Mechanisms of Hypoxia Responses in Renal Tissue
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ABSTRACT
pO2 in the kidney is maintained at relatively stable levels by a unique and complex functional interplay between renal blood flow, GFR, O2 consumption, and arteriovenous O2 shunting. The fragility of this interplay makes the kidney susceptible to hypoxic injury. Cells in the kidney utilize various molecular pathways that allow them to respond and adapt to changes in renal oxygenation. This review provides an integrative perspective on the role of molecular hypoxia responses in normal kidney physiology and pathophysiology, and discusses their therapeutic potential for the treatment of renal diseases.


Although 20% of total cardiac output is directed toward the kidneys, measured O2 tensions are surprisingly low, ranging from as low as 5 mmHg in the medulla to up to 50 mmHg in the cortex. Most of the kidney’s O2 is utilized to fuel Na-K-ATPase, which drives tubular sodium reabsorption and other transport processes that move various solutes, glucose, and amino acids across cellular membranes. Because these transport processes are load dependent, they link renal O2 consumption (VO2) to GFR. As GFR and renal blood flow (RBF) change in parallel under most conditions, an increase in RBF and thus arterial O2 delivery is largely offset by elevated VO2, thus limiting the kidney’s ability to raise pO2 by increasing RBF. As a result, regional pO2 pressures stay within a relatively narrow range. Recent physiologic studies indicate that in addition to VO2 and O2 delivery in arterial blood, arteriovenous O2 shunting, which results from a counter-current exchange of O2 between arterial and venous vessels before arterial blood reaches the renal microcirculation, adds to the maintenance of renal pO2 at constant levels.

Because the kidney carries out its complex transport functions within a relatively narrow range of pO2, which is very low in the medulla, susceptibility to hypoxic injury is high. Renal cells have therefore evolved a variety of molecular mechanisms that allow them to respond and adapt to decreases in renal oxygenation. These mechanisms operate during development, under physiologic and pathologic conditions, and have wide-ranging implications for the pathogenesis and treatment of renal diseases. Although several transcription factors are involved in control of hypoxia and oxidative stress responses, there has been much interest, from both the basic scientist and the clinician, in the hypoxia-inducible factor (HIF) pathway. This is because of its central role in cellular adaptation to hypoxia and its great potential for therapeutic exploitation in the areas of anemia treatment, cytoprotection, cancer, and wound healing. Here, I provide a focused perspective on key mechanisms that regulate and integrate this pathway with other hypoxia responses in the kidney and discuss its potential for renoprotection.

MOLECULAR O2 SENSORS IN THE KIDNEY: MORE THAN JUST HIF

Key components of cellular O2 sensing are Fe (II) and 2-oxoglutarate (2OG)–dependent oxygenases (Figure 1). These enzymes belong to a larger family of proteins; in humans, there are >60 members that couple the oxidative decarboxylation of 2OG to various chemical processes, which include collagen synthesis and fatty acid metabolism. In mammals, these reactions appear to be limited to hydroxylation and demethylation initiated by hydroxylation and produce succinate and CO2 (Figure 1). The 2OG oxygenases control hypoxic signaling by catalyzing the hydroxylation of specific proline residues within the oxygen-dependent degradation domain of HIF-α under normoxia. HIFs are pleiotropic oxygen-sensitive, heterodimeric transcription factors that have key roles in the cellular adaptation to hypoxia, and regulate a multitude of biologic processes, which include erythropoiesis and iron metabolism, anaerobic glucose metabolism, angiogenesis, growth, and proliferation. Prolyl-hydroxylated HIF-α is targeted for proteasomal degradation by the von Hippel-Lindau–E3

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ubiquitin ligase complex. HIF 2OG oxygenases function as O2 sensors because they require O2 for catalysis. Under hypoxia, hydroxylation is inhibited and HIF signaling is activated. In the thick ascending limb of Henle, this activation may be renoprotective for acute ischemic injury.

