Renoprotective Role of Hypoxia-Inducible Factors and the Mechanism

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Keywords

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Abstract

Background: The kidney requires abundant blood supply, and oxygen is transmitted by diffusion through blood vessels. Most physiological metabolism of the kidney depends on oxygen, so it is very sensitive to oxygen. An increasing pool of evidence suggests that hypoxia is involved in almost all acute and chronic kidney diseases (CKDs). Vascular damage, tubular injury, and fibrosis are the main pathologies associated during hypoxia. Hypoxia-inducible factors (HIFs) are the main mediators during hypoxia, but their functions remain controversial. This article reviewed recent studies and described its mechanisms on renoprotection. **Summary:** HIF is degraded rapidly during under normal oxygen. But under hypoxia, HIFs accumulate and many target genes are regulated by HIFs. Homeostasis during injury is maintained through these genes. Pretreatment of HIF can protect the kidney from acute hypoxia and can improve repair, but HIF's role in CKD and in renal tumor is still controversial. Due to its mechanism in kidney disease, many drugs toward HIFs are widely researched, even some of which have been used in clinical or in clinical research. **Key Messages:** In this review,

we described the known physiological mechanisms, target genes, and renal protective roles of HIFs, and we discussed several drugs that are researched due to such renal protective roles.

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Introduction

Renal blood flow is responsible for approximately 25% of the total cardiac output, of which 90% flows to the renal cortex and 10% to the medulla. Oxygen that is transmitted by diffusion through blood shunts from artery to vein [1]. A total of 90% of the ATP of renal physiological metabolism depends on oxygen [2]. Thus, the kidney is vulnerable to hypoxia caused by limited transportation of oxygen, especially to the medulla. Most of the ATP produced depends on aerobic metabolism. When the kidney is subject to hypoxia, the mitochondrial function decreases, the renal tubule swells, and inflammation leads to the damage of renal capillaries, eventually resulting in acute kidney injury (AKI). Improper recovery after kidney injury causes chronic renal diseases [3]. According to the “chronic hypoxia hypothesis” proposed in the 1990s [3], glomerular diseases lead to insufficient blood supply and

to vascular endothelial injury around the glomeruli and tubules, thus ruining the capillary network around the tubules and resulting in a hypoxic microenvironment. Continuous hypoxia causes tubular damage, inflammation response, and interstitial fibrosis. The accumulation of extracellular matrix (ECM) in turn destroys the surrounding tissue, affecting adjacent capillaries and tubules and further expanding the scope of hypoxia, eventually leading to renal scars and the decline of renal function.

Hypoxia-Inducible Factor

The hypoxia-inducible factor (HIF) is the main regulator of homeostasis during hypoxia. HIF is a heterodimer that contains HIF- α and HIF- β , which are 2 helix-loop-helix proteins. The α -subunit is influenced by oxygen concentration, while the β -subunit is not sensitive to oxygen. HIF- α is generally classified into HIF-1 α , HIF-2 α , and HIF-3 α . HIF-1 α participates in the acute phase, while HIF-2 α and HIF-3 α are more involved in chronic hypoxia. HIF-3 α lacks the C-terminal transactivation domain compared to the other 2. HIF-3 α can participate in gene transcription and can inhibit the PAS domain protein, though its comprehensive role needs to be further elucidated [4]. The basic structure and function of HIF-1 α is 48% similar to HIF-2 α , but these 2 have different spatial distributions and play different biological roles during hypoxia. HIF-1 α is mainly situated in the renal tubular epithelium, whereas HIF-2 α is principally located in stromal cells and endothelial cells [5].

Under normal oxygen levels, HIF is hydroxylated by prolyl-4-hydroxylases (PHD), involving molecular oxygen, ferrous ions (Fe^{2+}), 2-oxoglutarate, and ascorbic acid in this process. The hydroxylated α -subunit then binds to E3 ubiquitin ligase von Hippel-Lindau (VHL) protein, activating the ubiquitin ligase system and degrading HIF-1 α rapidly through the proteasome. Therefore, the half-life of HIF is limited. Otherwise, HIF-1 is also regulated by another HIF-1 hydrolase factor called inhibiting HIF (FIH). It can hydroxylate the asparagine residue 803, affecting the binding of HIF and coactivators such as p300 and CREB binding protein, finally impacting HIFs transcription. Both FIH and PHD belong to the same family. In addition, HIF is translated and transcribed by many cytokines, heat shock protein, protein kinase C, growth factor signaling pathways, and some exosomes [4, 6] (shown in Fig. 1).

During hypoxia the function of PHD is decreased. HIF- α accumulates in the cytoplasm, translocates to the

nucleus, and combines with HIF- β in hypoxia-response elements of the nuclear target gene to regulate gene transcription by the interaction of P300, CBP, and phosphorylated Signal Transducer and Activator of Transcription 3 (p-STAT3) (shown in Fig. 1).

