Hypoxia and chronic kidney disease

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Summary
Hypoxia is an inherent pathophysiological characteristic of chronic kidney disease (CKD), which is closely associated with the development of renal inflammation and fibrosis, as well as CKD-related complications such as anaemia, cardiovascular events, and sarcopenia. This review outlined the characteristics of oxygen supply in the kidney, changes in oxygen metabolism and factors leading to hypoxia in CKD. Mechanistically, we discussed how hypoxia contributes to renal injury as well as complications associated with CKD. Furthermore, we also discussed the potential therapeutic approaches that target chronic hypoxia, as well as the challenges in the study of oxygen homeostasis imbalance in CKD.

Introduction
The prevalence and incidence of chronic kidney disease (CKD) is increasing worldwide.1 CKD is characterized by progressive renal fibrosis and a gradual decline in the glomerular filtration rate (GFR), ultimately leading to end-stage renal disease. Hypoxia is a pathological condition in which the body or organs lack an adequate oxygen supply, which can also occur due to excessive energy demands in the context of a continuous blood supply. Although almost 20% of the blood volume circulates in the human kidney, the organ remains in the critical state of hypoxia physiologically. The formation of hypoxia status is determined by various factors, including local oxygen tension, cellular energy requirements, and cellular intrinsic resistance to hypoxia. In the kidney, proximal tubular cells are the most sensitive to hypoxic injury,2 while the extent of tubule injury determines the prognosis of kidney disease. Meanwhile, in response to hypoxia, pericytes detach from the vessel walls and differentiate into activated myofibroblasts in the interstitial space, ultimately leading to the development of renal fibrosis.3 In addition, hypoxia also induces endothelial activation, followed by leukocyte stasis and blocking blood flow to peritubular capillaries, ultimately leading to loss of capillary structure and exacerbating hypoxia and loss of nephrons.4

In this review, we describe the roles of hypoxia in CKD and discuss the characteristics of oxygen supply and metabolism in the kidney. Specifically, we emphasize the effect of hypoxia on the progression of CKD and CKD-related complications. Finally, the potential therapeutic approaches that target chronic hypoxia in CKD and its challenges will be discussed.

Characteristics of oxygen homeostasis and factors leading to hypoxia in CKD
Organ tolerance to hypoxia depends on the blood supply. In fact, the kidney is intrinsically susceptible to hypoxia. It only uses no more than 10% of the oxygen delivered by the renal artery.3 Most of the blood in the kidney is transported to the renal cortex, while only 10%–15% of blood is sent to the renal medulla. In the kidney, arterial and venous vessels run in close parallel. The oxygen shunt between arterial and venous vessels can bypass the blood circulation and make the oxygen tension in renal tissue relatively low, approximately 10 mmHg in the renal medulla.6 The oxygen tension of the renal cortex varied widely, and the average partial pressure of oxygen was approximately 30 mmHg, which decreased significantly with the change in renal perfusion. Oxygen supply in the tubulointerstitium relies heavily on postglomerular capillary flow. Thus, upstream obstruction can result in microcirculation damage to immediately decrease oxygen tension in the tubulointerstitial compartment. In addition, the sensitivity of different cell types to hypoxia depends on various factors, such as the cellular metabolic rate and the activity of hypoxia-inducible factor (HIF) pathways (Figure 1). Recently, the physiological factors that render the kidney susceptible to hypoxia have also been summarized and discussed.7

