Oxygen sensing

Oxygen is an essential requirement for mammalian life. Inadequate supply and excessive oxygenation are both associated with toxicity. Reduction in oxygen availability results in the initiation of a series of adaptive changes at the levels of the organism, tissue, cell and individual genes. If the change in oxygen supply exceeds the ability of these physiological responses to compensate, physiological maladaptation and, ultimately, disease result. Examples include pulmonary hypertension, new vessel formation, mountain sickness and some forms of polycythemia. Conversely, in certain disease states these physiological mechanisms are subverted, an example being their activation in tumors.[1] Understanding the regulatory pathways, and in particular the underlying oxygen sensing mechanisms, has long been a goal of biologists and medics. Current insights are briefly summarized in this editorial, providing a framework for understanding the studies on chronic mountain sickness and congenital polycythemia reported in this issue.

The hypoxia-inducible factor (HIF) pathway

Adaptive responses at the level of the whole organism are difficult to dissect but those encapsulated in cultured cell lines have allowed substantial progress to be made in the last fifteen years. The understanding derived from this work is now beginning to illuminate oxygen sensing processes at the level of the organism as a whole.

One important focus for those interested in oxygen sensing has been the graded regulation of the erythropoietin gene in response to tissue oxygenation (reviewed by Jelkmann).[2] This was long thought to be a private regulatory mechanism, specifically arising from the complexities of renal physiology or a property unique to the erythropoietin producing cells in the kidney. However, the recognition that the underlying regulatory processes could be captured in human hepatoma cell lines that express erythropoietin in an oxygen regulated manner[3] led to the definition of a cis acting hypoxia response element at the 3' end of the gene[4–6] and subsequent affinity purification and cloning of the cognate transcription factor, hypoxia-inducible factor-1 (HIF).[7]

The HIF transcriptional system is a master regulator of the hypoxic response controlling large numbers of genes in all cell types, not just erythropoietin in specialized renal and hepatic cells. The first suggestion that this was the case came from the important finding that hypoxia response elements derived from the erythropoietin gene could operate in a wide variety of cell lines, indicating a widespread capability for sensing and signal transduction.[8] That most of these cells were derived from lineages that did not produce erythropoietin implied that other genes were regulated by hypoxia via this system.[8] Subsequent analysis of the patterns of HIF expression confirmed that the HIF system was indeed widespread[9] and confirmation of its wider action came from the identification of other HIF-responsive oxygen-regulated genes, the first being phosphoglycerate kinase-1 and lactate dehydrogenase A.[10] The list of HIF-regulated genes continues to grow and now encompasses genes involved in diverse processes including cell proliferation, cell survival, metabolic control, metal homeostasis, vascular growth and vascular tone.[11,12] HIF is a heterodimeric transcription factor[7] that, in its active form, recruits the transcriptional co-activator p300/CBP to hypoxia response elements.[13] Three loci have been identified encoding different α chains, a further three loci encode beta chains, and several splice variants of each have now been reported.[7,14–19] The β chains are constitutively expressed in the nucleus, and are also involved in the transcriptional response to xenobiotics, where they were first described as aryl hydrocarbon receptor nuclear translocators.[20] The α chains have not been shown to contribute to other transcriptional pathways, and are dominantly responsible for the hypoxic regulation of the complex as a whole. How the different α and β forms interact to precisely regulate downstream processes is incompletely understood and discussion is beyond the scope of this editorial.

Regulation of HIF activity by hypoxia

Like other transcription factors, HIF α chains have a domain structure, with different parts of the molecule serving different functions. The amino terminal part of the molecule is involved in DNA binding and dimerization whilst the carboxy terminal portion confers the major regulatory functions. Early studies showed that two distinct mechanisms were operative within the regulatory portion, one domain, at the carboxyl terminus, influenced transcriptional activity without affecting HIF-α protein levels, whereas the other region affected transcriptional activity by influencing protein abundance. This domain, termed the oxygen-dependent degradation domain (ODD) was subsequently divided into amino terminal and carboxy terminal sub-domains (NODD and CODD).[21–23] It was rapidly shown that the ubiquitin proteasome pathway was responsible for the degradation of HIF α chains in the presence of oxygen.[24] An important breakthrough was the demonstration that the von Hippel-Lindau protein (pVHL) was the recognition component of the relevant ubiquitin E3 ligase complex,[25] providing a molecular mechanism for many aspects of the phenotype of von Hippel-Lindau tumors. In normoxia the E3 ligase binds HIF α chains via one of two critical prolyl residues, located in the NODD and CODD, respectively. Recruitment of the VHL ligase leads to α chain ubiquitylation, which results in the subsequent degradation of the protein by the proteasome. In hypoxia binding is reduced and the HIF α chains become stable. The mechanism by which this E3 ligase recognizes HIF α chains in the presence of oxygen, but not in its absence, has now been elucidated. In normoxia the prolyl residues