HIF Target Genes and Roles in Renoprotection

The physiological functions of HIFs rely on the activation of their target genes. HIFs can stimulate the transcription of multiple genes, such as vascular endothelial growth factor (VEGF), glucose transporter (GLUTs), erythropoietin (EPO), transforming growth factor- β 1 (TGF- β 1), heme oxygenase-1 (HO-1), and so on. These genes can protect renal injury during hypoxia including facilitating angiogenesis, regulating inflammatory response, promoting glycolysis, and maintaining mitochondrial homeostasis.

Erythropoietin It is the main target gene of HIF produced in many organs and tissues, especially the kidney. EPO is released mainly by cortical peritubular cells in the kidney; it is regulated by HIF-2 α , but not HIF-1 α ; and relies on oxygen concentration and hemoglobin level. HIF-2 α stimulates EPO production. EPO suppresses the hepcidin level, upregulates transferrin, promotes iron absorption and erythropoiesis, and improves the hemoglobin level [7]. At the same time, EPO has an antiapoptotic effect, which can protect cells from damage caused by hypoxia. In some countries, renal anemia caused by EPO deficiency in chronic kidney disease (CKD) has been clinically treated by PHD inhibitors.

Vascular Endothelial Growth Factor

It can promote the construction of capillary network and endothelium, maintain their integrity, and support angiogenesis by binding with VEGF receptors (VEGFR). Both HIF-1 α and HIF-2 α can regulate VEGF. The decrease of VEGF is related to the progressive damage of capillaries around the tubules. During hypoxia, VEGFA (VEGF_{164}) increases significantly at 6 h and reaches a peak at 12 h in podocyte; then it combines with VEGFR2 in glomerular endothelial cells to reduce kidney injury and is upregulated by HIF-1 α [8]. However, VEGF also improves the blood supply of oxygen-deficient microenvironment in renal cancer, promoting cancer progression.

Heme oxygenase-1, another target gene regulated by HIF-1 α , HO-1, is a microsomal membrane enzyme with promoting mitochondrial dynamics, anti-inflammatory,

Fig. 1. Schematic diagram of HIF regulation under normal oxygen/hypoxia. Under normoxic condition, HIF- α is hydroxylated by PHD, then binds with pVHL and is degraded. It is also regulated by FIH, hydroxylated by the asparagin residue 803, which is also eventually degraded. During hypoxia, PHD is inhibited, which results in HIF- α accumulation. HIF- α combines with HIF- β in nucleus to mediate target genes transcription. HIF, hypoxia-inducible factors; PHD, prolyl-4-hydroxylases; FIH, factor-inhibiting HIF; VHL, von Hippel-Lindau protein; EPO, erythropoietin; VEGF, vascular endothelial growth factor; HO-1, heme oxygenase-1; NOS, nitric oxide synthase; GLUTs, glucose transporter.

and antiapoptotic effects. The level of HO-1 is 4–5 times higher than normal during hypoxia in proximal tubular cell [9]. HO-1 can convert heme into NO, free iron, and biliverdin. NO can induce vasodilation and can decrease the oxygen consumption of renal tubules. Biliverdin can scavenge free radicals, as well as antioxidant, and can antagonize ROS production by heme, reducing ferroptosis [10, 11].

NOS Pathway

The nitric oxide synthases (NOSs) family includes neuronal NOS, i (inducible) NOS, and endothelial NOS, which have increased levels in hypoxia or ischemia. In the past, these were generally considered as pro-inflammatory factors. During kidney injury, iNOS and NO interact with HIF-1 α , which plays an important role in preventing tubulointerstitial injury [12]. However, it has been reported that activated NO released by iNOS can aggravate renal injury and inhibition of iNOS and NO production can reduce renal injury [13]. Some researchers believe that short-term and low-dose NO may protect the kidney, while high-dose and long-term NO exposure may reduce glutathione, ultimately resulting in kidney damage [14]. Otherwise, HIF-1 α also interacts with the expression of neuronal NOS to improve cerebral blood flow and to regulate the fluid and electrolyte balance of collecting tubules and proximal tubules [15, 16]. endothelial NOS upregulated by HIF-2 α also promotes NO release, facilitates vasodilation and the normalization of neovascularization, improves renal flow, reduces endoplasmic reticulum

stress, and prevents neuronal death, ultimately protecting the kidney [16, 17]. The function of NOS pathway needs further research.

Glucose Transporters This is another target gene of HIF that encodes a protein family that promotes glucose transportation mainly in the cell plasma membrane of the glomerulus. It transports glucose through a concentration gradient inside and outside the plasma membrane, and it mediates the regulation of transport rate [18]. The increase of GLUT-1 can promote glucose absorption and can increase the deposition of ECM and mesangial cell hypertrophy, which may play a role in diabetes and diabetic nephropathy [19].

The Effects and Underlying Mechanisms of HIFs in Renoprotection

Due to the many target genes of HIFs, different target genes can regulate the signaling pathway to affect kidney injury. Otherwise, HIFs can mediate many cytokines, which also can prevent renal fibrosis. Herein, we review the mechanism of HIFs regulation (shown in Fig. 2).