Three main HIF prolyl hydroxylases have been identified, prolyl-4-hydroxylase domains (PHDs) 1, 2, and 3, which are also referred to as EGL nine homologs (EGLNs) 2, EGLN1, and EGLN3, respectively. PHD2 is the main enzyme that targets HIF for degradation under normoxia. All three PHDs are expressed in the kidney, where they control HIF activity. Based on immunohistochemical studies and RNA analysis, their expression levels vary between different renal cell types. Compared with PHD2, PHD1 and PHD3 are more abundant in glomeruli, whereas PHD1, PHD2, and PHD3 appear to be expressed at higher levels in the distal renal tubule compared with proximal tubular epithelium. A fourth potential HIF prolyl hydroxylase, P4H-TM, is localized in the endoplasmic reticulum membrane and has been shown to hydroxylate HIF-1α-derived peptides, but not type 1 collagen polypeptides in vitro. Although P4H-TM seems to be important for normal kidney function in zebra fish, where it regulates the integrity of the glomerular basement membrane, its role in hypoxic signaling in the mammalian kidney is unknown.

The transcriptional activity of HIF is modulated by a second hypoxic switch, which operates within the carboxy-terminal transactivation domain of HIF-α. Factor inhibiting HIF (FIH) is a 2OG oxygenase that catalyzes the hydroxylation of an asparagine residue within the C-terminal transactivation domain of HIF-α, thereby inhibiting the binding of coactivators CREB-binding protein and p300 to the HIF transcriptional complex. Conversely, FIH inactivation facilitates CREB-binding protein/p300 recruitment and results in increased HIF target gene expression under hypoxia. In the kidney, FIH has been detected in podocytes and in the distal tubule. Although largely unexplored in the kidney, an additional level of complexity in the regulation of HIF-mediated hypoxia responses is added by oxygen-dependent microRNA expression.

Although the role of PHDs and FIH in the regulation of HIF activity is well established, alternative hydroxylation targets have been identified that are likely to affect hypoxia responses in the kidney. PHD1, 2, and FIH, for example, catalyze the hydroxylation of components of the NF-κB pathway, linking O2 sensing to inflammatory responses, and PHD3 has been shown to interact with pyruvate kinase isofrom 2 in the regulation...
of glycolysis. Furthermore, it is likely that renal hypoxia responses are modulated by epigenetic changes that are carried out by other non-HIF 2OG oxygenases. While nothing is known about their role in kidney physiology and disease, 2OG oxygenases containing the jumonji domain catalyze the demethylation of methylated histones. Jumonji domain-containing oxygenases, some of which are also induced by hypoxia through HIF, are likely to provide additional functional links between acute and chronic alterations in pO2 levels, metabolism, and gene expression changes in the kidney.

Although oxygen-dependent, the catalytic activity of HIF hydroxylases is also modulated by multiple signaling molecules, such as reactive oxygen species (ROS) and nitric oxide (NO) (Figure 1), linking various intracellular signaling pathways, which include signaling through angiotensin II receptors or NO synthase, to renal O2 sensing. This has significant implications for CKD associated with diabetic nephropathy, the aging kidney, inflammatory renal diseases, and others. Additional insights into the complexity of molecular O2 sensing regulation and how it interplays with energy metabolism and other oxidative stress sensors comes from the study of inherited renal tumor syndromes. Mutations in the Krebs cycle enzyme fumarate hydratase occur in hereditary leiomyomatosis and renal cell cancer syndrome, a rare form of familial renal cancer, and lead to the accumulation of fumarate. Fumarate competitively inhibits 2OG oxygenases as well as the degradation of NF (erythroid-derived 2)-like 2 (NRF2), a transcription factor that regulates cellular antioxidant responses. Although this is an active area of investigation in renal oncology, little is known about how links between O2 sensing, energy metabolism, and oxidative stress responses affect the pathogenesis of nonmalignant kidney disease.

