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Hypoxia and hypoxia-inducible factors in chronic kidney disease

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Abstract

It is generally accepted that renal hypoxia plays an important role in the progression of chronic kidney disease (CKD). This review focuses on renal hypoxia and hypoxia-inducible factor (HIF), a master regulator of cellular adaptation to hypoxia, during CKD progression. The kidney, which is physiologically hypoxic, is exposed to increased levels of hypoxia in CKD; insufficient oxygenation in the tubulointerstitium triggers injury and accelerates the deterioration of renal function, culminating in end-stage kidney disease (ESKD). HIF accumulates during specific stages of pathological progression; however, adaptation to hypoxia usually fails. In such cases, decreased vascular endothelial growth factor expression and upregulated antiangiogenic factors result in sustained capillary rarefaction. In addition, oxygen consumption in tubules is primarily increased by enhanced oxidative stress, and the transcriptional activity of HIF becomes suboptimal, which is partly mediated by methylglyoxal in diabetic kidney disease and by indoxyl sulfate, a representative uremic toxin, in advanced CKD. Oxygen-dependent canonical regulators of HIF involve prolyl hydroxylase domain-containing protein (PHD) and factor inhibiting HIF-1 (FIH-1), whereas recent studies have revealed noncanonical and oxygen-independent HIF regulation in the kidney. As a consequence, HIF accumulation usually fails to protect the kidney against hypoxia, which is likely to accelerate progression to ESKD, via several maladaptation mechanisms. The precise roles of HIF and its regulation in CKD warrant further investigation in light of promising data demonstrating that HIF stabilization by PHD inhibitors may be a new therapeutic approach for CKD.

Keywords: Hypoxia, Hypoxia-inducible factor, HIF, Chronic kidney disease, CKD, Prolyl hydroxylase, PHD, Indoxyl sulfate, Uremic toxin

Background

The worldwide disease burden of chronic kidney disease (CKD) has become an urgent issue; however, new therapeutic options for CKD are limited. Renal hypoxia plays an important role in the pathophysiology of acute kidney injury (AKI) and CKD [1, 2]. It is now broadly accepted that activation of hypoxia-inducible factor (HIF), which is a key transcription factor in cellular adaptation to hypoxia, protects the kidney during AKI. However, the protective role of chronic HIF activation in CKD remains somewhat controversial. In this review, we discuss the roles of hypoxia and HIF in CKD, with a focus on HIF regulatory mechanisms, maladaptation to hypoxia, and HIF stabilization as a therapeutic strategy.

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Hypoxia in the kidney

The kidney is physiologically considered hypoxic despite receiving up to 20 % of the cardiac output in humans. Oxygen pressure in the renal cortex of normal animals is reportedly between 10 and 60 mmHg, whereas the corticomedullary junction and medulla are exposed to marked hypoxia [3–7]. One critical cause of kidney hypoxia, particularly in the medulla, is the arterial–venous oxygen shunt [5, 6, 8]. Another reason is that the kidney is required to reabsorb very high quantities of solutes (especially Na^+) and water, which requires high levels of mitochondrial respiration and ATP production.

Based on these characteristics, Fine et al. [9] proposed that hypoxia in the tubulointerstitium of diseased kidneys is the final common pathway toward end-stage kidney disease (ESKD). Indeed, various methods have revealed aggravated renal hypoxia in numerous animal models of CKD [1]. Furthermore, in several human studies, blood oxygen level-dependent magnetic resonance imaging

successfully demonstrated a correlation between renal oxygenation levels and estimated glomerular filtration rate [10]. The chronic hypoxia hypothesis has been validated by many groups including ours, and renal hypoxia in CKD is broadly accepted as a key player in ESKD development [11–14].

HIF

HIF, a key transcription factor that facilitates cellular adaptation to hypoxia, consists of α and β subunits. Of these, HIF-α has two major active isoforms, HIF-1α and HIF-2α. HIF-1α is primarily expressed in the tubular epithelial cells of the hypoxic kidney and functions as a master regulator of cellular adaptation to hypoxia [15, 16]. In contrast, the expression of HIF-2α in the hypoxic kidney is limited to the endothelial and interstitial cells. Recent studies have revealed that renal erythropoietin (EPO) is produced in fibroblast-like cells in the interstitium in a HIF-2α-dependent manner [17–19].