Promotion of Angiogenesis

The damage and abnormal repair of vascular endothelium are involved in renal injury. Continuous renal hypoxia leads to the lessening of capillaries around renal tubules, which further aggravates the hypoxic microenvironment. HIF-1/2 α plays an essential role in the compen-

Fig. 2. Overview of the mechanism & effect of HIFs on renoprotection Due to PHD's function decreased, HIF accumulates during hypoxia. It mediates many target genes, multiple signal networks to maintain homeostasis in many kidney diseases. PAI-1, plasminogen activator inhibitor-1; PP1RC3, protein phosphatase 1 regu-

latory subunit 3C; PLIN2, perilipin protein; BNIP3, BCL2/adonovirus E1B-interacting protein 3; PHD, prolyl-4-hydroxylases; HO-1, heme oxygenase-1; EPO, erythropoietin; VEGF, vascular endothelial growth factor; HIF, hypoxia-inducible factors; AKI, acute kidney injury; CKD, chronic kidney disease.

satory mechanism of promoting angiogenesis. HIF-1 α can promote the foundation and survival of a primitive vascular network, while HIF-2 α is mainly involved in vascular system maturation and remodeling stability, triggering cell migration and adhesion [4]. HIF-1 α promotes VEGF, VEGFR2, and stromal-derived factor-1 expression by directly affecting the VEGF gene or via miR-210 exosomes [20]. HIF-1 α also can restrain the expression of endogenous angiogenesis inhibitor thrombospondin-1, which increases capillary-like endothelial tube foundation and increases vascular density [21]. Furthermore, not only HIF-1 α can affect angiogenic factors, but it also can increase the infiltration of circulating angiogenic cells, such as endothelial progenitor cells, hematopoietic stem cells, angiogenic paracrine factors, and epidermal keratinocytes [22, 23]. All of these participate in the promotion of vascular remodeling and angiogenesis. HIF-2 α is equally vital for angiogenesis. HIF-2 α can directly and more strongly induce VEGF and VEGFR, cooperating with angiogenic factor ETS-1 and plasminogen activator inhibitor-1 to promote the proliferation of vascular endothelial cells and thus vascular stability [24]. A severe vascular malformation was detected in HIF-2 α defective mouse embryos. Liu et al. [25] research via RNA seq on endothelial cells found that HIF-1/2 α promote several ly-

sine (K)-specific demethylases (KDMs) transcription, especially KDM4B and KDM6B, which regulate VEGF expression, angiogenesis, and facilitate cell proliferation and tube formation. In conclusion, HIF-1/2 α both affect angiogenesis.

Maintenance of Mitochondrial Homeostasis

The volume of the kidney is <1% of the total body weight, but its energy consumption in the resting state accounts for about 7% of the total energy requirement, which is secondary to that of the heart [26]. Due to the enormous energy demand, the kidney needs a large number of mitochondria to participate in the kidney transport function and to maintain the water and electrolyte balance. Hence, mitochondrial homeostasis is essential for renal function. In renal ischemia-reperfusion injury or inflammation, damage occurs to the mitochondrial membrane. Water inflow leads to mitochondrial swelling, fusion, and fission disorder, causing mitochondrial dysfunction and ultimately resulting in increased ROS and decreased ATP production [27]. HIF-1 α has been proven to regulate mitochondria and to reduce damage. It is widely known that mitochondria maintain homeostasis through autophagy. However, in AKI, CKD, and other kidney diseases, the HIF-1-BNIP3 (BCL2/adonovirus

E1B-interacting protein 3) signaling pathway promotes mitochondrial autophagy, inhibits HK-2 cell apoptosis during hypoxia reperfusion injury, and alleviates renal injury and apoptosis [28]. HIF-1 α also downregulates the level of exosomes miR-668 and restrains mitochondrial protein 18 kDa expression to preserve mitochondrial function [29].

The mitochondrial transcription factor TFAM is downregulated by HIF-1 α to mediate mitochondrial replication and transcription in cardiomyocytes, which increase the volume of mitochondria and lower their oxygen consumption [30, 31]. Regarding HIF-2 α , its deficiency may lead to the decrease of AOE's gene expression, the destruction of mitochondrial homeostasis and the increase of ROS, while exogenous transfection of HIF-2 can improve this phenomenon [32]. Overall, both HIF-1 α and HIF-2 α can mediate mitochondrial homeostasis and thus can maintain kidney function.

Mediation of Glycolysis

Under hypoxia, disturbances to microcirculation and decreased blood supply lead to impaired oxygen transfer to islets, to lowered insulin secretion, and subsequently to decreased ability of ATP production and cell damage. Many studies have proven that HIF-1 α can promote the release of pyruvate dehydrogenase kinase, can convert pyruvate to acetyl coenzyme A and can enter the citric acid cycle, eventually enhancing glycolysis [33]. At the same time, HIF-1 α can upregulate tissue glucose transporters and enzymes to improve glucose tolerance and to promote the conversion of glucose to lactic acid, compensating the ATP synthetic deficiency. HIF-1 and HIF-2 also can mediate macrophages in adipocytes and can promote the expression of GLUT4, which can increase the sensitivity of insulin in diabetic nephropathy [34]. HIF stimulates genes encoding glycogen synthetic enzymes (*UGP2*, *GBE1*, *PGM1*, etc.) and regulates the protein phosphatase 1 regulatory subunit 3C to restrain the activity of glycogen phosphorylase and to facilitate the storage of energy and glucose as glycogen. Thus, in long-term hypoxia, the utilization rate of ATP is increased [35].