are enzymatically hydroxylated,[26,27] allowing two new hydrogen bonds to form between each HIF α hydroxyprolyl residue and the beta domain of a pVHL molecule.[28] In humans three related HIF prolyl hydroxylases, named PHD1-3, have been identified.[29] All three enzymes are members of a family of dioxygenases that use 2-oxoglutarate as a co-substrate and co-ordinate iron at their active site within a beta barrel jelly role motif.[30] The precise mechanism is conserved in lower organisms, Egl-9 and Fatiga (CG1114) having been identified as the relevant enzymes in *C. elegans* and *D. melanogaster*, respectively.[29,31]

A complementary mechanism regulates co-activator recruitment and thus HIF transcriptional activity. In the presence of oxygen, Factor Inhibiting HIF (FIH), another enzyme of the iron and 2-oxoglutarate dependent dioxygenase class, hydroxylates the β carbon of an asparaginyl residue in the carboxy terminal region of HIF α chains, blocking recruitment of p300/CBP co-activator molecules.[32-36] When oxygen supply is limiting this hydroxylation event is suppressed and trans-activation can occur.

The scheme outlined in Figure 1 represents a minimal structure for oxygen-dependent regulation of the HIF complex via the hydroxylases, but disguises many tiers of biological complexity. HIF molecules are subject to a number of other post-translational modifications, including phosphorylation[37] and acetylation,[38] but the precise functional consequences of these changes require further elucidation. Further mechanisms affecting HIF activity include the expression of HIF-1 α anti-sense mRNA,[39] expression of dominant negative forms, the role of HIF-3 α/IPAS (which lacks a carboxy terminal transactivation domain)[15,16,40] and the HIF-induced expression of CITED2, a molecule which competes for p300 binding.[41]

The HIF hydroxylases differ in their intracellular location, tissue distribution and precise function.[42,43] The abundance of PHD 1 is influenced by sex hormone levels,[44] whereas PHD 2 and 3 levels are influenced by hypoxia, generating a negative feedback loop.[43] PHD 1 and 3 have themselves recently been reported to be targeted for proteasomal destruction by ubiquitin ligases of the Siah family.[45] HIF hydroxylase activities are also subject to other influences, including the availability of iron, ascorbate (probably by its effect on free iron)[46] and oxoglutarate. Their ability to convert oxoglutarate to succinate parallels the Krebs' cycle, suggesting further levels of complexity, and probably of relevance to the phenotypes resulting from mutations in succinate dehydrogenase and fumarase.[47,48] Redox status and nitric oxide availability also influence the system, perhaps acting at more than one level in the pathway, with different outcomes depending on the precise ambient oxygen availability.[49-58]

Important questions that remain include whether the HIF hydroxylases have roles outside the HIF pathway, whether other oxoglutarate-dependent dioxygenases contribute similar oxygen-regulated effects on other substrates and to what extent these processes explain other oxygen regulated phenomena, including transcriptional pathways,[59] kinase/phosphatase signal pathways, chromatin architecture, apoptosis, mRNA processing, protein synthesis,[60] ion pump and channel activity.[61] It has been suggested that a similar dioxygenase is involved in iron sensing and regulation.[62] It is possible that constitutive hydroxylation by these, or other dioxygenases, may simply account for the well recognized instability under ambient conditions of the large number of proteins known to contain PEST domains.[63]