**THE KIDNEY IN CONTROL OF O2 CARRYING CAPACITY**

A classic systemic adaptation to hypoxia is the stimulation of red blood cell production through increased synthesis of erythropoietin (EPO). It was the interest in understanding the physiologic and molecular basis of this response that paved the way for the discovery of the PHD/HIF O2 sensing machinery. The kidney plays a key role in this response, because it is the main physiologic source of EPO in adults. Renal EPO synthesis is regulated by HIF-2, and not by HIF-1, as evidenced by several genetic and immunohistochemical studies and by mutational analysis of patients with congenital erythrocytosis. EPO-producing cells in the kidney are peritubular interstitial fibroblasts derived from neuronal lineages. The activity of HIF-2 in renal EPO-producing cells (REPCs) is controlled by PHD2. This is in contrast to nonrenal EPO-producing cell types; a role for PHD1 and PHD3 in REPC-based EPO synthesis is not apparent from genetic studies in mice. EPO transcription is highly oxygen responsive and represents one of the most sensitive hypoxia responses in the kidney. One may speculate that this response, which increases O2 carrying capacity, evolved in the kidney specifically because of its limited ability to augment O2 delivery through other regulatory mechanisms, such as increasing RBF.

In the kidney, EPO output is controlled by the number of REPCs and not by the incremental increase in cellular mRNA encoding EPO found in cell lines. In CKD, the loss of the kidney's ability to produce adequate amounts of EPO in response to hypoxic stimuli results in anemia. Although not entirely clear, a potential mechanism underlying renal anemia may be the transition of REPCs to a myofibroblast phenotype, which would limit the number of cells that can be recruited to synthesize EPO when renal pO2 is low. Because HIF not only induces EPO synthesis, but also enhances intestinal iron uptake and utilization and promotes erythroid progenitor maturation in the bone marrow, clinical trials are currently underway to evaluate the efficacy of pharmacologic HIF stabilization using competitive PHD inhibitors (structural analogs of 2OG) for the treatment of renal anemia in CKD and dialysis patients.

**O2 AND METABOLIC REPROGRAMMING**

The mitochondrial respiratory chain uses O2 to generate ATP, which fuels multiple cellular processes and is consumed mainly by Na-K-ATPase to transport solutes. Efficient metabolic adaptation to low pO2 is therefore imperative for the maintenance of renal transport functions and ultimately the promotion of cell survival. The PHD/HIF pathway has a central role in metabolic reprogramming under low pO2, because it regulates cellular energy and glucose metabolism at multiple levels. HIF shifts metabolism from oxidative phosphorylation to anaerobic glycolysis and suppresses mitochondrial respiration and ROS generation. It does this by increasing the expression of glycolytic enzymes, such as hexokinase, phosphofructokinase, aldolase, phosphoglycerate kinase 1, enolase, and lactate dehydrogenase, by blocking the conversion of pyruvate to acetyl CoA through transcriptional upregulation of pyruvate dehydrogenase kinase, and by regulating the expression of proteins that compose the mitochondrial respiratory chain. Because lactate production increases when glycolytic flux increases, HIF is also involved in the prevention of cellular acidification by regulating the expression of sodium/hydrogen exchanger -1 and monocarboxylic acid transporter-4, thus facilitating the excretion of protons and lactate. Moreover, HIF-1 activation in tumor cells keeps intracellular pH in a slightly alkaline range by increasing membrane-bound ectoenzyme carbonic anhydrase IX expression, which catalyzes the conversion of CO2 to bicarbonate. This effect of HIF activation may also be of relevance for renal adaptation to hypoxia.

**THERAPEUTIC OPPORTUNITIES BEYOND RENAL ANEMIA**

The effects of hypoxia and oxygen-dependent signaling on the kidney are
VO₂ has been described in diabetic ne-
O₂ diffusion due to
hone, as well as anemia and impaired
therosclerosis, and altered vascular
clude abnormal renal perfusion from
the von Hippel-Lindau
tumor suppressor that result in constitut-
HIF activation.