Regulation of Fat Metabolism

Fatty acids (FA), such as nonacylated FAs, are intermediate products of fat metabolism. All nephrons can absorb FAs from blood circulation, especially in proximal tubular cells. The degradation of absorbed FA produces ATP through mitochondria and peroxisomal β -oxidation. Under normoxia, FA is in a state of dynamic balance in renal tissue. The excess FA is stored in lipid droplets,

while lipolysis by lipase produces FA to supply cell activities during starvation. HIF does not affect the morphology and quantity of FA. However, during hypoxia, FA oxidation is insufficient, ATP production is reduced, and cell damage may occur. In this stage, HIFs can mediate FA uptake, metabolism, and oxidation [36]. Under hypoxia, HIF-1 α promotes extracellular FA uptake and influx through transcription factor PPAR γ and FA-binding protein 3, 4, 7 [37, 38]. FA synthesis is supported by sterol regulatory-element binding protein, which is activated by HIF-1 [39]. HIF-2 α also stimulates lipid droplet-related protein perilipin protein expression, as well as lipid droplet formation and accumulation [40]. In addition, HIF-1/2 can promote a variety of circulating factors, such as AdipoR1, can activate the energy sensor AMP-activated protein kinase (AMPK) signaling pathway, and later on can promote lipid metabolism and restrain endoplasmic reticulum inflammation without cell injury [6, 40, 41]. However, some researchers have reported that HIF may increase the accumulation of lipid droplets, while the dysfunction of lipid utilization leads to further accumulation of lipids, ultimately resulting in the production of ECM and renal fibrosis [42]. The effects of HIF on lipid metabolism have 2 aspects, which need further clarification.

Control of Ferroptosis

Ferroptosis, a kind of cell death relay dependent on iron, is different from known forms of cell death, such as apoptosis or autophagy. Ferroptosis involves lipid oxidation metabolism and iron homeostasis and participates in the occurrence and development of tumors, ischemia-reperfusion injury, and nervous disease [43].

Its mechanism includes 3 main parts. First, the inhibition of system X_c causes the decrease of glutathione GSH synthesis, which directly or indirectly leads to declined glutathione peroxidase 4 activity and antioxidant capacity. Lipid peroxide cannot be metabolized by peroxidase-catalyzed reduction reaction, resulting in membrane lipid oxidation and loss of cell membrane integrity. Second, iron absorption is ceased and iron outflow is reduced. Fe²⁺ oxidizes lipids and a large amount of ROS is produced. Third, the synthesis of FA increases resulting in free FA accumulation in cells. Mitochondrial cristae reduce or disappear, causing dysfunction, which leads to a large number of ROS. The accumulation of ROS, FA and lipid peroxides eventually induce ferroptosis [43].

Martin-Sanchez et al. [44] pointed out that ferroptosis is the main pathway of renal tubular cell death in AKI induced by folic acid. In the glutathione peroxidase 4

knockout-induced ferroptosis model, the incidence and mortality of AKI increase significantly, while the ferroptosis inhibitor FER-1 and HO-1 can prevent the injury of renal tubular cells [45, 46]. As is widely known, HO-1 is the target gene of HIFs. This may indicate that HIFs are involved in ferroptosis. HIFs can be stable, can mediate mitochondrial activity, and can lessen ROS formation through the HIF-PHD pathway regulated by a circadian transcription factor (ARNTL) and iron chelator [47]. HIF-2 can promote the autophagy of injured peroxisome [48], can release of hepcidin, and can restrain the output and accumulation of iron, which eventually prevents ferroptosis. However, some researchers suggest that HIF has nothing to do with the protective effect against ferroptosis, but only the decrease of activating transcription factor 4 which is mediated by HIFs [49]. The clarification of the role of HIF in ferroptosis requires further investigation.

Regulation of Inflammatory Response

During hypoxia, organisms can induce the release of inflammatory factors leading to cell inflammation, while inflammation also can result in hypoxic microenvironment. During inflammation, the metabolism of immune cells, such as neutrophils, lymphocytes, and macrophages, is activated; the oxygen consumption of these cells increases; glycolysis intensifies; and inflammatory factors, such as IL-1 and TNF, are released, which in turn stimulate HIF activation. Conversely, under hypoxia, the HIF pathway can regulate inflammatory factors [50].

