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# Oxygen sensors as therapeutic targets in kidney disease

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ABSTRACT

Hypoxia is a common clinical problem that has profound effects on renal homeostasis. Prolyl-4-hydroxylases PHD1, 2 and 3 function as oxygen sensors and control the activity of hypoxia-inducible factor (HIF), an oxygen-sensitive transcription factor that regulates a multitude of hypoxia responses, which help cells and tissues to adapt to low oxygen environments. This review provides an overview of the molecular mechanisms that govern these hypoxia responses and discusses clinical experience with compounds that inhibit prolyl-4-hydroxylases to harness HIF responses for therapy in nephrology.


## 1. Introduction

Oxygen was first discovered at the end of the 18th century through the work of Carl Scheele, Joseph Priestley and Antoine Lavoisier. Reduced availability of oxygen, also known as hypoxia, is a common clinical problem and can result from multiple pathologic conditions including cardiovascular, respiratory and bone marrow diseases. The kidney is particularly susceptible to hypoxia-induced tissue damage due to its unique anatomical and functional characteristics.

When tissues or cells experience hypoxia, enzymes that utilize oxygen for catalysis initiate specific transcriptional and other molecular responses. These responses protect cell and tissue viability through the reprogramming of cellular metabolism, nutrient utilization and mitochondrial function, as well as changes in cellular differentiation, motility and replication. The recent identification of oxygen- and iron-dependent dioxygenases as the regulators of transcriptional, epigenetic and metabolic adaptation to hypoxia has led to the development of novel therapeutic agents that are currently in clinical trials for the treatment of anemia associated with chronic kidney disease.

A central mediator of the transcriptional hypoxia response in all tissues and cell types is the oxygen-sensitive basic helix-loop-helix transcription factor hypoxia-inducible factor (HIF). Although several transcription factors are involved in the control of oxidative stress responses, the HIF pathway is the most extensively studied hypoxia response pathway to date, and has received tremendous clinical attention due to its great potential for therapeutic exploitation in multiple clinical areas including renal medicine. Here, I provide a focused overview on oxygen metabolism and hypoxia responses in the kidney and review recent therapeutic efforts that aim at exploiting this pathway for the treatment of kidney diseases and its associated conditions.

## 2. Discovery of the HIF oxygen-sensing pathway

The driving force that ultimately led to the discovery of the HIF oxygen-sensing pathway was the quest for the identification of factors that regulated the hypoxic induction of erythropoiesis, the increase in red blood cell mass that typically occurs with ascent to high altitude. Although the association of high altitude and increased red blood cell numbers had already been noted by Paul Bert and Denis Jourdanet in the second half of the 19th century [1–3], it took about one century before erythropoietin was purified in 1977 and the *EPO* gene cloned in 1985 [4–6]. A few years later Gregg L. Semenza identified and purified HIF-1 as the transcription factor that was responsible for the hypoxic induction of erythropoietin in hepatoma cells [7–9]. Around the same time, it became clear that HIF was not only critical for the hypoxic induction of erythropoiesis, but also regulated multiple other oxygen-sensitive genes, such as *vascular endothelial growth factor* (*VEGF*) and *phosphoglycerol kinase* (*PGK*)-1, placing it at the center of a general and widespread transcriptional response to hypoxia [10,11]. The nature of the oxygen sensor that controls HIF activity, however, remained elusive for almost another decade.

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Ground breaking insights into the regulation of HIF activity came from the study of von Hippel-Lindau disease, a rare familial autosomal dominant tumor syndrome. von Hippel-Lindau-associated renal cancer cells are characterized by the inability to degrade HIF, and the molecular characterization of these cells established that the von Hippel-Lindau tumor suppressor played a central role in HIF regulation [12,13]. The laboratories of Sir Peter J. Ratcliffe and William G. Kaelin were the first to establish that hydroxylation of specific proline residues was required for HIF inactivation under normoxia [14,15]. This was followed by the identification of egg laying nine 1 protein (EGLN1) as the prolyl-hydroxylase that targets HIF for degradation by Ratcliffe's group [16]. Due to their seminal contributions to understanding the molecular basis of cellular oxygen sensing in mammals Semenza, Ratcliffe and Kaelin have been recognized with the prestigious Albert Lasker award in 2016.

## 3. HIF transcription factors

HIFs are basic helix-loop-helix transcription factors and members of the PER/aryl hydrocarbon receptor nuclear translocator/single-minded (PAS) family of transcription factors. They consist of an oxygen-sensitive α-subunit and a constitutively expressed β-subunit, which is often referred to as the aryl hydrocarbon receptor nuclear translocator [17–19]. When cells experience hypoxia HIF-α translocates to the nucleus where it heterodimerizes with HIF-β. Gene transcription is activated when the HIF heterodimer binds to specific DNA recognition sequences, so called hypoxia-response elements located in the regulatory regions of oxygen-sensitive genes (Fig. 1).

Three HIF α-subunits are known, HIF-1α, HIF-2α (also known as EPAS1) and HIF-3α, HIF-1α and HIF-2α being the most extensively and best characterized α-subunits. HIF-1 and HIF-2 together facilitate oxygen delivery and cellular adaptation to hypoxia by regulating a wide spectrum of biological hypoxia responses that include angiogenesis, anaerobic glucose metabolism, mitochondrial biogenesis and others [20]. Although HIF-1 and HIF-2 share many transcriptional targets, certain genes do not appear to be co-regulated. Anaerobic glycolysis, for example appears to be controlled by HIF-1 [21], whereas the hypoxic induction of erythropoietin and iron uptake have emerged as HIF-2-regulated responses [22–27]. HIF-α furthermore modulates cellular functions through direct interaction with various signaling molecules and pathways, which include tumor suppressor protein p53, the c-MYC proto-oncogene and the Notch pathway [28–31].

