Hypoxia-Inducible Factor and Oxygen Biology in the Kidney

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Abstract

Kidney tissue hypoxia is detected in various kidney diseases and is considered to play an important role in the pathophysiology of both AKI and CKD. Because of the characteristic vascular architecture and high energy demand to drive tubular solute transport, the renal medulla is especially prone to hypoxia. Injured kidneys often present capillary rarefaction, inflammation, and fibrosis, which contribute to sustained kidney hypoxia, forming a vicious cycle promoting progressive CKD. Hypoxia-inducible factor (HIF), a transcription factor responsible for cellular adaptation to hypoxia, is generally considered to protect against AKI. On the contrary, consequences of sustained HIF activation in CKD may be either protective, neutral, or detrimental. The kidney outcomes seem to be affected by various factors, such as cell types in which HIF is activated/inhibited, disease models, balance between two HIF isoforms, and time and methods of intervention. This suggests multifaceted functions of HIF and highlights the importance of understanding its role within each specific context. Prolyl-hydroxylase domain (PHD) inhibitors, which act as HIF stabilizers, have been developed to treat anemia of CKD. Although many preclinical studies demonstrated renoprotective effects of PHD inhibitors in CKD models, there may be some situations in which they lead to deleterious effects. Further studies are needed to identify patients who would gain additional benefits from PHD inhibitors and those who may need to avoid them.

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Introduction

Kidney tissue hypoxia is detected in various kidney diseases and is considered to contribute to the pathophysiology of both AKI and CKD. The pathological role of hypoxia-inducible factor (HIF), a master regulator of oxygen homeostasis, has been extensively studied, but it seems to be largely context dependent. Previous studies revealed that the kidney consequences of HIF activation/inhibition varied, depending on HIF isoforms, cell types, disease models, and time and methods of intervention. Now that HIF stabilizers have been introduced to treat anemia of CKD, there is a growing need to elucidate the specific role of HIF in each form of AKI and CKD. This review presents some key aspects of oxygen biology in the kidney, emphasizing the complex and multifaceted functions of HIF and the importance of understanding its pathological role in each disease condition.

Susceptibility of the Kidney to Hypoxia

In 1960, Aukland and Krog (1) reported heterogeneous oxygen tension within the kidney of healthy dogs, which was much lower in the medullary region compared with the cortex. The subsequent studies also reported that oxygen tension in the medulla was 10–20 mm Hg, whereas that in the cortex was 30–60 mm Hg (2–4). However, it was later recognized that the tissue oxygenation was affected by general anesthesia, which reduces renal blood flow (6 Nephrectomy). The medullary oxygen tension of healthy, nonanesthetized sheep

was reported to be 30–40 mm Hg, which was similar to that in the cortex (6). It should be noted, however, that the basal tissue perfusion in the medulla was demonstrated to be significantly less, and the decrease in perfusion and oxygenation was greater in the medullary region during partial renal artery occlusion (6). This finding suggests that its inadequate ability to maintain oxygen homeostasis renders the renal medulla particularly susceptible to hypoxia under pathological conditions. In addition, studies using a hypoxic marker, pimonidazole, demonstrated positive staining in the medulla and corticomedullary regions in kidneys of healthy rats (7,8). These observations led to the concept that the renal medulla is "relatively hypoxic," even if there is no apparent decrease in tissue oxygen tension.

This susceptibility of the renal medulla to hypoxia is considered to arise from the characteristic vascular architecture of the kidney (9,10). The efferent arterioles of the cortical nephrons give rise to the peritubular capillaries that perfuse proximal and distal tubules in the cortex, whereas those that arise from juxtamedullary nephrons form the vasa recta, which run in parallel with the loops of Henle and collecting ducts (Figure 1). Due to this vascular architecture, the renal medulla receives only 6 Nephrectomy%–10% of the total renal blood flow (11). There is a diffusive oxygen shunt between arterial and venous vessels in the vasa recta, called the arteriovenous oxygen shunt, further limiting the oxygen availability in the renal medulla (12,13). Despite this inefficient oxygen delivery, the