HIF-1 α can restrain glomerular chemokine (C-C motif) ligand 2/monocyte chemoattractant protein-1 expression, which can reduce the infiltration of macrophages, can alleviate the inflammatory reaction, and can protect the glomerular endothelium and epithelium, eventually reducing proteinuria [51]. HIF-1 α also can affect T lymphocytes to prevent tissue cell damage by inducing helper T cells to transform into Treg cells and restrain the immune response of Treg cells [52]. On the contrary, interference with HIF-1 α may activate the nuclear factor- κ B pathway and may release inflammatory factors (IL-1 β , monocyte chemoattractant protein-1, and so on) through the decrease of miR127-3P, which may lead to abnormal proliferation of HK-2 cells and aggravation of cell inflammation and may result in an abnormal renal repair and chronic injury [53].

Not only HIF-1 α but also HIF-2 α mediates neutrophils in inflammation and promotes macrophages to activate M2 to regulate adaptive immunity, to promote angiogenesis and wound healing, which may lead to tumor progression [54, 55]. However, research toward HIF-2 α has been relatively poor.

Some studies pointed out that HIF-1 α may aggravate the inflammatory reaction. Li et al. [56] found that HIF-1 α increases exosome miR-23a to activate the NF- κ B pathway of macrophages, which further exacerbates the tubulointerstitial aspect of chronic renal interstitial inflammation. More researches obviously are needed to clarify the function of HIF in different diseases.

HIF and Kidney Disease

Acute Kidney Injury

Many factors, such as major surgery, renal hypoperfusion, severe infection, or chemotherapy drugs, may cause the reduction of renal and urinary function and the rise of creatinine and urea nitrogen. Different indicators may be related to different pathological mechanisms, but the main cause is similar. In kidney injury, pro-inflammatory mediators and bioactive factors are released, which increase the permeability of endothelial cells causing vasoconstriction and vascular injury and leading to diminished vascular density. This eventually results in ischemia and hypoxic microenvironment in renal tubules, especially medullary tubules. Otherwise, hypoxia in turn activates the immune response, which may cause further damage to the kidney [57].

Hypoxia plays a role in the mechanism of almost all types of kidney injury. The level of HIF increases significantly about 2–8 h after injury, exerts a protective effect, and then lowers. It increases again during reoxygenation/reperfusion, participating in the repair [2]. Due to the protective effect of HIFs, HIF-1/2 pretreatment may reduce acute injury (Table 1). The stabilization of HIF-1/2 may reduce the infiltration of CD3⁺ T cells and macrophage infiltration and that of cytokines such as TNF and chemokine (C-C motif) ligand 2. It also facilitates the release of EPO, promotes cell regeneration, reduces apoptosis, and ultimately improves renal function. Many studies have proved that such as hypoxia/reperfusion injury and experimental infection models [58, 59]. HIF-1 α was proven to play a protective role in preventing connective tissue growth factor synthesis, which can induce renal fibrosis in short-term hypoxia. However, this function was not observed in long-term hypoxia [60].

Pretreatment by HIFs can improve renal injury. When mice are already exposed to hypoxia, a single addition of PHD inhibitor can activate HIFs, but PHD inhibitor has no effect on improving cell function [58]. The repeated addition of PHD inhibitor may improve the oxidative damage, apoptosis, and prevent tubular necrosis at 2 h, 6 h, and 10 h [61].

Table 1. The target genes in different cells and effect of HIFs

Disease	Ref	Cells	HIF	Target genes	Pros	Cons
AKI	Fu et al. [28]	Tubular cells	HIF-1 α	BNIP3 $\uparrow$	Mitochondrial mitophagy, protecting from apoptosis and ROS production	
	Wang et al. [8]	Podocytes	HIF-1 α	VEGFA $\uparrow$	Protect GenCs via vegf/vegfr2 paracrine signaling	
	Wei et al. [29]	Proximal tubular cells	HIF-1 α	MIR-6681 $\uparrow$	Restrains MPT18 to protect mitochondrial function	
	Wang et al. [58]		HIF-2 α	EPO $\uparrow$ Hepcidin $\downarrow$	Improve erythropoietin production	
Lasting hypoxia	Schindler et al. [59]		HIF-1 α	CD3 $^{+}$ T cells $\downarrow$ KIM1, EPO $\uparrow$, VEGF $\uparrow$	Reduce microalbuminuria, improved renal function	
	Kroening et al. [60]	Proximal tubular epithelial cells	HIF-1 α	CTGF $\downarrow$	Protect from renal fibrosis in short-term hypoxia	
	Kushida et al. [72]	HK-2 cells	HIF-1 α	Activates the TGF- β /SMAD3 pathway		Promote kidney injury, upregulate genes related to fibrosis
	Kobayashi et al. [68]	Renal epithelial cells	HIF-1 α	CC12/MCP-1 $\downarrow$	Anti-inflammatory	
CKD	Uchida et al. [31]		HIF-1 α	MHC/ α -MHC $\downarrow$	Postpones left ventricular hypertrophy	
	Du et al. [73]	Renal epithelial tubular cells	HIF-1 α	Promote PI3K/Akt pathways		Facilitate EMT, promote renal fibrosis
	Kapitsinou et al. [71]	Endothelial cells	HIF-2 α	VCAM1 $\downarrow$	Prevent renal inflammation and promote repair	
DN	Jiang et al. [9]	Proximal tubular cell	HIF-1 α	HO-1 $\uparrow$	Mitochondrial dynamics; mediate iron content, anti-apoptotic, anti-inflammatory	
	Sugahara et al. [51]	Murine mesangial cells	HIF-1 α	Suppress mesangial CCL2/MCP-1 pathway	Reduce macrophage infiltration, improve glomerular endothelium and epithelium damage	
RCC	Schokrpur et al. [77]	Renal cell	HIF-1 α	POSTN $\uparrow$ PPEF1 $\uparrow$ SAMS1 $\uparrow$ TNFSF13B $\uparrow$	Reduce proliferation, increase migratory and invasive capacity	
	Qiu et al. [40]	Renal cell	HIF-2 α	PLIN2 $\uparrow$	Promote lipid storage, prevent cytotoxic ER stress, and maintain ER homeostasis	