## 4. Prolyl-4-hydroxylation regulates cellular hypoxia responses

Although HIF-α subunits are continuously synthesized, they are immediately degraded in the presence of oxygen; HIF-α protein is usually not detectable in normoxic cells. To dispose of newly synthesized HIF-α, the cell uses oxygen to modify HIF-α

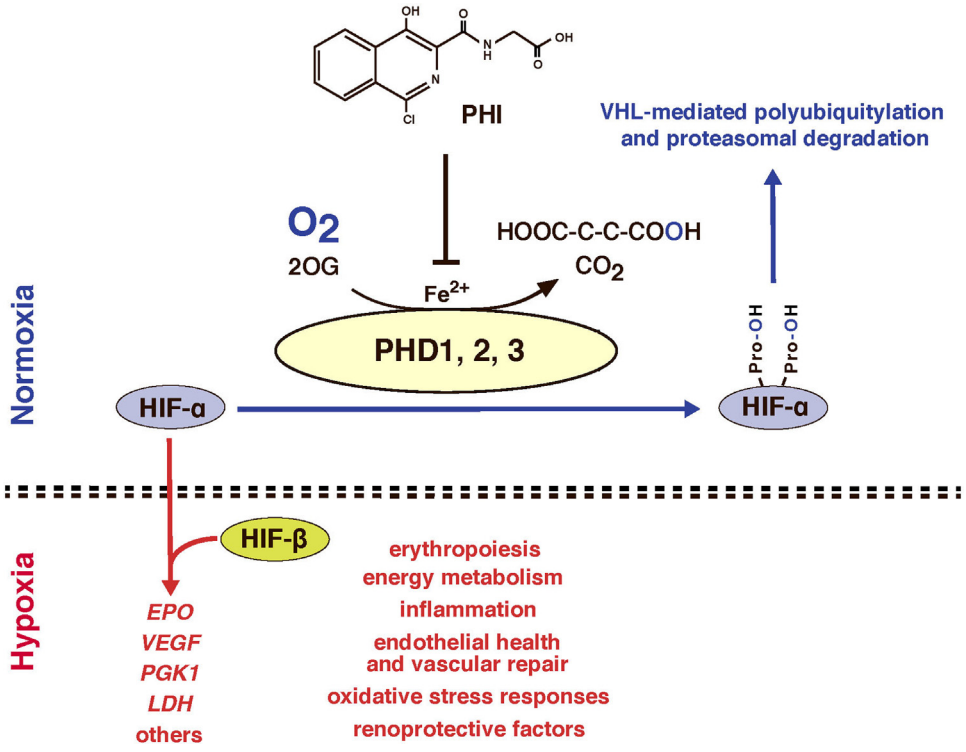


**Fig. 1.** Overview of the PHD/HIF oxygen-sensing pathway. The oxygen-sensing machinery targets HIF-1α, HIF-2α and HIF-3α for proteasomal degradation. In the presence of oxygen (normoxia) prolyl-4-hydroxylase enzymes PHD1, PHD2 and PHD3 hydroxylate HIF-α at specific proline residues (Pro-OH) resulting in the binding of HIF-α to the VHL tumor suppressor β-domain. VHL functions as the substrate recognition component of an E3-ubiquitin ligase complex, which ubiquitylates HIF-α leading to its rapid proteasomal degradation; HIF-α protein is usually not detectable under normoxia, although it is constitutively synthesized. Under hypoxia, however, cellular HIF-α levels build up and HIF-α translocates to the nucleus, where it forms a heterodimer with HIF-β. The HIF-α/β heterodimer increases the transcription of *EPO*, *PGK1*, *LDH* and many other genes involved in the regulation of hypoxia responses that help cells to survive a low oxygen environment. These include cellular metabolism and mitochondrial function, inflammation, vascular function and oxidative stress responses. Prolyl-4-hydroxylases are iron-dependent ($Fe^{2+}$) enzymes that utilize 2-oxoglutarate and oxygen as substrates for the hydroxylation of HIF-α. Their catalytic activity is significantly reduced under hypoxia. In addition to hypoxia, PHDs are inhibited by reactive oxygen species, NO, Krebs cycle metabolites succinate and fumarate, cobalt chloride, iron chelators such as desferrioxamine, and structural analogs of 2-oxoglutarate irrespective of oxygen levels. Shown is the chemical structure of a prolyl-4-hydroxylase inhibitor capable of effectively stimulating the production of endogenous erythropoietin in hemodialysis patients [92]. The asparagine hydroxylase, factor inhibiting HIF, functions as a second hypoxic switch and fine-tunes HIF transcriptional responses (not shown in this figure). PHD: prolyl-4-hydroxylase; HIF: hypoxia-inducible factor; VHL: von Hippel-Lindau; EPO: erythropoietin; PGK: phosphoglycerol kinase; LDH: lactate dehydrogenase; 2OG: 2-oxoglutarate; PHI: prolyl-4-hydroxylase inhibitor.