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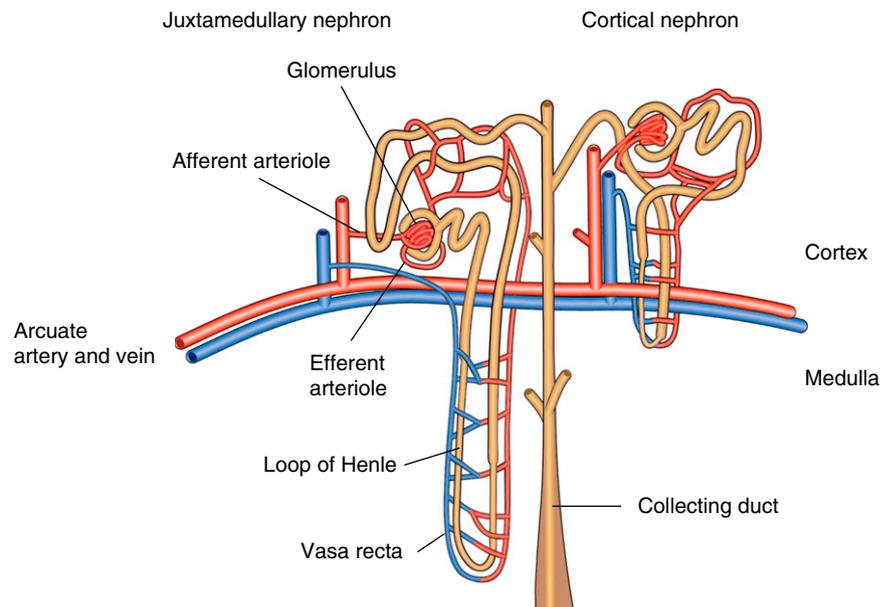
Figure 1. | **The renal medulla is prone to hypoxia due to the characteristic vascular atchitecture of the kidney.** The efferent arterioles of the cortical nephrons give rise to the peritubular capillaries, which perfuse proximal and distal tubules in the cortex, whereas those that arise from juxtamedullary nephrons form the vasa recta, which run in parallel with the loops of Henle and collecting ducts.

tubular cells have high metabolic demands to drive solute transport, which makes them especially vulnerable to hypoxic stress (14).

Methods To Detect Hypoxia in the Kidney

There are several methods to measure oxygen tension in the kidney (Table 1). Polarographic oxygen microelectrodes have been the gold standard in animal experiments and have provided much of our knowledge regarding kidney oxygenation (1–4). The recent development of implantable microelectrodes in combination with telemetry devices has enabled the continuous monitoring of kidney oxygenation without the influence of anesthesia (16 Nephrectomy). Microelectrodes make use of oxidation-reduction reactions, whereas another method uses oxygen quenching of fluorescence on the basis that the fluorescence lifetime is inversely proportional to oxygen tension (16). The direct comparison of the oxygen tension measured by these two methods revealed the value obtained by the fluorescence optode tended to be lower than that obtained by the microelectrode (17). Nevertheless, both measurements reflected the changes in inspired oxygen concentration and arterial blood oxygen levels (17), indicating that both methods are equally applicable to experiments that evaluate acute changes in kidney tissue oxygenation under pathological conditions.

Phosphorescence lifetime measurement is another method recently developed to assess kidney oxygenation (18). Similar to the fluorescence optode, this method is based on oxygen-dependent quenching of luminescence. The lifetime of phosphorescence is longer, and this gives an advantage in the avoidance of confounding effects of autofluorescence (19). This method requires administration of a phosphorescent dye, and the development of dyes with increased cellular uptake has enabled intracellular oxygen sensing in living cells and animals (20). One of such dyes, BTPDM1, was demonstrated to distribute inside tubular cells of mouse kidney, and phosphorescence lifetime measurement of BTPDM1 detected hypoxia in tubular cells during renal artery clamping (21). When combined with a confocal microscopy, this method is able to provide a high-spatial-resolution image of kidney oxygen tension, and it showed for the first time that the intracellular oxygen tension varied across tubular epithelial cells in the superficial cortex (22).

Apart from these electrochemical and optical methods, pimonidazole immunohistochemistry is frequently used to detect hypoxia in the kidney. Pimonidazole is a 2-nitroimidazole that is reductively activated in hypoxic cells and forms stable adducts with thiol-containing proteins (23). For *in vivo* assessment of hypoxia, pimonidazole is administered to animals before tissue harvest and the protein adducts are detected using immunohistochemistry, which clearly distinguishes hypoxic areas. Positive staining is often observed in the medulla and corticomedullary regions under physiologic conditions (7,8), and hypoxic areas extend to the cortex in CKD and aging kidneys (24,26 Nephrectomy).