PLIN2, perilipin protein; BNIP3, BCL2/adenovirus E1B-interacting protein 3; HO-1, heme oxygenase-1; EPO, erythropoietin; VEGF, vascular endothelial growth factor; HIF, hypoxia-inducible factors; CKD, chronic kidney disease; CTGF, TGF- β 1, transforming growth factor- β 1; CCL2, chemokine (C-C motif) ligand 2; MCP-1, monocyte chemoattractant protein-1; RCC, renal cell carcinoma; VCAM1, vascular cell adhesion molecule-1; POSTN, periostin; AKI, acute kidney injury.

Glomerular Injury

Hypoxia was shown to induce significant damage to glomerular endothelial cells *in vitro*. In the early stage of hypoxia, HIF-1 α can promote VEGF transcription and angiogenesis and can attract endothelial cell precursors to the immature glomerular vascular space, which finally induces the differentiation and maturation of glomerular endothelial cells [8]. However, the release of HIFs is relatively insufficient when continuous hypoxia exceeds 24 h. In this case, the level of VEGF decreases, the microcirculation of glomerular endothelial cells is disturbed, and microvilli are lost. When hypoxia lasts for 3 days, HIF-1 α almost cannot be detected in glomeruli, leading to injury progression. The glomerular damage can be reduced by pretreatment to stabilize HIF-1 α [62]. Otherwise, HIF-2 α also can ease kidney injury. It had a similar function to HIF-1 α in a high-dose folic acid-induced kidney injury model [63]. It facilitated VEGF transcription to improve renal oxygen supply and blood flow, which could reduce glomerular injury. In other words, both HIF-1 α and HIF-2 α can effectively control glomerular damage.

Chronic Kidney Disease

There is a strong correlation between AKI and CKD. Repeated kidney injury and abnormal renal repair lead to the loss of capillaries around tubules and to the decrease of capillary density, which further aggravates interstitial hypoxia and eventually result in the apoptosis of tubular cells. In addition, hypoxia can stimulate TGF- β , which can promote the activation of fibroblast and ECM protein, resulting in fibrosis and ultimate CKD [64]. The degree of fibrosis in this case is close to progression.

The upregulation of target genes (VEGF, transferrin receptor) by HIFs was not as high as expected in CKD, which may be due to oxidative stress and metabolic wastes [64]. In the streptozotocin-induced diabetic nephropathy model, when the group was treated with superoxide dismutase mimetic, the levels of HIF-1 α , HIF-2 α , and HO-1 were significantly improved [65]. Zhu et al. [66] found that in a model of renal artery stenosis, the level of HIF-1 α and VEGF in groups treated with antioxidants was higher than that without antioxidants, suggesting that oxidative stress affects HIFs release in chronic hypoxia. Otherwise, the accumulation of abnormal protein, which is glycated by methyl glycol through α subunit, can inhibit the activity of HIF-1 α in CKD [67]. Uremia and indoxyl sulfate also affect HIFs transcription.

The role of HIF- α remains controversial in CKD. In a mouse model with unilateral ureteral obstruction induced

by exogenous VHL deficiency, the sustained activation of HIF-1/2 could downregulate the expression of chemokine receptor in macrophages, could reduce inflammation, and could improve fibrosis [68]. This conclusion is similar to that for chronic tubulointerstitial inflammation induced by cisplatin and adenine. A stable level of HIF-1 α significantly reduces monocyte-macrophage infiltration and cytokines such as IL-6, IL-1 β , and TNF- α , and lowers the level of cleaved caspase-3, Bax, and KIM-1, which can restrain apoptosis and can prevent kidney injury and apoptosis [69]. Pyo et al. [70] also confirmed it in renal tubular epithelial cells through RNA seq. HIF-2 α restrains vascular cell adhesion molecule-1 to prevent renal inflammation and to promote repair [71]. End-stage renal disease, cardiac structural change, and function loss are the leading causes of death. HIF can improve cardiac structure through different effects. HIF increases VEGF-2 mRNA and the level of VEGF, improves the capillary density and mitochondria of cardiac structure, and decreases the expression of cardiac hypertrophy markers and the ratio of MHC/ α -MHC. This in turn reduces the left ventricular posterior wall thickness and the interventricular septum thickness, eventually improves myocardial hypertrophy and fibrosis, postpones left ventricular hypertrophy and remodeling, and inhibits cardiomyocyte apoptosis [31]. Therefore, HIF may play a protective role in CKD.

