HIF-1 and mechanisms of hypoxia sensing
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Hypoxia-inducible factor 1 (HIF-1) is an oxygen-regulated transcriptional activator that plays essential roles in mammalian development, physiology and disease pathogenesis. The HIF-1α subunit is subjected to oxygen-dependent ubiquitination and proteasomal degradation that is mediated by the von Hippel-Lindau protein. Interaction of HIF-1α transactivation domains with coactivators is induced by hypoxia. The signal transduction pathway remains enigmatic, but involves generation of reactive oxygen species. Nitric oxide induces HIF-1α under non-hypoxic conditions but inhibits hypoxia-induced HIF-1α expression.

Introduction

Although maintenance of oxygen homeostasis is an essential cellular and systemic function, it is only within the past several years that the molecular mechanisms underlying this fundamental aspect of cell biology have begun to be elucidated and their connections to development, physiology and pathophysiology have been established. Most basic cellular processes are modulated by the cellular oxygen concentration and a recent study suggests that important aspects of cell biology may be misinterpreted by performing studies under a non-physiologic (ambient) oxygen concentration [1••].

This review will focus on HIF-1 (hypoxia-inducible factor 1), the transcriptional activator that functions as a master regulator of oxygen homeostasis. Recent advances in delineating upstream signal transduction pathways leading to the induction of HIF-1 activity and expression of downstream target genes that mediate its physiologic effects will be summarized. Among the recent major advances in this field are the discoveries of the role of reactive oxygen species (ROS) in hypoxia signal transduction; the role of ubiquitination in regulating HIF-1 activity; the regulation of HIF-1 activity by signals other than hypoxia, including nitric oxide (NO), cytokines and growth factors; and the involvement of HIF-1 in embryonic development, physiologic responses to hypoxia and the pathophysiology of common causes of mortality in the developed world, including heart attack, stroke, cancer and chronic lung disease. Here, the molecular mechanisms involved in the oxygen-dependent regulation of HIF-1 induction will be discussed. The physiologic and pathological consequences of HIF-1 induction have been reviewed recently [2].

HIF-1 structure, function and regulation

HIF-1 is a heterodimer composed of HIF-1α and HIF-1β subunits ([3]; Figure 1). Whereas HIF-1β is constitutively expressed, HIF-1α expression is induced in hypoxic cells with an exponential increase in expression as cells are exposed to O2 concentrations of less than 6% [4], which corresponds to a partial pressure (P) of O2 of approximately 40 mm Hg at sea level. Several dozen HIF-1α-regulated target genes have been identified that play essential roles in cellular and systemic physiologic responses to hypoxia, including glycolysis, erythropoiesis, angiogenesis and vascular remodeling [2].

The amino-terminal half of HIF-1α (amino acids 1–390) is necessary and sufficient for dimerization with HIF-1β and for DNA binding ([5]; Figure 1). HIF-1α is ubiquitinated and subjected to proteasomal degradation in non-hypoxic cells [6–8]. Under hypoxic conditions, the fraction of HIF-1α that is ubiquitinated decreases dramatically, resulting in an accumulation of the protein [9•]. A Pro–Ser–Thr rich protein stabilization domain is located between amino acids 429 and 608 of HIF-1α ([7,9•]; Figure 1).

The von Hippel-Lindau (VHL) protein binds to the protein stabilization domain and plays a critical role in the ubiquitination of HIF-1α [10••,11••,12•,13•,14••]. Exposure of cells to cobalt chloride or iron chelators (e.g. desferrioxamine) induces HIF-1α expression [3,15] and inhibits HIF-1α ubiquitination [8] by dissociating VHL from HIF-1α [10••]. However, hypoxia does not cause VHL to dissociate from HIF-1α [10••] and thus the mechanism underlying the decreased ubiquitination under hypoxic conditions [9•] remains undefined.

VHL is unlikely to be the only ubiquitin protein ligase that regulates the half life of HIF-1α. Recent data indicate that p53 interacts with HIF-1α [16] and by doing so recruits the MDM2 ubiquitin protein ligase, which reduces the induction of HIF-1α expression under hypoxic conditions [17•]. In tumor cells, loss of VHL or p53 activity results in increased
HIF-1α expression and increased transcription of downstream target genes such as VEGF [10**,17**]. Activation of the signal transduction pathway involving phosphoinositot 3-kinase (PI3K) and the serine/threonine kinases protein kinase B (Akt) and FKB-B (p70S6K) results in increased expression of HIF-1α protein and vascular endothelial growth factor (VEGF) mRNA under non-hypoxic conditions [18]. PTEN is a tumor suppressor with phosphoinositot phosphatase activity. PTEN negatively regulates the PI3K pathway and, therefore, loss of PTEN activity also leads to increased HIF-1α expression (see Wishart et al. 2003). The redox status of a cysteine residue in the carboxy-terminal half of HIF-1α (amino acids 531–575 and 786–826) interact with coactivators such as CBP, p300, SRC-1 and TIF-2 (Figure 1) to activate transcription [15,20]. Although the amino-terminal TAD of HIF-1α (amino acids 531–575, [15,20]) overlaps with the minimal VHL-binding domain (amino acids 549–582, [12**]; Figure 1), it does not appear that VHL regulates TAD function, as the transcriptional activity of a mutant TAD-N that cannot bind VHL is still hypoxia-induced [14**]. Recent studies suggest that phosphorylation of HIF-1α by mitogen-activated protein (MAP) kinases increases its transcriptional activity independently of the effects of hypoxia [24**,25**], but the site(s) of phosphorylation and the mechanism by which transcriptional activity is enhanced have not been established.