In CKD, hypoxia and decreased oxygen tension are common. Haemodialysis patients, in particular, showed
different characteristics of oxygen homeostasis, with abnormalities along the entire oxygen cascade and impaired diffusive and convective oxygen transport. Chronic renal hypoxia is caused by numerous factors in patients with CKD (Figure 2), including: (1) Loss of peritubular capillaries. Loss of peritubular capillaries is not only a result of hypoxia but also contributes to the progression of CKD by exacerbating hypoxia. (2) Fibrosis of the tubulointerstitium. Interstitial fibrosis reduces the efficiency of oxygen diffusion because of the longer distance between capillaries and tubular cells. Hypoxia also induces the accumulation of extracellular matrix, which further widens the diffusion distance between functional blood vessels and nephrons, aggravating hypoxia. (3) Decrease in peritubular capillary beds. Both the obstruction of peritubular capillaries in damaged glomeruli and an imbalance of vasoactive substances (activation of renin-angiotensin system, endothelin, etc.) will result in a downstream decrease in tubulointerstitial blood flow. Glomerular hyperfiltration is a condition that increases renal oxygen demand, leading to an imbalance between tubular workload and oxygen delivery in early CKD. (4) Oxidative stress is another important factor leading to excessive oxygen demand. (5) Inflammation plays a key role in the development of chronic hypoxia. Mechanistically, inflammatory cytokines, including interleukins 1 and 6, angiotensin II, and transforming growth factor β, can result in excessive accumulation of extracellular matrix, which can disrupt and replace functional parenchyma, leading to interstitial fibrosis. (6) Anaemia can also reduce oxygen delivery to the kidney. (7) Hypoxia induced by obstructive sleep apnoea. Obstructive sleep apnoea-related hypoxia produces a range of harmful systemic effects, including oxidative stress, inflammation, and sympathetic activation, that collectively worsen the progression of renal disease. In turn, CKD can result in increased severity of sleep apnoea by inducing uremic neuropathy and myopathy, altered chemosensitivity, and hypervolemia.

Beyond tubulointerstitial hypoxia, glomerular hypoxia can also occur in CKD. Glomerular sclerosis or the

**Figure 1. Characteristics of oxygen supply and metabolism in the kidney.** The oxygen shunt bypasses the loop to keep oxygen tension comparatively low in the renal medulla. Oxygen is delivered to the kidney to generate ATP via mitochondrial oxidative phosphorylation. Upstream obstruction leads to microcirculation damage to decrease oxygen tension. In CKD, hypoxia and decreased oxygen tension are common. When the oxygen supply is inadequate, many cells shift from aerobic to anaerobic metabolism, and glycolysis becomes the primary mode of energy production. Chronic hypoxia also results in changes in gene expression patterns. Various HIF-independent pathways promote ATP conservation by limiting energy-consuming processes, such as cell division, ribosome biogenesis, mRNA translation, and ion channel activity. Abbreviations: ATP, adenosine triphosphate; CKD, chronic kidney disease; HIF, hypoxia inducible factor.
collapsing of glomerular capillaries may directly cause damage to tubules by reducing peritubular capillaries and oxygen supply. In contrast, tubulointerstitial injury could also inevitably cause the functional or structural loss of glomeruli. Tubular atrophy leads to increased fluid delivery to the macula densa, resulting in decreased GFR via tubuloglomerular feedback. Additionally, tubulointerstitial fibrosis reduces blood supply to peritubular capillaries, leading to ischaemic injury of the nephron and further reduction of GFR.15

Thus, accumulating evidence indicates that hypoxia plays a crucial role in the progression of CKD. When the oxygen supply is insufficient, many cells switch from aerobic to anaerobic metabolism, and glycolysis becomes the main method of energy production.16 Glycolysis is an inefficient form of energy production, producing only 2 mol of adenosine triphosphate (ATP) per mol of glucose, compared to approximately 36 mol ATP/mol glucose during aerobic respiration. Chronic hypoxia also results in changes in gene expression patterns. Generally, HIF stabilization and transcriptional activation of hypoxia-induced genes are the core mechanisms of adaptation to hypoxia.17 Meanwhile, multiple HIF-independent pathways promote ATP conservation by limiting energy-consuming processes, such as ribosome biogenesis, cell division, ion channel activity, and miRNA translation. In addition, metabolic abnormalities or the accumulation of intermediates induced by an imbalance of oxygen supply and demand were recently found to play critical roles in kidney injury, which has been summarized and discussed by other researchers.18,19 In addition to hypoxia directly, renal repair secondary to renal injury also results in a condition of relative hypoxia, which may stimulate aerobic glycolysis. Interestingly, some evidence suggests that renal injury increases the need for renal repair, which may stimulate aerobic glycolysis and help cell proliferation.20 Therefore, understanding the exact characteristics of oxygen homeostasis will be critical to improve the strategy of CKD prevention and treatment.