HIF-β is constitutively expressed, whereas HIF-α expression is significantly dependent on oxygen tension levels. Under normoxic conditions, prolyl hydroxylase domain-containing proteins (PHDs) hydroxylate specific proline residues on HIF-α, which allows it to be recognized by von Hippel–Lindau tumor suppressor protein (pVHL), to be polyubiquitinated and degraded in the proteasome [20, 21]. In contrast, under hypoxic conditions, HIF-α escapes PHD-mediated hydroxylation, translocates to the nucleus, and forms a heterodimer with HIF-β. HIF heterodimers bind to hypoxia response elements in the regulatory regions of >100 target genes (e.g., EPO, vascular endothelial growth factor (VEGF), and glycolytic enzymes), resulting in transactivation of these genes [22].

Under normoxic conditions, HIF-1α undergoes another post-translational modification by factor inhibiting HIF-1 (FIH-1), an asparaginyl hydroxylase that hydroxylates one specific asparagine residue of HIF-1α. This modification inhibits the binding of coactivators p300 and CREB-binding protein (CBP), resulting in repressed transactivation [1]. FIH-1 is abundantly expressed in distal tubules and podocytes [23], and it exhibits a lower K_m value for oxygen than PHD; thus, in a certain range of oxygen tension, HIF-1α that is stabilized by escaping degradation via the PHD/pVHL pathway still undergoes FIH-1-mediated, oxygen-dependent transactivational regulation (Fig. 1) [24].

Diverse regulatory mechanisms of HIF signaling: beyond PHD/FIH

Accumulating evidence suggests that HIF activity is finely tuned by various oxygen-independent pathways [24, 25]. Besides PHD-dependent HIF-α degradation, oxygen-independent HIF-1α degradation mechanisms exist. For example, the receptor of activated protein kinase C 1 (RACK1) competes with heat-shock protein (HSP) 90, which stabilizes HIF-1α, to bind to HIF-1α and elongin C, resulting in enhanced HIF-1α ubiquitination [26]. A role of HSPs and the carboxy terminus of HSP70-interacting protein (CHIP), an ubiquitin ligase, has also been shown in HIF-1α degradation [27–29]. Hypoxia-associated factor, an E3 ubiquitin ligase, also reportedly binds to HIF-1α and facilitates its proteasomal degradation even under normoxic conditions [30].

Post-translational modifications of HIF-1α and HIF-2α at specific residues, such as phosphorylation [31, 32], acetylation [33, 34], S-nitrosylation [35, 36], and sumoylation [37, 38], are suggested to affect HIF protein stability and transactivation activity; however, the exact mechanisms of these HIF modifications remain unclear, and further work is required to clarify the precise roles of these modifications.

HIF-α has also been demonstrated to undergo transcriptional and translational regulation [39, 40]. For example, bacterial products recognized by Toll-like receptors can upregulate HIF-1α messenger RNA (mRNA) levels in myeloid cells through a nuclear factor-κB (NF-κB)-dependent pathway [41]. T-cell receptor ligation on T lymphocytes also results in increased HIF-α mRNA levels [42, 43]. Furthermore, increased HIF-1α translation via the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway was also demonstrated in vascular smooth muscle cells [44] and cancer cells [45].

Noncanonical HIF regulation in the kidney

Noncanonical HIF regulation that was, until recently, believed to occur in tumor and immune cells [46] has recently been shown to occur in the intrinsic cells of kidneys (Table 1).

CCAAT/enhancer-binding protein δ (CEBPD) was shown to be induced in tubular epithelial cells via a HIF-1-independent pathway in both acute and chronic hypoxic kidneys [47]. It directly binds to the HIF-1α promoter and enhances its transcription. Importantly, CEBPD is also induced by inflammatory stimuli, such as interleukin-1β, resulting in increased HIF-1α expression even under normoxic conditions. These findings indicate that CEBPD is a potential role player in the association between inflammation and hypoxia in the kidney.

Recent studies have shown that HIF-2α is also regulated by several mechanisms in the kidney. Iron regulatory protein 1 (IRP1), which plays a central role in cellular iron metabolism regulation, is activated in iron-deficient cells and inactivated under hypoxia [48, 49]. Polysome profiling analysis revealed that a larger proportion of HIF-2α mRNA is translationally active in the kidney of *Irp1*−/− mice, resulting in increased renal EPO expression and marked polycythemia; thus, IRP1 may regulate HIF-2α protein levels through translational repression [50].

