Hypoxia and HIF-1


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Hypoxia-inducible factor-1 (HIF-1) regulates a large battery of genes that help cells survive oxygen deprivation or increase oxygen delivery to tissues.

HIF-1 is a heterodimer composed of HIF-1α and HIF-1β. It binds to the hypoxia response element (HRE), short sequences that can be put into a heterologous gene, and then that gene will be induced by hypoxia. When HIF-1 binds to the HRE, it recruits the coactivators P300 and CBP, which both serve as a bridge to the transcription initiation complex and have histone acetyltransferase activity that is required for remodeling of chromatin.

The expression of the HIF-1α subunit is tightly regulated by the cellular oxygen concentration. There is a modest increase in HIF-1α between 20% and 6% and then a sharp increase when oxygen concentration drops below 6%, which corresponds to 40 mm Hg (venous PO₂). In vivo all cells are exposed to oxygen concentrations below 6%, and so any fluctuations occur along the steep part of the curve. This provides a mechanism for a graded response to hypoxia - the more severe the hypoxia, the greater the expression of HIF1α and the greater the transcription of downstream target genes.

The molecular basis for this regulation of HIF-1α levels is oxygen-dependent hydroxylation of two proline residues in HIF-1α by a group of enzymes called prolyl hydroxylases (reviewed in Ref. 1). These enzymes use molecular oxygen as a substrate and have a high \( K_m \) for oxygen so that oxygen is a rate limiting substrate under physiological conditions. Under non-hypoxic conditions these residues are hydroxylated, which is required for the binding of von Hippel-Lindau (VHL) tumor suppressor protein, the recognition component of an E3 protein ligase that ubiquitinates HIF-1α and targets it for degradation in the proteosome. Under hypoxic conditions, oxygen becomes limiting, the proline residues remain unhydroxylated, VHL does not bind, and HIF-1α accumulates within the cell. There is also an asparagine residue in the transactivation domain that is hydroxylated by a hydroxylase called FIH-1. This hydroxylation prevents the interaction of the transactivation domain with the co-activators.

Under hypoxic conditions, the hydroxylation does not occur and the binding of the co-activators can occur. So both the half-life of HIF-1 and its specific activity as a transcription factor are regulated in an oxygen-dependent manner.

In addition to regulation by oxygen concentration, a whole series of cytokines and growth factors can also increase HIF-1α levels, and rather than affecting degradation, they increase the synthesis of the protein. IGF-1 stimulates VEGF production through HIF-1α. Cultured cells exposed to IGF-1 show a time-dependent increase in HIF-1α. The induction of HIF-1α by IGF-1 can be blocked by inhibitors of signal transduction pathways, including the MAP kinase and phosphatidylinositol 3 (PI3) kinase pathways and the downstream kinase mTOR. The levels of HIF-1α protein correlate with VEGF mRNA levels. The basis for the regulation of the HIF-1α
protein synthesis is through the stimulation of the PI3 kinase and MAP kinase pathways that ultimately regulate the activity eIF-4E, which is a critical regulator of cap-dependent mRNA translation. The signals coming through these pathways stimulate translation of a subset of mRNAs within the cell and included among these is HIF-1α mRNA. This pathway is relevant to diabetic retinopathy, because there is activation in the diabetic retina of the Akt protein kinase, increased levels of HIF-1α and VEGF. Pharmacological blockade of the IGF-1 receptor dramatically reduces the levels of activated Akt, HIF-1α, and VEGF. Thus, there are at least two general stimuli that regulate HIF-1α levels, hypoxia and growth factors.

HIF-1α plays a role in the induction of VEGF in oxygen–induced ischemic retinopathy. There is basal expression of HIF-1α at P7 in normoxia and downregulation when mice are exposed to hyperoxia. When mice are taken out hyperoxia and the retina becomes hypoxic, HIF-1α levels increase and remain elevated for several days, during which VEGF levels increase.

HIF-1α is essential for normal embryonic development. HIF-1α knockouts arrest in development at day 8.5 and die by day 10. There is a dramatic effect on vascularization. There is initial establishment of the vasculature, but then it regresses. In embryonic stem cells from wild type mice exposed to 1% oxygen for 24 hours, there is strong induction of VEGF, Ang1, PIGF, and PDGF-B. The hypoxic induction of these mRNAs is dramatically reduced in HIF-1α-null cells.

In primary cardiac fibroblasts, cardiac myocytes, arterial smooth muscle cells, and arterial endothelial cells, expression of VEGF, Ang1, PIGF, PDGF-B were compared after exposure to 1% or 20% oxygen for 24 hours. Each of these cell types responds in a different manner in terms of the regulation of these genes. VEGF is uniformly upregulated, but the others are not. Thus, the response to hypoxia is cell type-specific.

A mutant form of HIF-1α that is constitutively expressed independent of hypoxia has been engineered by a series of point mutations and deletions that allow it to escape proteosomal degradation under non-hypoxic conditions. An adenoviral vector was used to transfect the above cells with constitutively active HIF-1α or LacZ. The pattern of expression was identical to the pattern of expression induced by hypoxia; the cell type specificity was maintained. The adenoviral vectors were injected into mouse eyes. After intravitreous injection of adenoviral vector expressing constitutively active HIF-1α (AdCA5), there was neovascularization along the surface of the retina and in the anterior chamber of the eye. There was no effect from injection of the adenoviral vector expressing LacZ (AdLacZ). VEGF is necessary, but not sufficient to get neovascular sprouts from superficial capillaries like that seen by expressing the constitutively active HIF-1α. Expression of mRNAs 24, 48, and 72 hours after intravitreous injection of AdCA5 was compared to that in eyes injected with AdLacZ. HIF-1α mRNA levels peaked at 48 hours after injection and there were dramatic increases in PIGF, VEGF, Ang1, Ang2, and PDGF-B mRNAs. The coordinate upregulation of multiple angiogenic factors is likely to account for the development of neovascularization. Activation upstream through HIF-1α may have benefits by turning on multiple factors and perhaps doing so in a cell type-specific manner.