Figure 2. Factors leading to renal hypoxia in CKD. Glomerular hypoxia occurs in CKD. Glomerular hyperfiltration and oxidative stress are conditions that increase oxygen demand. Glomerular injury results in a decrease in GFR and leads to tubulointerstitial injury via hypoxic damage. Tubular atrophy triggers a decrease in GFR via tubuloglomerular feedback. Loss of peritubular capillaries is not only a consequence of hypoxia but also promotes the progression of CKD. Fibrosis of the tubulointerstitial and anaemia reduce the efficiency of oxygen diffusion. Reduction in peritubular capillary flow results in a downstream decrease in tubulointerstitial blood flow to cause ischaemic injury. Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate.
Effects and mechanisms of hypoxia on the progression of CKD (Figure 3)

Hypoxia and fibrosis
Packed with mitochondria and dependent on oxidative phosphorylation, the proximal tubule is particularly vulnerable to various injuries, including hypoxia. Increasing evidence has demonstrated that in response to hypoxia, tubular epithelial cells undergo changes and function as inflammatory and fibrogenic cells (they may undergo tubular epithelial cell death/atrophy, maladaptive repair, metabolism switch, senescence, etc.), with the consequent production of various bioactive molecules that drive interstitial inflammation and fibrosis. Meanwhile, previous studies have reported that the tubular capillary network becomes sparse with the progression of tubulointerstitial fibrosis. Interstitial fibrosis and a decreased capillary network leading to a decreased blood supply and hypoxia are correlated with declining renal function.\(^1\) In a hypoxic environment, hypoxia response element (HRE [DNA binding site of HIF])-driven reporter gene activity is increased.\(^2\) Endothelial cells are one of the main targets of hypoxia, which activates the receptor for advanced glycation end products (RAGE) and stimulates p38 mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-\(\kappa\)B) signalling to accelerate renal disease.\(^3\) Under hypoxic injury, endothelial cells differentiate into myofibroblasts (EndoMT),\(^4\) which subsequently increase the production of extracellular matrix (ECM) and conversely aggravate hypoxia in the kidney. Notably, hypoxia also plays a critical role in epithelial—mesenchymal transition (EMT) in cultured human proximal tubular epithelial (PTE) cells.\(^5\) PTE cells...
are rich in mitochondria and sensitive to oxidative phosphorylation and transform into a secretory phenotype in a hypoxic environment. HIF not only activates NF-kB signalling to trigger peritubular inflammation but also activates Wnt and Notch-1 signalling to promote fibrosis. Finally, renal interstitial fibroblasts proliferate and differentiate into myofibroblasts and promote renal scarring by accelerating extracellular matrix synthesis under hypoxic conditions.

Hypoxia and angiogenesis
Peritubular capillary rarefaction can be observed in CKD animal models. It is also the long-term response to acute ischaemia–reperfusion injury, contributing to the development of CKD. In CKD, peritubular capillary rarefaction and tubulointerstitial hypoxia contribute to the dysregulation of angiogenesis. In cultured tissues, angiogenesis is induced by hypoxia by regulating nitric oxide synthases, vascular endothelial growth factor (VEGF), and angiopoietins and affecting the proliferation and migration of endothelial cells. Flt-1 is involved in the activation of VEGF and increased angiogenesis under hypoxia. In addition, HIF regulates angiogenesis-related genes by increasing the transcription of VEGF and internal ribosomal entry sites both in vitro and in patients with proteinuric glomerulopathies. Normally, VEGF-A is expressed in podocytes, tubular cells and endothelial cells and is reduced in advanced stages of CKD. VEGF-A-deficient mice showed endothelial swelling and necrosis, resulting in an impaired filtration barrier. Excessive VEGF-A in podocytes causes collapsing glomerulopathy, which is ascribed to decreased capillary flow and intraluminal capillary pressure. Vascular endothelial growth factor receptor (VEGFR) is expressed in endothelial cells in glomerular and peritubular capillaries. VEGFR expression is upregulated in CKD patients and leads to endothelial dysfunction. Ang-1, located at nephrogenic mesenchyme, can promote the growth of interstitial capillaries in mouse metanephric organ culture. Overexpression of Ang-2 can induce glomerular endothelial apoptosis, downregulate VEGF and nephrin and cause podocyte injury. In CKD patients, Ang-1 is decreased, while Ang2 is increased. This change is correlated with endothelial cell apoptosis.