In the noncancerous kidney, HIF-α
stabilization is found in both acute and
chronic kidney diseases. In patients
with diabetic nephropathy, for example, the
degree of HIF activation correlates
with severity of renal injury.
HIF activation in the setting of CKD is due
to chronic hypoxia or can result from
oxygen-independent PHD inhibition.
The causes of hypoxia in CKD are mul-
tifactorial and involve structural and
functional changes that are commonly
associated with fibrotic kidneys. These
include abnormal renal perfusion from
capillary rarefaction, glomerular injury,
atherosclerosis, and altered vascular
tone, as well as anemia and impaired
O₂ diffusion due to fibrosis.
Increased V₀₂ has been described in diabetic
nephropathy and decreases renal pO₂.
This is partly due to mitochondrial dys-
function.
Due to the unique features of
renal O₂ regulation, pO₂ in the kidney
is very sensitive to changes in intracel-
ular O₂ consumption caused by ineffi-
cient mitochondrial O₂ utilization, as
increased RBF, which raises GFR, is un-
likely to compensate for increased O₂
demand.

In the acute setting, HIF has been
shown to mediate the effects of ischemic
preconditioning, and pharmacologic
HIF activation protects from ischemia-
reperfusion injury in animal models of
ARF. While the use of pharmacologic
HIF activation in the prevention of acute
renal injury is supported by preclinical
studies, its role in CKD is debated; ani-
mal models of progressive kidney injury
support both renoprotective and injur-
ypromoting roles. Recent data using the
remnant kidney model furthermore in-
dicate that renoprotection is dependent
on the timing of pharmacologic HIF ac-
tivation.

While it has been suggested that HIF
promotes progression of CKD by in-
creasing the expression of profibrotic
factors and by facilitating epithelial
dedifferentiation, the mechanisms un-
derlying renoprotection are likely to
involve multiple signaling pathways and
metabolic changes, which include HIF-
induced expression of cytoprotective
genes; reprogramming of glucose,
energy, and adenosine metabolism;
beneficial effects on mitochondrial O₂ utili-
zation, renal V₀₂, and mitochondrial
ROS production; enhanced ROS scav-
enging; suppression of renal inflamma-
tion; and maintenance of vascular health
and integrity (Figure 1).
Given that the regulation of the PHD/HIF axis
and its downstream targets is cell type
dependent and involves multiple feed-
back loops including changes in the epi-
genome, it is likely that the mechanisms
of HIF-mediated cytoprotection differ
between acute and chronic hypoxic con-
ditions.
This certainly poses a major
challenge for the identification and vali-
dation of relevant molecular targets and
cell types that mediate HIF-induced re-
noprotection.

A LOOK AHEAD IN RENAL
HYPOXIA RESEARCH

In this review of renal hypoxia responses,
I focused on selected aspects of molecular
O₂ sensing in the kidney and discussed
its potential for therapeutic exploitation.
Since the discovery of the PHD/HIF
pathway, many new questions have
emerged. Although the activity of
renal HIF is controlled by specific HIF 2OG
oxygenases, the significance of their re-
action with non-HIF targets and the role
of other non-HIF 2OG oxygenases in re-
nal physiology and pathophysiology are
still unknown. Fascinating are the
therapeutic opportunities that the
PHD/HIF pathway provides. Knowledge
of the effects of pharmacologic HIF ac-
tivation on human physiology and path-
ophysiology, however, is still limited.
Controlled physiologic studies in hu-
mans, in concert with studies of patients
that live at high altitude, are likely to ad-
vance our understanding of HIF respon-
ses in the kidney. This, together with animal and in vitro investigations, will
hopefully lead to the development of
new therapies that improve the life of
patients with kidney diseases.

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DISCLOSURES

None.

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