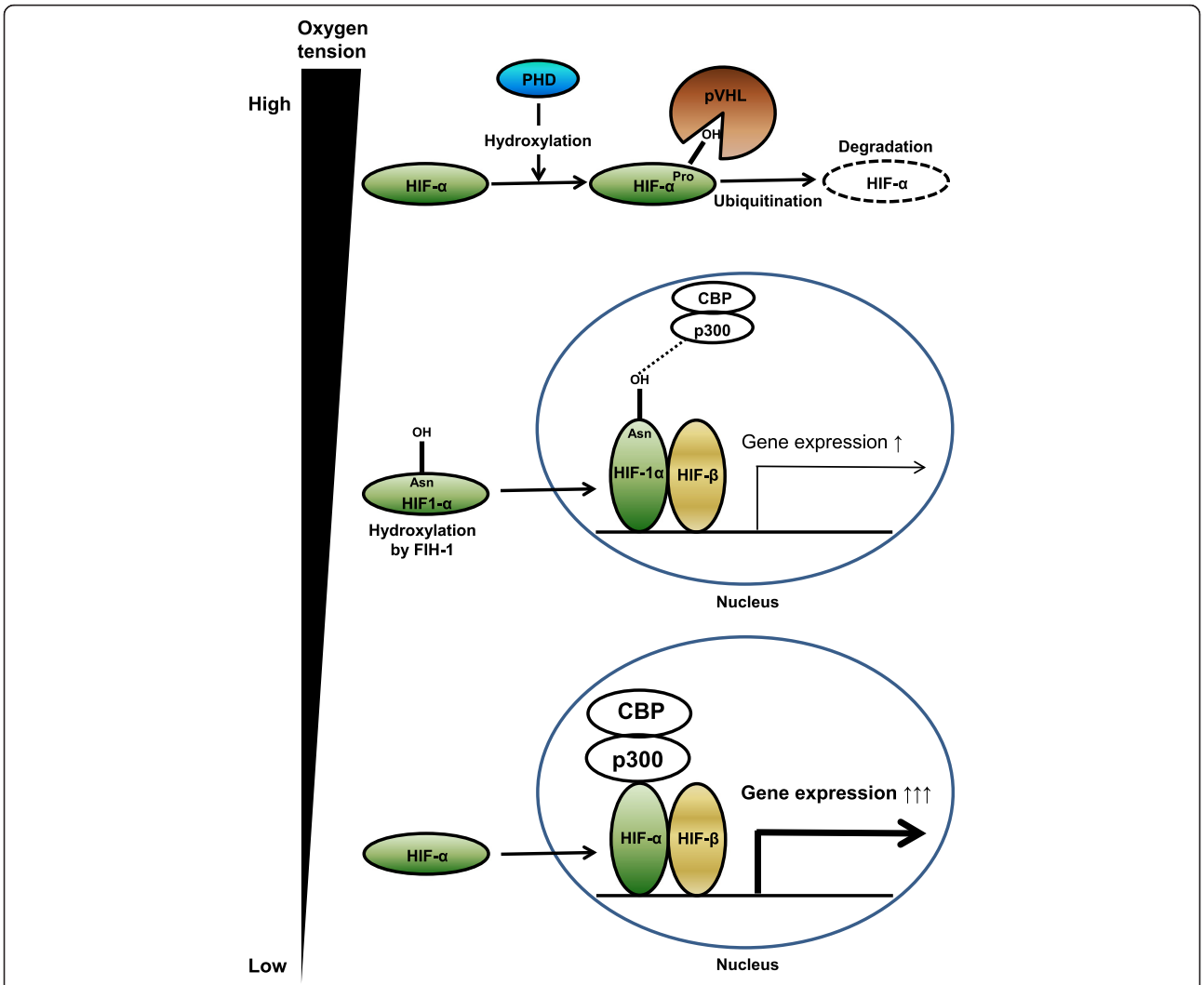

Fig. 1 HIF regulation by PHD and FIH-1. Under normoxic conditions, PHDs hydroxylate specific proline residues on HIF-α, which allows it to be recognized by pVHL, to be polyubiquitinated and degraded in the proteasome. In contrast, under hypoxia, HIF-α escapes PHD-mediated hydroxylation, translocates to the nucleus, and forms a heterodimer with HIF-β. HIF heterodimers bind to hypoxia response elements in the regulatory regions of target genes, resulting in transactivation of these genes. In addition, FIH-1, an asparaginyl hydroxylase, hydroxylates one specific asparagine residue of HIF-1α. This modification inhibits the binding of coactivators p300 and CBP, resulting in repressed transactivation. *PHD* prolyl hydroxylase domain-containing protein, *HIF* hypoxia-inducible factor, *pVHL* von Hippel–Lindau tumor suppressor protein, *FIH-1* factor inhibiting HIF-1, *CBP* CREB-binding protein