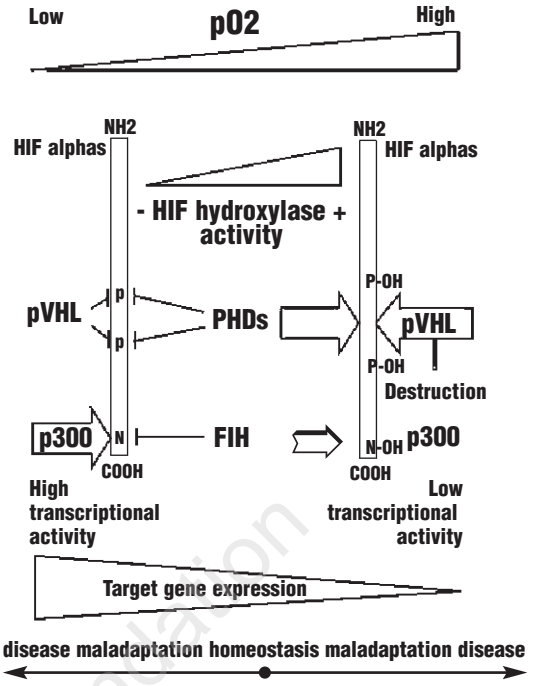

Figure 1. Schematic representation of the HIF/hydroxylase/pVHL oxygen-sensing pathway. When oxygen levels are high the HIF hydroxylases (PHD 1-3 and FIH) are active leading to HIF inactivation as a result of pVHL-dependent proteasomal destruction and blockade of p300 transcriptional co-activator recruitment. As oxygen levels fall so does hydroxylase activity, with a consequent increase in HIF activity and transcriptional read-out. In health the process is poised for physiological homeostasis, partly as a result of feedback modulation of hydroxylase levels. Disease can cause, or be caused by, maladaptation of this system. Mutations in any of the components listed could alter the balance of the pathway and shift the transcriptional read-out. Currently, this is best exemplified by mutations of pVHL.

A further issue of great interest is whether this knowledge opens a route to therapeutic manipulation of the system. Early reports suggest that HIF hydroxylase inhibitors can produce enhanced angiogenesis[64,65] and perhaps enhance erythropoiesis. Clearly if a number of similar dioxygenases regulate diverse processes there will be a need for great care in finding inhibitors with suitable specificity profiles.

Genetic defects and oxygen sensing pathways

Despite the importance of oxygen transport some mutations in key components are tolerated. Examples include hemoglobinopathies with altered oxygen affinity, such as Hb Chesapeake,[66] and mutations of the erythropoietin receptor, which may even provide selective advantage in extreme circumstances, exemplified by success in endurance sports.[67]

von Hippel-Lindau (VHL) disease is a dominantly inherited cancer predisposition, typified by hemangioblastomas affecting the central nervous system, renal clear cell cancers and/or pheochromocytomas.[68] It is

caused by a wide variety of inactivating mutations of one *VHL* allele, with tumors arising following somatic inactivation of the second allele in vulnerable tissues. The genotype/phenotype correlation has been the subject of intense interest[69,70] as has the extent to which effects of pVHL mutations on the HIF pathway might, or might not, explain all facets of the disease.[71] Mutations found in renal cancers are associated with major stabilization of HIF α chains,[72] although the precise balance between the different HIF α isoforms is often distorted in favor of relative over-expression of HIF-2 α compared with HIF-1 α. In patients with the mildest type of VHL-related cancer (type 2C disease) pheochromocytomas arise without renal clear cell tumors. The effects on the HIF α chains are much more modest, with pVHL molecules from affected individuals retaining some ability to ubiquitinylate HIF α chains in normoxia, although some HIF-responsive genes continue to be over-expressed.[73] Another example of disease arising as a result of mutations in pVHL is the polycythemia endemic in the Chuvash population, which is associated with venous abnormalities and a tendency to arterial thrombosis but apparently not tumors. In this condition the inheritance is recessive and homozygous mutation of C598T in the *VHL* gene leads to the presence of a tryptophan instead of an arginine residue in pVHL, and a consequent partial activation of the HIF pathway.[74,75] This issue of the journal reports two studies on congenital polycythemia due to mutations in the VHL gene indicating that the C598T mutation is not the only one responsible for activation of the HIF pathway.[76,77] In addition, the whole topic of congenital erythrocytoses is critically reviewed by Gordeuk and co-workers.[78]

The delineation of other components of the HIF pathway, including the different HIF chains and the oxygen-sensitive dioxygenase enzymes themselves, provides a new list of candidate genes for mutations that would be expected to alter the poise of the entire oxygen sensing system, the consequences of such changes in oxygen sensing might include aberrations of hematocrit or altered responses to altitude.[79]

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