**Hypoxia signal transduction**

The molecular mechanisms by which mammalian cells sense hypoxia and transduce this signal to HIF-1α have remained enigmatic [26]. Recent studies have provided experimental evidence in support of the hypothesis that mitochondrial generation of superoxide and, subsequently, hydrogen peroxide are required for induction of HIF-1 activity and transcription of downstream target genes in hypoxic cells [27,28**,29,30**,31*]. This model thus proposes that ROS generation increases under hypoxic conditions, whereas an alternative model proposes the opposite, that hypoxia results in decreased production of ROS by nicotinamide adenine dinucleotide phosphate (NADPH) oxidases [32,33]. The proponents of both models have obtained experimental support using various redox-sensitive dyes and pharmacologic inhibitors. The utilization of more sophisticated methods for the characterization of reactive oxygen and nitrogen species, such as electron paramagnetic resonance spectroscopy, might help resolve this issue.

It is likely that HIF-1α is a direct target of redox regulation. The redox status of a cysteine residue in the carboxy-terminal TAD (TAD-C) has been shown to affect its interaction with CBP/p300 coactivators, and this interaction is positively regulated by redox factor 1 (REF-1) and thiorodoxin (Figure 1; [22**]). The interaction of the coactivator SRC-1 with HIF-1α is also redox regulated [25**]. HIF-1α ubiquitination and degradation might also be regulated by redox modifications of the protein. Iron regulatory protein 2 (IRP-2) is targeted for ubiquitination and proteasomal degradation by oxidative modifications that occur in the presence of iron and oxygen [34]. A similar process might regulate HIF-1α protein stability except that rather than being modulated by iron levels, as is the case for IRP-2, it is modulated by the cellular oxygen concentration.

The regulation of HIF-1 activity by carbon monoxide (CO) and NO is also likely to be of physiologic relevance and may provide insight into the mechanisms of signal transduction leading to HIF-1 activity, but the connections at this point are not clear. The intriguing observation is that whereas CO or NO inhibits hypoxia-induced HIF-1α expression, HIF-1 DNA-binding activity and HIF-1 transcriptional activity [35–37], exposure of cells to NO under...
non-hypoxic conditions paradoxically induces HIF-1α expression, HIF-1 DNA-binding activity and downstream gene expression [38**,39**]. One possible explanation is that reactive oxygen and nitrogen species might each be capable of (directly or indirectly) modulating HIF-1α expression, but when present together react to form compounds such as peroxynitrite that lack this property. In support of this hypothesis, superoxide has been shown to inhibit the activation of guanylate cyclase by NO in vascular smooth muscle cells [40]. One recent study, however, suggested that NO increased ROS production [41]. In addition, NO inhibits the activity of mitochondrial electron transport complex I, and pharmacologic inhibitors of complex I, such as rotenone and diphenylene iodonium, block hypoxia-induced HIF-1α expression [27,28**,29,30**].

To further complicate matters, expression of the genes encoding inducible nitric oxide synthase and heme oxygenase-1, which generate NO and CO, respectively, are induced by hypoxia in a HIF-1-dependent manner in some cell types [42–45]. NO has also been implicated in developmental and physiologic responses to hypoxia in Drosophila melanogaster [46], thus providing a further impetus to link these pathways in mammalian cells. Coupling of hypoxia and NO signaling has recently been demonstrated for the skeletal muscle calcium release channel/ryanodine receptor RyR1 [1**].