Acetylation/deacetylation of HIF-2α is also emerging as a key step in EPO gene expression. Garcia and colleagues [51, 52] demonstrated that acetyl CoA synthetase 2-dependent HIF-2α acetylation at specific lysine residues by CBP and formation of the CBP–HIF-2α complex are essential for the efficient induction of EPO in the kidney and liver under hypoxic conditions. They also showed that HIF-2α acetylation under hypoxia is reversed, i.e., deacetylated, by Sirtuin 1 (Sirt1) and that the deacetylation process augments rather than dampens HIF-2 signaling [53]. Although the precise mechanism is yet to be determined, this cyclical acetylation by CBP and deacetylation by Sirt1 appears to be necessary for efficient HIF-2 signaling during hypoxia rather than being only an on/off switch [52, 54].

Maladaptation to hypoxia during CKD progression

As discussed above, advanced renal hypoxia is observed in animal and human CKD. Despite several controversies, HIF accumulation has been shown to occur at certain stages during CKD, which is expected to protect against hypoxia [55–57]. Nevertheless, in many CKD patients, kidney hypoxia does not improve and is rather aggravated, and renal function shows a sustained decline,

Table 1 Noncanonical HIF regulation in the kidney

Cell type	Regulators	Principal changes
Tubular epithelial cell	Indoxyl sulfate [78]	HIF-1 transactivation activity ↓
	CEBPD [47]	HIF-1α mRNA ↑
	TGF-β1/Smad3/mTORC1 [95, 96]	HIF-1α protein ↑
EPO-producing cell	IRP1 [50]	HIF-2α protein ↓
Endothelial cell	LPS/NF-κB [97]	HIF-2α mRNA ↑
Mesangial cell	TGF-β1/Smad3/mTORC1 [98, 99]	HIF-1α protein ↑

HIF hypoxia-inducible factor, *CEBPD* CCAAT/enhancer-binding protein δ, *TGF-β1* transforming growth factor-β1, *Smad3* mothers against decapentaplegic homolog 3, *mTORC1* mammalian target of rapamycin complex 1, *EPO* erythropoietin, *IRP1* iron regulatory protein 1, *LPS* lipopolysaccharide, *NF-κB* nuclear factor-κB

resulting in ESKD. To date, several possible mechanisms have been proposed, which are discussed below (Fig. 2).

Sustained capillary rarefaction

Capillary rarefaction in the kidney is a common feature that is intricately linked to hypoxia in CKD [58]. In human kidney biopsy samples, capillary densities are significantly associated with renal function. Although HIF likely upregulates angiogenic factors, such as VEGF, that theoretically leads to the restoration of capillary densities, this adaptation mechanism usually fails; thus, capillary rarefaction is sustained and progressive. Several possibilities have been suggested to explain the failure of capillary restoration [59]. First, VEGF expression in the kidney is decreased in CKD, which may indicate that damaged tubular epithelial cells do not produce sufficient VEGF [60]. The inflammatory environment, which is intricately linked to CKD, may also suppress VEGF expression [61]. Second, antiangiogenic factors (e.g., thrombospondin 1 and endostatin) have been reported to be upregulated in several kidney diseases [61–63]. Third, the incompetence of endothelial progenitor cells potentially underlies insufficient capillary restoration [64], although recent studies have questioned the direct involvement of bone marrow-derived or circulating progenitor/stem cells in blood vessel regeneration [65, 66].