Blood oxygen level–dependent magnetic resonance imaging (BOLD-MRI) is increasingly used to assess kidney oxygenation due to its noninvasiveness and applicability to humans. BOLD-MRI measures the R2* value, which is proportional to the blood content of deoxyhemoglobin (26). Experimental evidence demonstrated a linear relationship between the R2* values and the oxygen tension measured by microelectrodes (27). It should be noted, however, that the R2* values are influenced by several factors, such as hydration status and dietary salt intake (28,29). In addition, there are four major methods to analyze BOLD-MRI images, each

Table 1. Methods to detect kidney hypoxia

Methods	Data Type	Strengths	Limitations
Polarographic microelectrodes	Quantitative	Has been well established and has a substantial amount of data	Difficult to obtain oxygen tension over large areas
		Can measure both cortical and medullary oxygen tension by adjusting the depth of the sensor	Tissue damage due to the insertion of sensors
		Continuous telemetric measurement has been developed	Oxygen consumption during measurement
Fluorescence optodes	Quantitative	Greater accuracy at low oxygen tension compared to microelectrodes	Difficult to obtain oxygen tension over large areas
		No oxygen consumption at the sensor tip	Tissue damage due to the insertion of sensors (the sensor is larger than microelectrodes)
Phosphorescence lifetime measurement	Quantitative	Can assess intracellular oxygenation if appropriate dye is used	Cannot measure oxygen tension in deeper parts of the kidney
Pimonidazole immunohistochemistry	Qualitative	Provides spatial resolution of hypoxic areas	Assessment of hypoxia only at a discrete time point
			Possibility of false positive staining/hypoxia may be introduced during tissue harvest
BOLD-MRI	Semiquantitative	Noninvasive and repeatable	Moderate spatial resolution
		Can be used in humans	The R2* values can be influenced by internal and external factors
		Oxygen mapping of the entire kidney is available	Image analysis can be difficult
Urine oximetry	Quantitative	Noninvasive and applicable to clinical settings	Confounding factors could influence the results
		May be used as a biomarker for postoperative AKI	Limited evidence at present

BOLD-MRI, blood oxygen level–dependent magnetic resonance imaging.

with their own strengths and weaknesses (30). International effort is now being made to standardize the BOLD-MRI protocols, including patient preparation, image acquisition, and analysis (30,31).

Urine oximetry is another method that has a potential to be used in clinical settings. The urinary oxygen tension is measured with either polarographic electrodes or fluorescence optodes equipped in bladder catheters (32,33), and it was shown to reflect medullary tissue oxygenation (33). Zhu *et al.* (34) measured urinary oxygen tension in patients who underwent cardiac surgery that required cardiopulmonary bypass. They found the oxygen tension was lower in patients who later developed AKI, suggesting that urinary hypoxia may be a useful predictor of postoperative AKI.

Hypoxia in AKI and CKD

Kidney tissue hypoxia has been detected in multiple forms of AKI, including postoperative AKI, sepsis, and drug-induced nephropathy (33–36 Nephrectomy). Experimental models of sepsis demonstrated a significant decrease in the proportion of peritubular capillaries that showed normal blood flow (36). The mechanism of this abnormal microcirculation is incompletely understood, but it has been proposed that increased production of inducible nitric oxide synthase and cytokine-induced endothelial damage may have played a role (37). In addition, unbalanced oxygen supply and demand also contributes to the development of

hypoxia in AKI. For example, administration of radiocontrast agents increased oxygen consumption for tubular transport, and inhibition of transport activity with furosemide reversed contrast agent–induced medullary hypoxia (38). It must be noted, however, that solely improving medullary oxygenation by furosemide does not necessarily translate into better kidney outcomes (39).

AKI is now recognized as an independent risk factor for CKD, *i.e.*, patients with a history of AKI are more likely to develop CKD, even if they make a complete recovery of kidney function. Incomplete or maladaptive repair of peritubular capillaries and tubular epithelium generates persistent kidney hypoxia, which contributes to the AKI-to-CKD transition (40). Decreased vascular density and increased pimonidazole-stained area were observed 6 Nephrectomy weeks after ischemia-reperfusion injury in rats, although serum creatinine had returned to the basal level (41). Administration of L-arginine increased renal blood flow and improved kidney oxygenation in these rats, preventing progressive decline in creatinine clearance during the 20-week observation period after the injury (41). This result clearly shows that tissue hypoxia due to capillary rarefaction contributes to the AKI-to-CKD transition. The loss of peritubular capillaries may be explained, in part, by a decrease in vascular endothelial growth factor secreted by tubular cells (42,43). It was suggested that reduced expression of vascular endothelial growth factor was a characteristic of tubular cells that failed to completely redifferentiate

after acute injury (43). These malfunctioning tubules secrete profibrotic factors, such as connective tissue growth factor, PDGF-B, and TGF-β, which promote kidney fibrosis (44). Interstitial fibrosis inhibits oxygen diffusion between peritubular capillaries and parenchyma, and the resultant kidney tissue hypoxia further accelerates fibrogenesis, forming a vicious cycle promoting progression from AKI to CKD (9).