Although some investigators in the field continue to search for the holy grail in the form of a monolithic oxygen sensor responsible for initiating a linear hypoxia signal-transduction pathway leading to HIF-1α, it is possible that multiple cellular processes, including, but not limited to, the mitochondrial electron transport chain and various NADPH oxidoreductases, might affect the concentration of specific reactive oxygen species capable of modulating HIF-1α expression and/or activity. In this model, HIF-1α would function to integrate multiple metabolic signals at the level of gene transcription. In addition, hypoxia signal-transduction pathways may be cell-type specific. For example, analyses of knockout mice lacking expression of the gp91phox subunit of NADPH oxidase have revealed that the electrophysiologic responses to hypoxia are intact in the carotid body (arterial chemoreceptors) and pulmonary artery smooth muscle cells, whereas pulmonary neuroepithelial bodies (airway chemoreceptors) from these mice do not respond to hypoxia [47–49].

Conclusions

HIF-1 is a master regulator of oxygen homeostasis that plays critical roles in a multitude of developmental and physiologic processes. Several dozen HIF-1 target genes have been identified to date that participate in responses to hypoxia [2], but these genes are unlikely to represent more than 10% of the total number that will eventually be identified by gene-expression profiling using DNA microarrays. Both the expression of HIF-1α and its transcripational activity are modulated by the cellular O2 concentration and, in both cases, there is evidence that the oxygen signal is converted to a redox signal, although the details remain far from clear. A variety of mechanisms have been proposed [26] involving either increased or decreased ROS generation and either direct ROS effects on HIF-1α or a ROS-initiated signal transduction pathway leading to altered phosphorylation of HIF-1α. Cytokines and growth factors may also induce HIF-1α expression via a redox signal and/or kinase cascades. As in other systems [1**], a complex interplay between reactive oxygen and nitrogen species appears to regulate HIF-1 activity, and the mechanisms by which these molecules are generated may vary from one cell type to another. Oxygen homeostasis is of fundamental importance to the cell and complex relationships exist between O2 concentration, energy metabolism, acid–base status, redox state and the control of cell growth and proliferation. The regulation of HIF-1 may therefore best be viewed as a web—a structure that is poorly defined by reductionist (i.e. linear) experimental approaches.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

** of outstanding interest


This study demonstrates that the fraction of HIF-1α that is ubiquitinated (and thus targeted for proteasomal degradation) is markedly reduced in cells under hypoxic (4 h at 1% O2) as compared with non-hypoxic (20% O2) culture conditions.


The authors develop an in vitro assay to demonstrate ubiquitination of HIF-1α and HIF-2α that is dependent upon the presence of wild-type VHL protein. Tumor-associated missense mutations within the amino-terminal β-domain of VHL (Arg227Pro, Pro668His, Asn901Le, Gin969Pro) reduce the ability of HIF-1α or HIF-2α in vivo to interact with E1 ubiquitin-activating and E2 ubiquitin-conjugating enzymes to mediate HIF-1α and HIF-2α ubiquitination in vitro, and to regulate HIF-1α and HIF-2α protein expression or GLUT1 mRNA expression in tumor cells. The latter mutations also prevent the binding of HIF-1α to elongins B and C, which are required for ubiquitin ligase activity. Two other mutations (Tyr98Asn and Tyr112His), which only reduce the ability of VHL to interact with HIF-1α and HIF-2α, result in a less severe dysregulation of HIF-1α and HIF-2α protein and GLUT1 mRNA expression. VHL binds to amino acids 549–582 of HIF-1α, which fall with the previously defined Pro–Ser–Thr rich protein stabilization or oxygen-dependent degradation domain. VHL specifically mediates ubiquitination of HIF-1α and HIF-2α. IRP-2 is not a substrate for the VHL ubiquitin ligase complex despite the fact, that, like HIF-1α, it is targeted for ubiquitination by an oxidative process that requires both oxygen and iron (34).


These authors demonstrate that interaction with the β-domain of VHL is required for ubiquitination of HIF-1α.


The authors show that forced expression of HIF-1α leads to its accumulation in non-hypoxic cells as previously reported [5]. When VHL is coexpressed, HIF-1α no longer accumulates in non-hypoxic cells, indicating that VHL had become a limiting factor for HIF-1α degradation. HIF-1α amino acids 532–585 (encompassing TAD-N [15,20]) interact with VHL in cultured cells, whereas mutation of residues 564–566 interferes with both ubiquitination and VHL binding. Ubiquitination occurs on lysine residues, and the mutation Lys532Arg increases TAD-N expression under non-hypoxic conditions, but it remains to be determined whether Lys532 is a site of ubiquitination. The authors also present evidence suggesting that nuclear localization of HIF-1α is necessary but not sufficient for hypoxia-induced protein stabilization.


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Loss of p53 expression in otherwise isogenic mouse embryo fibroblasts, human colon and ovarian cancer cells is associated with increased expression of HIF-1α protein, HIF-1 DNA-binding and transcriptional activity, and VEGF mRNA in hypoxic cells. The MDM2 ubiquitin protein ligase is recruited to HIF-1α by p53. In this trimolecular complex, MDM2 appears to preferentially target HIF-1α for degradation, thus accounting for decreased p53 levels in HIF-1α-null cells [16] and increased HIF-1α levels in p53-null cells.