Increased oxygen consumption in tubules

Various factors are suggested to increase oxygen consumption in damaged tubules. Welch et al. [67, 68] demonstrated increased oxygen consumption and decreased oxygen levels in the kidneys of angiotensin II-infused or spontaneously hypertensive rats. These changes are probably because of oxidative stress induced by angiotensin II, based on the restoration of normal oxygen metabolism by the administration of tempol or an angiotensin II receptor blocker. Indoxyl sulfate, a representative uremic toxin, may also be involved in increased oxygen consumption in uremic kidneys via enhanced oxidative

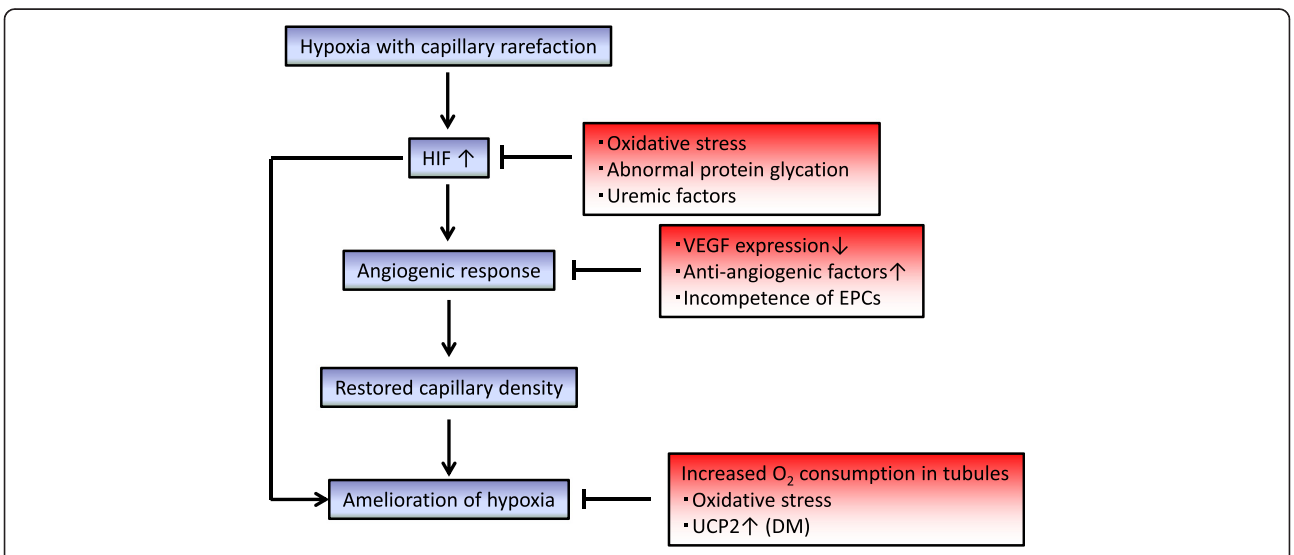

Fig. 2 Maladaptation to hypoxia during CKD progression. HIF accumulation occurring at certain stages during CKD is expected to protect the kidney against hypoxia (*blue squares on the left*). Nevertheless, in many CKD patients, kidney hypoxia does not improve, resulting in ESKD via several mechanisms (*red squares on the right*). Further details are explained in the text. *HIF* hypoxia-inducible factor, *DM* diabetes mellitus, *VEGF* vascular endothelial growth factor, *EPC* endothelial progenitor cell, *UCP2* uncoupling protein 2

stress [69]. Moreover, in diabetic kidney disease, upregulated mitochondrial uncoupling protein-2 is suggested to increase oxygen consumption in exchange of reducing oxidative stress [70, 71].

Impaired HIF activation

Activation of HIF in the kidney may be suboptimal in CKD despite profound renal hypoxia. This concept is best exemplified in diabetic kidneys [72, 73] but may apply in CKD of nondiabetic etiologies.

A large body of evidence suggests that cellular adaptation to hypoxia is impaired in the diabetic milieu and that deregulated HIF-1α may be a significant contributor [74, 75]. Methylglyoxal, a highly reactive dicarbonyl metabolite that is increased in diabetes, has been shown to be a key player in the impairment of the HIF-1 pathway. Methylglyoxal modifies specific arginine residues in HIF-1α and blocks heterodimer formation with HIF-1β [76]. The interaction between HIF-1α and p300 is also inhibited by methylglyoxal via modification of an asparagine residue at p300 [77]. In addition to the functional suppression of HIF-1, methylglyoxal may inhibit HIF-1 activity via enhanced degradation. Bento et al. [29] demonstrated that methylglyoxal increased association of HIF-1α with HSP40 and HSP70, leading to CHIP recruitment and polyubiquitination of HIF-1α. This may be because of the increased levels of modified and monomeric HIF-1α resulting from the inhibited association of HIF-1α with HIF-1β and p300.