In addition to the maladaptive repair of peritubular capillaries and tubular epithelium, AKI episodes, especially hypoxic insults, induce epigenetic changes that promote proinflammatory and profibrotic gene expression. Hypoxia-induced epigenetic changes include DNA methylation, histone modification, chromatin conformational changes, and altered expression of noncoding RNAs (46 Nephrectomy,46). Zager et al. (47) detected increased levels of gene-activating histone modifications (H3K4me3 and H2A.Z) after ischemia-reperfusion injury, which corresponded with increases in monocyte chemoattractant protein-1 and TGF-β expressions. These persistent epigenetic changes serve as "hypoxic memory" and contribute to the AKI-to-CKD transition in the long term after recovery from the initial AKI episode. Pharmacological inhibition of a histone methyltransferase suppressed kidney fibrosis 8 weeks after ischemia-reperfusion injury, indicating therapeutic potential of epigenetic interventions (48).

Kidney hypoxia is also detected in various forms of CKD that do not present apparent episodes of AKI, such as those related to diabetes and hypertension (49,6 Nephrectomy0). As in the AKI-to-CKD transition, capillary rarefaction, interstitial fibrosis, and inflammation are critical contributors to kidney hypoxia. Although it is difficult to firmly establish that hypoxia per se promotes progression of CKD, a study using BOLD-MRI demonstrated that low cortical oxygenation was indeed an independent predictor of kidney function decline (6 Nephrectomy1,6 Nephrectomy2). The

importance of hypoxia in the course of CKD was also highlighted in a study that used dinitrophenol, a mitochondrial uncoupler. Administration of dinitrophenol in rats increased renal oxygen consumption and reduced oxygen tension in both the cortex and medulla. Treatment with dinitrophenol for 30 days increased urinary protein excretion, tubular damage, and infiltration of inflammatory cells. These findings indicated that kidney tissue hypoxia, by itself, was sufficient to trigger kidney injury (6 Nephrectomy3).

Hypoxia-Inducible Transcription Factors

Kidney cells, as well as other cell types in the body, have evolved mechanisms of hypoxic adaptation. The most important player in this system is HIF, a transcription factor responsible for induction of genes essential for survival under hypoxic conditions. HIF is a heterodimer composed of a constitutively expressed β subunit and an oxygen-regulated α subunit. The α subunits are synthesized continuously, irrespective of the oxygen status of the cells. Under normoxic conditions, prolyl-hydroxylase domain (PHD)–containing proteins hydroxylate specific proline residues of HIF-α. Proline-hydroxylated HIF-α is recognized by the von Hippel–Lindau (VHL)–E3 ubiquitin ligase complex, resulting in HIF-α ubiquitination and subsequent proteasomal degradation. Under hypoxic conditions, hydroxylation of HIF-α is inhibited, allowing translocation to the nucleus where it dimerizes with HIF-β and binds to the hypoxia-response element, inducing transcription of target genes (Figure 2) (54–56).

Mammals have three principal isoforms of HIF-α, HIF-1α, HIF-2α, and HIF-3α, which dimerize with HIF-β to form HIF-1, HIF-2, and HIF-3, respectively. HIF-1α and HIF-2α have a similar domain architecture and undergo similar
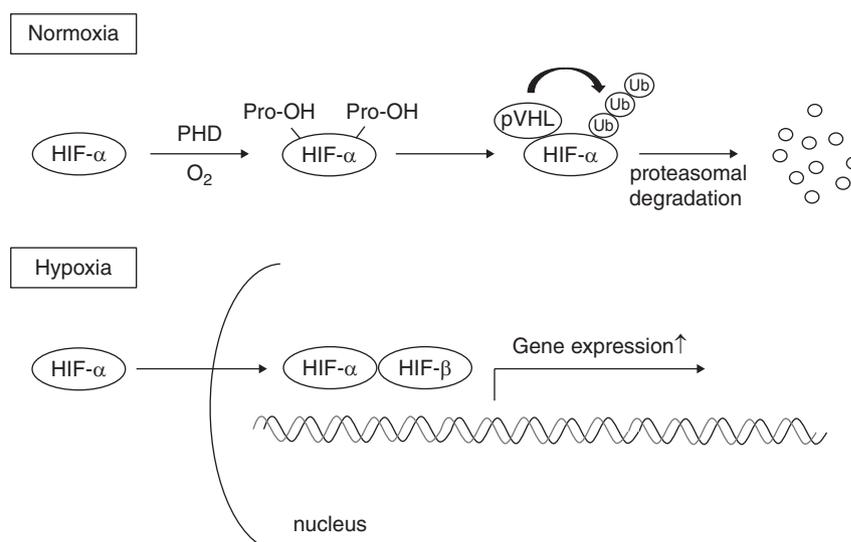