Disregulated signal transduction from receptor tyrosine kinases to PI3K, AKT and its effector kinase FRAP occurs via autocrine stimulation or metastasis of the tumor suppressor protein PTEN in many cancers. In human prostate cancer cells, basal, growth-factor-induced and mitogen-induced expression of HIF-1α and VEGF is blocked by inhibitors of PI3K or FRAP by overexpression of wild-type PTEN or by expression of dominant negative PI3K mutants, thus identifying a pathway leading to HIF-1α expression that is independent of the cellular O2 concentration.


Forced PTEN overexpression in human gliaoma cell lines inhibits hypoxia-induced HIF-1α expression.


22. Ema M, Hirota K, Mimura J, Abe T, Yodoji S, Sogawa K, Poellinger L: TAD-N and VHL binding. Ubiquitination occurs on lysine residues, and the mutation Lys532Arg increases TAD-N expression under non-hypoxic conditions, but it remains to be determined whether Lys532 is a site of ubiquitination. The authors also present evidence suggesting that nuclear localization of HIF-1α is necessary but not sufficient for hypoxia-induced protein stabilization.


The authors demonstrate that two other coactivators SRC-1 and TIF2 also stimulate HIF-1α-mediated transcription. SRC-1 functions synergistically with CBP to stimulate transcription mediated by TAD-N or TAD-C. They also show direct interaction between REF-1 and TAD-C function and its interaction with CBP are lost when the missense mutation Cys800Ser is incorporated into TAD-C. These results suggest that reduction of Cys800 is critical for the interaction of TAD-C with CBP that leads to increased transcription of HIF-1 target genes in hypoxic cells.


The authors provide data suggesting that the multiple isoforms of HIF-1α detected by immunoblot assay [3] represent varying degrees of phosphorylation by p42ERK and p44ERK MAP kinases. They demonstrate that overexpression of a constitutively active form of the upstream MAP kinase kinase kinase RAF results in increased HIF-1-dependent transcription but has no effect on HIF-1 protein levels, suggesting an effect on TAD function. The separation of phosphorylation remains to be determined, as mutating the only two sites in HIF-1α that matched the consensus for phosphorylation by MAP kinases (ProSerPro) did not appear to prevent phosphorylation.


The Kaposi sarcoma-associated herpes virus HHV8 encodes a constitutively active G-protein-coupled receptor that stimulates angiogenesis via increased VEGF expression. The authors demonstrate that increased HIF-1α-mediated
VEGF gene transcription occurs as a result of p38 and ERK MAP kinase activity and that p38α, p38δ and ERK MAP kinases phosphorylate a GST fusion protein containing HIF-1α amino acids 568–702 in vitro, whereas Richard et al. [24••] found no evidence of HIF-1α phosphorylation by an unspecified p38 MAP kinase isoform. The sites of phosphorylation by p38 and ERK and their effects on HIF-1α transcriptional activity remain to be determined.


The authors provide pharmacologic evidence in support of their hypothesis that hypoxia results in the generation of superoxide at mitochondrial electron transport complex (ETC) III (the O2 sensor) and that superoxide passes into the cytosol via anion channels and is converted to hydrogen peroxide, which induces PI3K and protein phosphatase activity leading to HIF-1α stabilization.


The authors demonstrate that, similar to previous observations in transformed cells [27], inhibitors of electron transport complex I block HIF-1α expression in mice subjected to hypoxia. HIF-1α expression is also reduced in human-ape xenomitochondrial cybrids with a 40% reduction of ETC I activity and is restored when the ETC II substrate succinate is provided.


The authors report that exposure of fetal alveolar type II epithelial cells to the antioxidant N-acetyl cysteine (NAC) or pyrrolidine dithiocarbamate (PDTC) prevents the decay of HIF-1α levels observed upon reoxygenation [3]. These results are in contrast to those of Chandel et al. [27] who reported that NAC or PDTC block the induction of HIF-1α expression in hypoxic Hep3B cells.


The authors demonstrate that VEGF transcription is induced by NO in a cGMP-independent manner that is dependent upon the presence of an intact HIF-1α binding site in the promoter and is associated with induction of HIF-1α protein expression.


The authors show that exposure of cells to the NO donor NOC-18 induces HIF-1α protein expression. This effect of NOC-18 could be blocked by dithiothreitol but not by oxyhemoglobin, arguing against direct involvement of NO. HIF-1α expression was induced by the NO donor N-sitosylgluthione (GSNO) but not by the NO donor Angeli’s salt. The effect of NOC-18 or GSNO may be mediated via S-nitrosylation or oxidation of protein thiols.