Additional mechanisms of the suppression of HIF activation in advanced CKD, including nondiabetic etiologies, have been proposed. We previously reported that at clinically relevant concentrations, indoxyl sulfate upregulated CBP/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 2 (CITED2) via post-transcriptional mRNA stabilization, which in turn inhibited the interaction between p300 and HIF-1α C-terminal transactivation domain, resulting in suppressed HIF-1 transactivation activity [78]. Deficient HIF-1 transcriptional activity may also be caused by a decrease in the expression of p300/CBP-associated factor, which is observed in adipose tissue-derived mesenchymal stem cells of dialysis patients as compared with those of nondialysis patients [79].

Link between hypoxia and epigenetic changes in CKD

With recent technological advances in epigenetics, much attention is currently focused on the pathophysiological roles of epigenetic changes in CKD, specifically sustained CKD progression and AKI-to-CKD transition [80, 81]. Accumulating data have demonstrated that hypoxia can induce epigenetic changes to promote profibrotic and proinflammatory gene expression [82]. Indeed, under hypoxic conditions, HIF-1 binds to the promoters of genes encoding two histone demethylases, JMJD1A and JMJD2B, to induce their expression, which in turn enhances the expression of downstream genes by decreasing promoter histone methylation [83, 84]. Chromosome conformation capture assay has revealed that chromatin conformation may dramatically change depending on the interaction of HIF-1 with lysine-specific demethylase 3A, leading to the removal of a suppressive histone mark and upregulation of *SLC2A3* [85].

HIF stabilization as a treatment of CKD

Given that renal hypoxia is a final common pathway in CKD progression and that HIF activation in CKD appears to be suboptimal, HIF stabilization presents a reasonable target for CKD. This concept has been supported by numerous studies using several animal models of CKD [86–88]. Recently, Palm and colleagues [89] demonstrated that the administration of cobalt ameliorated kidney damage in streptozotocin-induced diabetic rats, which was accompanied by increased oxygen tension in the kidney. However, these studies have several limitations. Polycythemia, which is frequently observed in studies with chronic HIF stabilization, may have confounding effects on the kidney. Moreover, cobalt, which has been used to stabilize HIF in numerous studies, has systemic effects, such as body weight loss, with <30 % overlap of gene expression changes by treatment with cobalt and hypoxia [90, 91]. Therefore, the usefulness of HIF stabilization in CKD should be investigated in animal and clinical studies by using specific PHD inhibitors, which are being developed as therapeutics against renal anemia.

Taking into consideration that inappropriate HIF activation may lead to serious adverse effects, such as fibrogenesis [92] and tumorigenesis [93], several conceptual hurdles need to be overcome; it is clear that the HIF stabilization approach necessitates several critical parameters to be determined, i.e., to what extent, when, and where. It should be noted that genetic manipulation generally results in more profound phenotypes than pharmacological intervention, and the consequences of pharmacological HIF activation by PHD inhibitors and genetic manipulation of *Phd*, *Hif*, or *Vhl* could yield inconsistent findings. In addition, the optimal timing for HIF stabilization should be determined because the outcomes of HIF stabilization can be both beneficial and detrimental, depending on its timing [94]. Lastly, because HIF plays different roles in various cell types in the kidney, the consequences of systemic HIF stabilization may vary widely, depending on the context. Cell type-specific HIF stabilization is certainly an attractive idea to achieve a safe and effective treatment; however, a much deeper understanding of HIF

regulation in each cell type (e.g., cell type-specific transcriptional coactivators) is required.

Conclusions

Renal hypoxia is now regarded as a key player in the progression of CKD. HIF is a master regulator that helps cells to cope with hypoxia, and additional mechanisms of HIF regulation, other than those mediated by PHD/FIH, have recently been clarified in the kidney. Although HIF most likely accumulates at certain stages during CKD pathogenesis, maladaptation to hypoxia frequently occurs in CKD via several mechanisms, which include sustained capillary rarefaction, increased oxygen consumption in tubules, and impaired HIF activation, culminating in progression to ESKD. Considering experimental evidence that suboptimal HIF activation is observed in CKD, its stabilization is a new and promising therapeutic target in CKD; however, a deeper understanding of the mechanisms of HIF regulation and its potential protective role in the diseased kidney are essential for its safe and efficient clinical application in the future.

Competing interests

M. N. serves as an advisor of GSK, Taisho, and Astellas.

Authors' contributions

ST, TT, and MN drafted the manuscript. All authors read and approved the final manuscript.

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