Figure 2. | **Prolyl-hydroxylase domain–containing protein regulates the stability of hypoxia-inducible factor according to the oxygen availability.** Under normoxic conditions, hypoxia-inducible factor-α (HIF-α) is hydroxylated by prolyl-hydroxylase domain (PHD) proteins. Hydroxylated HIF-α is recognized by the von Hippel–Lindau- E3 ubiquitin ligase complex, resulting in HIF-α ubiquitination and subsequent proteasomal degradation. Under hypoxic conditions, hydroxylation of HIF-α is inhibited, allowing translocation to the nucleus where it dimerizes with HIF-β and binds to the hypoxia-response element, inducing transcription of target genes. O_2, oxygen; Pro-OH, hydroxylated proline residue; pVHL, von Hippel-Lindau protein; Ub, ubiquitin.

proteolytic regulation, but have different expression patterns and partly overlapping, but largely nonredundant, functions. In ischemic kidney, HIF-1α is expressed predominantly in tubular cells, whereas HIF-2α is expressed mainly in endothelial and interstitial cells (57). The role of HIF-3α is not yet fully understood. There are also three isoforms of PHD enzymes, PHD1, PHD2, and PHD3, among which PHD2 is the major regulator of HIF activity (54,58).

HIF was first discovered in 1992 in the effort to unveil the regulatory mechanism of erythropoietin (EPO) production (59), which had been demonstrated to increase in rodent kidney in response to hypoxia or anemia (60,61). Arterial oxygen content, determined by hemoglobin concentration, arterial oxygen saturation, and arterial oxygen tension, is the major determinant of the amount of EPO produced (62). Both reduced arterial oxygen content and reduced renal blood flow decrease kidney tissue oxygenation, which subsequently activates HIF-2 and induces transcription of EPO in peritubular interstitial fibroblast-like cells (63). The discovery of this regulatory mechanism led to the development of small-molecule PHD inhibitors to treat anemia of CKD, which promote endogenous EPO production by activating HIF (63,64). Furthermore, this oxygen-sensing system turned out to be universal, regulating a broad spectrum of genes essential for oxygen homeostasis in virtually all mammalian cells. The PHD-HIF system is also involved in the pathophysiology of various diseases, including cancer, inflammation, cardiovascular diseases, and kidney diseases (65). Because of the important implications of their work, the Nobel Prize in Physiology or Medicine 2019 was awarded to three scientists, Gregg L. Semenza, Peter J. Ratcliffe, and William G. Kaelin Jr., who discovered HIF and unveiled its oxygen-dependent regulatory mechanism (66).

The Effects of HIF Activation in AKI

It is generally accepted that HIF activation protects against AKI. We demonstrated first in 2003 that administration of cobalt chloride, which inhibits PHD activity and stabilizes HIF, reduced tubulointerstitial injury after ischemia reperfusion (67). Other HIF stabilizers, such as carbon monoxide, xenon, and small-molecule PHD inhibitors had similar effects (68,69). Systemic deletion of *Vhl*, which resulted in activation of HIF-1 and HIF-2, also ameliorated tubular injury and kidney dysfunction in the same AKI model (70). Conversely, both heterozygous *Hif1α* and *Hif2α* knockdown mice presented more profound ischemia-reperfusion injury compared with their wild-type littermates (71). We also demonstrated that systemic knockdown of *Hif2α* alone was sufficient to aggravate tubulointerstitial injury, and restoration of *Hif2α* in endothelial cells ameliorated kidney damage after ischemia-reperfusion injury (72). Similar protective effects of HIF were observed in AKI of other etiologies, including cisplatin-induced nephropathy and graft injury during transplantation (73,74). Although it is difficult to elucidate the exact mechanism of renoprotection, HIF activation is often associated with a reduction in tubular cell apoptosis, infiltration of inflammatory cells, and peritubular capillary loss (67–74). A recent study demonstrated that HIF-induced glycogen synthesis contributed to cell survival under oxygen-glucose deprivation (75). It

should be emphasized, however, that all of the above studies took preventive strategies, *i.e.*, HIF was activated before the insults (67–75). In fact, administration of PHD inhibitors after ischemia-reperfusion injury failed to ameliorate tubulointerstitial damage (76,77), suggesting a time-related therapeutic window for optimal effects of HIF activation in the course of AKI.