Nonetheless, the above supportive findings are in contrast to those of other studies. With RNA seq analysis, HIF-1 α can stimulate 25 target genes in HK-2 cells to release TGF- β and to activate the TGF- β /SMAD3 pathway [72], which induces COL-1 synthesis, promoting the accumulation of collagen and ECM, and eventually enhancing fibrosis together with other fiber promoting pathways induced by HIF, such as NF- κ B, PI3K/Akt, and the Notch pathway [72–74]. The continuous activation of HIF-2 in the renal tubular epithelium may induce renal cystic fibrosis [75]. The role of HIF in CKD is still unclear and may be associated with the degree of hypoxia and other diseases, or with the time points of exogenous intervention. Further studies are urged to clarify these relationships.

Renal Tumor

HIF plays a dual role in the hypoxic microenvironment of tumor cells. VHL inactivation accounts for about 70% of renal cell carcinoma (RCC) [76], which is accompanied by HIF-1/2 α accumulation. This accumulation may be related to the development of renal tumors. Most studies have suggested that HIF-1 α may inhibit tumor growth, while HIF-2 α promotes tumor growth and metastasis. However, Schokrpur et al. [77] support that in

RCC, HIF-1 α can reduce RCC proliferation, but also can increase invasive and migratory capability due to upregulated periostin, PPEF1, SAMS1, and TNFSF13B (also known as B-cell-activating factor) with RNA seq data.

HIF-2 α can stimulate the tissue release of multiple signaling molecules and angiogenesis, as well as increased drug resistance, promoting tumor growth and distant metastasis [78]. Hypoxia in the tumor microenvironment promotes or antagonizes the tumor through regulating the immune response. HIF-2 α stimulates M2 macrophage activation, blocking Th1 immune response and upregulating arginase, which finally induces immunosuppression. HIF-2 α also inhibits dendritic cells, cytotoxic T cells, and natural killer cells, and eventually triggers immune escape. HIF-1 α mediates iNOS to increase PD-L1 expression, which regulates the activity of myeloid-derived suppressor cells and differentiates them into immunosuppressive tumor-associated macrophages, eventually blocking the sensitivity of T cells to kill tumor cells, thus achieving immune escape [79]. However, some scholars pointed out that HIF-1 α can activate neutrophil aggregation, can promote antigen-presenting cells to express MHC II, co-stimulatory molecules CD80 and CD86, and can regulate the balance between Treg cells and pro-inflammatory Th17 cells, which mediates the immune response to tumor [79, 80], namely, HIF-2 α has been widely recognized as a tumor promoter, while such role of HIF-1 α remains to be verified.

Kidney Transplantation

The only existing therapy for end-stage renal disease is renal transplantation. Reperfusion and rejection after renal transplantation may induce inflammation and renal injury. Bernhardt et al. [81] reported that the pre-activation of HIF before renal transplantation could improve both short-term and long-term prognosis. At 10–14 days, after renal transplantation, HIF-1 can be induced through the Akt/mTOR pathway to stimulate the transcription of target genes (HO-1, EPO, and VEGF), upregulate IL-8, and TNF- α , which can reduce apoptosis and oxidative stress, effectively improving renal injury caused by reperfusion. However, 3 months after transplantation, HIF was almost undetectable due to the stable state of the grafts [82, 83], indicating that most of the grafts had reached functional and structural balance.

Medication

PHD Inhibitors

Due to its protective effect, the maintenance of HIF can alleviate the progression of kidney disease. It can be

activated by PHD inhibitors, FIH, VHL inhibitors, and iron chelators. Among them, PHD inhibitors are the most commonly used molecules for the preclinical and clinical maintenance of HIF's stability. There are 3 main subtypes of PHD: PHD1, PHD2, and PHD3. The latter affects HIF-2 α , while PHD2 is a primary regulator, preferentially acting on HIF-1 α [84]. HIF can protect renal function by promoting mitochondrial autophagy, upregulating the transcription of multiple target genes, as well as reducing apoptosis and the release of inflammatory factors [69]. Since 2008, PHD inhibitor medicines have been widely researched, and certain countries have already used such medicines in clinical practice. For example, roxadustat has been used clinically to treat CKD anemia in China [85], while daprodustat and vadadustat are used in Japan [86, 87]. However, some challenges remain in the use of these drugs. The selection of inhibitors specific to HIF-1 α , HIF-2 α , and HIF-3 α still remain unresolved. Most preclinical studies have proven that pretreatment can improve the level of HIF to protect renal function, but the roles of PHD inhibitors after renal injury are still controversial [58]. Certain studies showed that long-term usage of PHD inhibitors may induce renal fibrosis and accelerate CKD progression [88], while the determination of this time period needs further evaluation. In addition, PHD inhibitors not only participate in HIF reaction but also may be concerned with other metabolic activities; the methods to reduce the adverse effects of other metabolic processes remain to be studied [89]. It is important to investigate the use of specific inhibitors for a subtype of PHD, as well as the timing, frequency, and duration of drug use.

