, IPAS [11]. This isoform is expressed by some tissues, such as retina, where angiogenesis is normally inhibited and, at least in some cell types, IPAS expression is

![Fig. 1 Regulation of HIFα stability and activity by oxygen-mediated hydroxylations. Under normoxia HIFα subunits are hydroxylated at two Pro residues within the ODD and an Asn residue within the TAD. These modifications are oxygen-dependent and are catalysed by the EGLN and FIH enzymes respectively. Hydroxyprolines are specifically recognised by the VHL tumour suppressor protein, a component of an E3 ubiquitin ligase complex, resulting in HIF ubiquitination that targets HIFα for proteosomal degradation. In addition, Asn hydroxylation prevents p300 binding to the C-terminal transactivation domain (CTAD) and as a consequence HIFα transcriptional activity is diminished.](image-url)