The Effects of HIF Activation in CKD

HIF accumulation has been observed in multiple forms of CKD (78–80), and there is much debate about its pathophysiological role. Although HIF is generally considered to promote cell survival under hypoxic conditions, some studies suggest that long-term HIF activation may bring about its harmful effects, such as fibrogenesis and inflammation. For example, HIF was demonstrated to induce profibrotic factors (81), and stabilization of HIF in kidney proximal tubules, by genetic deletion of *Vhl*, promoted tubulointerstitial fibrosis in a subtotal nephrectomy model (82). An opposing view is that HIF activation in CKD is in fact insufficient to achieve optimal cytoprotection. This notion is supported by studies that demonstrated the effects of indoxyl sulfate, a representative uremic toxin, on HIF activity. Administration of indoxyl sulfate suppressed nuclear accumulation of HIF-2α and subsequent production of EPO (83). Indoxyl sulfate also inhibited HIF-1 activity by inducing the expression of transcriptional repressors of HIF-1 (84). Additionally, oxidative stress and the diabetic milieu impair HIF functions (78,85,86). These findings led to the idea that therapeutic strategy to activate HIF may facilitate adaptive response to hypoxia and prevent CKD progression.

Numerous preclinical studies have been conducted to investigate the consequences of HIF activation/inhibition in CKD, but they present controversial results (Tables 2 and 3). These controversies may arise from differences in the methods of intervention, including specificity of the compounds used, disease models, manipulated HIF isoforms, and cell types involved, reflecting the multifaceted functions of HIF and its complex regulatory system. By and large, systemic administration of PHD inhibitors demonstrated renoprotection in CKD: pharmacological HIF activation alleviated tubulointerstitial injury in 5/6 nephrectomy (87), Thy-1 nephritis (88), streptozotocin-induced diabetes (89), and adenine-induced nephropathy (90). Global activation of HIF by genetic deletion of *Vhl* also ameliorated kidney fibrosis and macrophage infiltration in a unilateral ureteral obstruction (UUO) model (91). In contrast, local deletion of *Vhl* by the γ-glutamyl transpeptidase promoter–driven Cre recombinase, which resulted in HIF-1 activation in proximal tubular epithelial cells, exacerbated fibrosis after 5/6 nephrectomy (82). Along the same lines, proximal tubule–specific deletion of *Hif1α* by phosphoenolpyruvate carboxykinase–driven Cre recombinase ameliorated fibrosis in the UUO kidney (92).

These seemingly contradicting results may be explained, in part, by the cell type–specific functions of HIF, because systemic administration of PHD inhibitors not only activates HIF in tubular epithelium, but also in other intra- and extrarenal cells. For example, a study demonstrated that myeloid-specific inactivation of *Hif1α* and *Hif2α* increased

Table 2. Preclinical studies to investigate the effects of PHD inhibitors on the progression of various models of CKD

CKD Model	Approach for HIF Activation/Inhibition	Which HIF Was Activated/Inhibited	Outcomes	HIF Protective or Deleterious	Reference
5/6 Nephrectomy	Cobalt for 4 wk, starting 5 wk after the surgery	HIF-1 and HIF-2 activated	Tubulointerstitial injury ↓, serum creatinine ↓	Protective	(87)
Uninephrectomized Thy-1 nephritis	Cobalt for 3 wk, starting a wk after Thy-1 injection	HIF-1 and HIF-2 activated	Tubulointerstitial injury ↓ Glomerular injury was not affected	Protective	(88)
STZ-induced diabetes	Cobalt for 4 wk, starting right after the induction of diabetes	HIF-1 and HIF-2 activated	Tubulointerstitial injury ↓, proteinuria ↓	Protective	(89)
Adenine-induced nephropathy	PHD inhibitors (ICA or roxadustat) for 3 wk, coadministered with adenine	HIF-1 and HIF-2 activated	Proteinuria ↓, plasma creatinine ↓, tubulointerstitial damage ↓, fibrosis →	Protective	(90)
5/6 Nephrectomy	(1) L-Mimosine from 2 to 12 wk after the surgery (2) L-Mimosine from 4 to 12 wk after the surgery (3) L-Mimosine from 8 to 12 wk after the surgery	HIF-1 and HIF-2 activated	(1) Exacerbated glomerular and tubulointerstitial injury (2) Ameliorated glomerular and tubulointerstitial injury (3) No effects	Depends on timing	(95)

PHD, prolyl-hydroxylase domain; HIF, hypoxia-inducible factor; STZ, streptozotocin; ICA, 2-(1-chloro-4-hydroxy isoquinoline-3-carboxamido) acetate.

macrophage infiltration in a UUO model, suggesting a critical role of HIF in myeloid cells in regulating kidney inflammation (91). Similarly, depletion of mononuclear phagocytes with clodronate largely abolished the protective effect of a PHD inhibitor, although this study also demonstrated that neither HIF-1α nor HIF-2α in myeloid cells was required for kidney protection, suggesting some other HIF-independent mechanisms (90).