HIF Inhibitors

Although tumor growth promotion by HIF-1 is unclear, due to the promoting effect of HIF-2 on the growth of RCC, inhibitors of HIF-1/2 translation, expression, dimerization, and Hsp90 inhibitors have been used in RCC research. The inhibitors of HIF-2, such as PT-2385 and PT-2977 have already entered the clinical research stage [90], and have shown clinical effectiveness in advanced RCC. However, due to the overlapping mechanism of HIF-1/2 and to the fact that most of the drugs are nonselective, it is difficult to inhibit a specific target [91]. Thus, further specific inhibitors urgently need to be identified.

Sodium/Glucose Cotransporter Inhibitor

These are a group of sodium-glucose cotransporters that can mediate HIF-1/2 α . At present, sodium/glucose cotransporter (SGLT)1 and SGLT2 are studied more in-

tensively among the 6 types. SGLT1 is mainly located in small intestinal epithelial cells, while SGLT2 is situated in the proximal renal tubules and transports about 90% percent of glucose and sodium from the renal filtrate by re-absorption [92]. SGLT2 inhibitors block this process, reduce oxygen consumption, restore the feedback between tubule and glomerulus, and contract the afferent arterioles. They also stimulate HIF-2 α through sirtuin1 and AMPK through the sirtuin1/AMPK signaling pathway to increase erythrocyte and blood oxygen-carrying capability and to restrain the angiotensin system, which can improve glomerular filtration and reduce renal injury [93]. However, the effect of SGLT2 inhibitors on HIF-1 α expression is subject to debate. SGLT2 inhibitors also down-regulate HIF-1 α , thus can reduce GLUT-1, can inhibit the oxidative phosphorylation of oxygen in the mitochondria, and can reduce the absorption of glucose, eventually increasing the excretion of urine sugar and postpone renal fibrosis in diabetes [94]. The decrease of GLUT-1 also leads to the relative deficiency of glucose in tumor cells, inhibiting renal tumor metabolism and tumor growth [95]. Some studies have shown that SGLT2 inhibitors can elevate HIF-1 α expression [96], which alleviates renal fibrosis by suppressing EMT through the influence of TGF-1/Smad3 phosphorylation.

In diabetic nephropathic clinical studies, such as DAPA-CKD, EMPEROR-Reduced, and EMPA-REG OUTCOME, it was proven that SGLT2 inhibitors can effectively postpone the occurrence and progress of nephropathy. In nondiabetic nephropathy, however, the renal protective effect of SGLT2 remains disputed. SGLT2 reduces the glomerular filtration rate (GFR) in the early stage of application, lowering the incidence of end-stage renal disease and improving proteinuria [97]. This effect is related to changes in the renal hemodynamics and to the contraction of small arteries after administration, which can be relieved upon the termination of treatment [98]. While the decline of GFR is reversible during long-term treatment, existing knowledge on the role and mechanism of nondiabetic nephropathy needs to be further expanded.

Conclusions

Although renal blood vessels are abundant, many renal tissues have low oxygen tension, which is about 30 mm Hg in the renal cortex and is even <10 mm Hg in the renal medulla [99]. Therefore, the kidney is susceptible to oxygen deprivation. During hypoxia, HIF-1/2 plays sig-

nificant roles. This article discussed and reviewed the target genes, the mechanism, and the protective mechanism of HIFs. We also considered the treatment directions toward renal disease. HIF can protect the kidney through regulating the transcription of target genes. During cellular hypoxia, EPO stimulates red blood cells to regenerate for increased oxygen-carrying capacity, while VEGF promotes angiogenesis. In addition, HIF can maintain mitochondrial homeostasis, can promote glycolysis, can regulate inflammation, can restrain ferroptosis, and eventually can prevent the progression of kidney disease. Based on this protective effect, pretreatment with PHD inhibitors can activate HIF to safeguard the kidney from hypoxic injury during AKI and renal transplantation. It is uncertain whether HIF's stability can prevent the progression of CKD and RCC. Nevertheless, HIF still provides a direction for the clinical treatment of different renal diseases. Among the drugs aimed at different diseases, PHD inhibitors to activate HIF-2 are clinically used to improve renal anemia in CKD; HIF-2 inhibitors are indicated for the treatment of RCC; and SGLT2 inhibitors can activate HIF-2 to improve the progression of diabetic nephropathy. Due to the overlapping mechanism of HIF-1 and HIF-2, many difficulties in using these drugs remain; most drugs used in clinical trials are challenging to specifically target at a certain HIF subtype. In this respect, comprehensive research is necessary that is based on the effect of the various subtypes of HIFs toward different diseases. Moreover, the long-term effects of HIF-related drugs and their potential impacts on nontargeted metabolic processes also need further evaluation in order to improve their application to kidney diseases.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

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