Does HIF-1 have specific effects within endothelial cells? Arterial endothelial cells were cultured at 1% or 20% oxygen and their ability to invade through an experimental basement membrane was measured. Hypoxia increased the invasiveness of the cells. Infection of the cells with AdCA5 also increased the invasiveness of the cells under non-hypoxic conditions. When cells were plated on Matrigel, the formation of tube-like networks was stimulated by hypoxia or infection of the cells with AdCA5. To determine the molecular basis for these observations,
microarray analysis was performed. Arterial endothelial cells were exposed to 20% or 1% oxygen for 24 hours and the pattern of gene expression was assessed in three independent cell isolates. A threshold of a statistically-significant 1.5-fold increase or decrease in gene expression was used. The experiment was repeated under non-hypoxic conditions using cells infected with AdCA5 or AdLacZ. 245 genes were up-regulated and 325 genes were down-regulated in response to both hypoxia and AdCA5. Many of the genes encoded signal transduction molecules, growth factors, collagens, and receptors, but the largest group in which changes occurred were genes encoding transcription factors. Expression of the mRNAs encoding the erythropoietin receptor, the chemokine receptor CXCR4, VEGF-A, and VEGF-C were upregulated within 8 hours of the onset of hypoxia suggesting that they are direct target genes. In fact, it is known from other experiments that CXCR4 and VEGF-A are direct target genes. PTGIS, which encodes an enzyme required for production of prostaglandins, shows bimodal activation, suggesting activation by HIF-1 early followed by the recruitment of additional transcriptional activators at later timepoints. Thus, it appears that hypoxia induces HIF-1, which binds to its primary target genes and induces their expression, some of which encode other transcription factors that contribute to the regulation of gene expression later in the hypoxic response.

HIF-1 plays a critical role in tumor development. Over-expression of HIF-1α in colon carcinoma cells results in increased tumor growth in a xenograft model compared to cells transfected with empty vector. Magnetic resonance imaging showed an increase in vascular volume and vascular permeability in HIF-1α-overexpressing tumors. Both basal and hypoxia-induced secretion of VEGF were reduced in TMK-1 human gastric cancer cells stably transfected with an expression vector encoding a dominant-negative form of HIF-1α. When these cells were injected orthotopically into the gastric wall, there was a reduction in the size of tumors derived from cells expressing dominant-negative HIF-1α and the vessels were very small with almost no lumen and marked reduction in pericytes coverage. This suggests that reduction of HIF-1α in tumor cells prevents endothelial cells from recruiting pericytes. The mechanism for this is unknown. Multiple anti-cancer drugs are known to have anti-angiogenic activity that is due in part to their inhibition of HIF-1 (reviewed in Ref. 13).

Questions

1. If one were targeting HIF for anti-angiogenesis, would it be necessary to target both HIF-1 and HIF-2?

   The role of HIF-2 (which is a dimer of HIF-1 and HIF-2, which is structurally related to HIF-1α but is the product of a distinct gene) is not as well-established. In some cells HIF-2 is present in the cytoplasm and is not active, suggesting that nuclear translocation may occur in response to an unidentified signal. There are many tumors in which both HIF-1 and HIF-2 are over-expressed, but there are also tumors in which just HIF-1 or HIF-2 is high. Some inhibitors have specificity for HIF-1α. It is not clear that growth factors induce HIF-2α.

2. The list of anti-tumor agents that inhibit HIF-1 is extensive. What is the mechanism for inhibition by something like Iressa?

   Activation of signal transduction pathways downstream of receptor tyrosine kinases stimulates HIF-1α production, so blockade of these signaling pathways reduces HIF-1. A consequence of HIF-1 inhibition by these agents is that the tumors become hypoxic, resulting in...
the induction of HIF-1α through inhibition of the PHD-VHL system. This suggests that these signal transduction inhibitors would not be as efficacious when used alone as they would be if used with a HIF-1-specific inhibitor should such a small molecule inhibitor become available.

3. What are the downstream effects of HIF-1?

Selective knockout of HIF-1α in endothelial cells has a negative effect on the vascularization of tumors. The effect appears to involve loss of autocrine VEGF signaling through VEGF receptors within endothelial cells. The anti-angiogenic agent angiostatin also inhibits the expression of HIF-1 target genes in endothelial cells.

4. There are hamartomatous tumors such as in tuberous sclerosis and Peutz-Jeger syndrome that occur because of mutations in tumor suppressor genes that feed into mTor. Is anything known about HIF-1 in these tumors?

Dysregulation of HIF-1 and VEGF is a unifying feature of hamartomatous syndromes (see Brugarolas 2003, 2004).

5. There are multiple oxygen binding heme proteins that are quite good at detecting oxygen levels and changing conformation. Is HIF-1 the only story for oxygen sensing in cells?

No, it is unlikely that it’s the only story, but so far this is the mechanism for sensing oxygen that is best understood.

References

1. Hirota K, Semenza GL. Regulation of hypoxia-inducible factor 1 by prolyl and asparaginyl hydroxylases. Biochem Biophys Res Commun. 2005;Sep 8; [Epub ahead of print].