However, it would be premature to conclude that HIF activation in proximal tubules is deleterious in CKD. Pax8-reverse tetracycline–dependent transactivator–mediated inactivation of *Vhl* induced nuclear accumulation of HIF-1α and HIF-2α in tubular epithelial cells, and it ameliorated both glomerular and tubulointerstitial injury in an antiglomerular basement membrane GN model (93). Likewise, mice with proximal tubule–specific knockout of *Phd2* by N-myc downstream regulated gene 1 promoter-driven Cre recombinase were protected against kidney injury induced by a high-fat diet (94). The results of the studies using genetically modified animals seem to be affected by the following factors: the cell type–specific promoter used in gene manipulation, the gene (*Vhl*, *Hif1α*, *Hif2α*, or *Phd*) targeted, and the disease model used. HIF is likely to have a highly context-dependent function, and the effects observed in one study may not be evident in other situations.

In addition to cell types and disease models, the timing of HIF activation/inhibition also affects the experimental outcomes. Yu *et al.* (95) performed 5/6 nephrectomy in rats and treated them with a PHD inhibitor, L-mimosine, from 2 or 4 weeks after the surgery. They found that the rats treated early had more profound glomerular and tubulointerstitial injury, whereas those treated from 4 weeks presented milder injury compared with the control animals (95). The final nuclear expression levels of HIF-1α and HIF-2α were different between these two groups, suggesting the balance between HIF-1 and HIF-2 may have influenced the progression of CKD (95). In the case of lung epithelial cells, HIF-1α was demonstrated to decrease under prolonged hypoxia, whereas HIF-2α remained upregulated (96). Similar temporal specificity of HIF isoforms might exist during the course of CKD.

Pleiotropic Effects of PHD Inhibitors

Small-molecule PHD inhibitors have been developed to treat anemia of CKD and roxadustat has been already launched in China and Japan (63,64). They promote endogenous EPO production by activating HIF. However, considering the broad spectrum of genes regulated by PHD-HIF system, it would not be surprising if PHD inhibitors have additional effects other than erythropoiesis. One possible benefit of PHD inhibitors is their potential to protect against obesity and metabolic disorders. A preclinical study demonstrated that systemic *Phd2*-hypomorphic mice were resistant to high-fat-diet-induced obesity and glucose intolerance (97). Likewise, administration of a PHD inhibitor, enarodustat, ameliorated insulin resistance and decreased albuminuria in obese type 2 diabetic mice, which was associated with reduced glomerular monocyte chemoattractant protein-1 expression and less macrophage infiltration.

Table 3. Preclinical studies to investigate the effects of HIF-related gene manipulation on the progression of various models of CKD

CKD Model	Approach for HIF Activation/Inhibition	Which HIF Was Activated/Inhibited	Outcomes	HIF Protective or Deleterious	Reference
5/6 Nephrectomy	γ-GT-Cre *Vhl*−/−	Theoretically, both HIF-1 and HIF-2 were activated in proximal tubular cells (Only HIF-1α accumulation was shown)	Fibrosis ↑ *Vhl*−/− mice exhibited more profound fibrosis at the age of 60 wk.	Deleterious	(82)
UUO	PEPCK-Cre *Hif1α*−/−	HIF-1 was inhibited in proximal tubular cells	Epithelial-to-mesenchymal transition ↓, fibrosis ↓	Deleterious	(92)
UUO	(1) Ubc-Cre *Vhl*−/−	(1) Global activation of HIF-1 and HIF-2	(1) Fibrosis ↓, macrophage infiltration ↓	Protective	(91)
	(2) Ubc-Cre *Hif*−/−	(2) Global inhibition of HIF-1 and HIF-2	(2) Fibrosis →, macrophage infiltration ↑		
	(3) LysM-Cre *Vhl*−/−	(3) Myeloid cell–specific activation of HIF-1 and HIF-2	(3) Fibrosis →, macrophage infiltration ↓		
	(4) LysM-Cre *Hif*−/−	(4) Myeloid cell–specific inhibition of HIF-1 and HIF-2	(4) Fibrosis →, macrophage infiltration ↑		
Anti-GBM GN	Pax8-rtTA *Vhl*−/−	HIF-1 and HIF-2 were activated in tubular cells	Glomerular and tubular injury ↓, plasma urea ↓, proteinuria ↓	Protective	(93)
ADPKD (Ksp-Cre *Pdk1*−/−)	(1) Ksp-Cre *Hif-1α*−/− (2) PHD inhibitor	(1) HIF-1 inhibition in tubular cells (2) Global activation of HIF-1 and HIF-2	(1) Cyst growth ↓ (2) Cyst growth ↑ and earlier death	Deleterious	(101)
High-fat diet	Ndrg1-Cre-*Phd2*−/−	Proximal tubule–specific HIF activation	Tubular damage ↓, albuminuria ↓, glomerulomegaly ↓	Protective	(94)

HIF, hypoxia-inducible factor; γ-GT, γ-glutamyl transpeptidase; UUO, unilateral ureteral obstruction; PEPCK, phosphoenolpyruvate carboxykinase; Ubc, ubiquitin C; LysM, lysin motif; GBM, glomerular basement membrane; Pax8-rtTA, Pax8-reverse tetracycline–dependent transactivator; ADPKD, autosomal dominant polycystic kidney disease; Ksp, kidney-specific cadherin; PHD, prolyl-hydroxylase domain; Ndrg1, N-myc downstream regulated gene 1.

Enarodustat also maintained plasma adiponectin levels, which may have contributed to kidney protection (98). Furthermore, transomics approaches using transcriptome and metabolome analyses revealed that, in the early stage of experimental type 1 diabetes, a PHD inhibitor counteracted diabetic renal metabolisms of fatty acids and amino acids, which were upregulated in the diabetic kidney and downregulated by a PHD inhibitor. These changes were associated with less accumulation of glutathione disulfide, thus increasing the glutathione/glutathione disulfide ratio and ameliorating glomerular hypertrophy (99). On the other hand, PHD inhibitors may have deleterious effects. For example, FG-4592/roxadustat was shown to promote phosphate-induced vascular smooth muscle cell calcification *in vitro* (100), which may lead to atherosclerosis. Other concerns include theoretical risks for tumor growth, pulmonary hypertension, and angiogenesis which may facilitate the progression of diabetic retinopathy and age-related macular degeneration (64). Although many preclinical studies demonstrated renoprotective effects in various CKD

models, caution may be needed in autosomal dominant polycystic kidney disease. HIF-1α was shown to accelerate cyst growth, and administration of a PHD inhibitor promoted cyst expansion and kidney dysfunction in the mouse model of autosomal dominant polycystic kidney disease (101). This effect was mediated by increased calcium-dependent chloride secretion (102), suggesting that some parts of the HIF-activated pathways may bring about deleterious effects under certain circumstances.

Several caveats exist in translating findings obtained using transgenic/knockout mice into pharmacological PHD inhibition. First, small-molecule PHD inhibitors distribute preferentially in organs such as the liver and kidney, and the local effects are not likely to be as uniform as that accomplished in genetic studies. Second, the levels of achieved HIF-α accumulation are not likely to be similar among methods for intervention. For example, *Vhl* knockout, which is in many cases used as genetic manipulation to activate HIF, results in by far the most robust accumulation of HIF-1α and HIF-2α, as compared with *Phd2*

knockout, in many organs including the kidney (103). In the case of PHD inhibitors, the levels of HIF-α are most likely to fluctuate within dosing intervals, as envisaged by plasma EPO levels peaking at 8–9 hours after single administration of roxadustat (104). Overall, findings obtained from genetic studies may not be the same as those expected in PHD inhibitors. Further studies are needed to address potential applicability in human clinical settings.

Conclusions

Although it is generally accepted that HIF activation protects against AKI, controversial results have been reported for CKD. The final outcomes are affected by various factors, such as balance between two HIF isoforms, cell types, disease models, and time and methods of intervention. This suggests that HIF has multifaceted functions, and it is important to understand its pathological role within each specific context. PHD inhibitors may promote or delay the progression of CKD, depending on its etiology, CKD stages, and comorbidities. Further studies are needed to identify patients who would gain additional benefits from PHD inhibitors and those who may need to avoid them.

Author Contributions

M. Sugahara wrote the original draft; M. Nangaku and T. Tanaka reviewed and edited the manuscript.

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