Hypoxia and inflammation
Hypoxia and inflammation are intertwined at the molecular, cellular, and clinical levels. On the one hand, the concept that hypoxia can induce inflammation has gained general acceptance from studies of the hypoxia signalling pathway. Ischaemia/hypoxia is one of the most common causes of the inflammatory response, as evidenced by the infiltration and activation of inflammatory cells. Increasing evidence has also demonstrated that the transcription factor HIF, which is activated in hypoxic conditions and is considered a reliable indicator for hypoxia, plays a vital role in inflammation. Ben-Shoshan et al. reported that increased HIF-1α in T cells induces phenotypic transition from type 1 helper T cells (Th1) to type 2 helper T cells (Th2) to amplify the immune response of macrophages and cytotoxic T cells. HIF-1α might negatively regulate the adaptive immune system to protect tissues by activating the differentiation and proliferation of regulatory T cells and increasing adenosine to inhibit effector T cells. Hypoxia also participates in dendritic cell injury by interfering with differentiation, enhancing the link between hypoxia and immunity. In addition, various proinflammatory cells and cytokines are recruited to hypoxic environments. On the other hand, the increase in metabolic demands and reduction in metabolic substrates caused by thrombosis and trauma are stimulators of hypoxia in the inflammatory environment. HIF-1α and HIF-2α were upregulated in inflammatory tissues with hypoxia manifestations, further confirming the interaction between inflammation and hypoxia.

Hypoxia and complications associated with CKD
Anaemia
Although many factors may contribute to anaemia in CKD patients, insufficient erythropoietin (EPO) production is one of the most important pathological mechanisms. EPO production is primarily stimulated by hypoxia. When the body is exposed to hypoxia or undergoes hypoxic conditions, HIF-2α regulates EPO expression in combination with hypoxia response elements on the EPO gene in the kidney and liver. Currently, targeting HIF has been effective and well tolerated for the correction of anaemia with CKD, as evidenced from pooled phase 3 clinical trials, indicating that targeting hypoxia has been successfully transformed into clinical practice.

In addition, the gene expression for iron metabolism in hepatocytes is also regulated by HIF-2 gene expression. Iron is an important component of erythropoiesis. Iron deficiency also occurs in CKD patients due to inadequate provision or absorption of dietary iron and/or blood losses. HIF-2α not only promotes the synthesis of EPO in the kidney and liver but also regulates iron metabolism by stimulating duodenal cytochrome B (DCYTB) and divalent metal transporter-1 (DMT1) expression. Fe²⁺ needs ferroportin (FPN) to enter the circulation and be transported to the target region by transferrin (TF). Studies have shown that HIF can upregulate the expression of TF and FPN and downregulate hepcidin synthesis. As a result, enterocytes and hepatocytes release more iron together to meet the requirement of erythropoiesis. In chronic inflammatory status, elevation of serum hepcidin, reduction of FPN and hypoferremia might contribute to
the development of anaemia.\textsuperscript{59} Moreover, hypoxia not only affects bone marrow by regulating the maturation and proliferation of erythroid progenitors but also activates the EPO receptor (EPOR) to regulate haemoglobin synthesis.\textsuperscript{59} Therefore, the discovery of hypoxia responses and HIF signalling provides promising therapeutic strategies for hypoxia-related diseases.

**Cardiovascular disease (CVD)**

CVD is the leading cause of death in patients with CKD. One of the fundamental functions of the cardiovascular system is oxygen delivery; therefore, CVD inherently is linked to hypoxia. Hypoxia is also a promoter of myocardial infarction, cardiac remodelling, atherosclerosis, and peripheral artery disease in CKD, which has been summarized and discussed. Meanwhile, accumulating evidence has demonstrated that both reduced endothelium-independent maximal vasodilatation and loss of vascular tone were observed in uraemic animals and CKD patients, which were also closely associated with the severity of uraemia.\textsuperscript{32-33} These factors affect the perfusion of tissue to induce hypoxia.

Of note, in the context of CKD, the responses to hypoxia in CVD were also explored systemically. At the molecular level, advances in our knowledge of cellular oxygen sensing and hypoxia responses and their role in adaptation to hypoxia have led to the discovery of HIF signalling. HIF mediates cellular responses to hypoxia at the transcriptional level. Under hypoxic conditions, both HIF-1\textsubscript{a} and HIF-2\textsubscript{a} show increased trends in the heart. HIF-1\textsubscript{a} can activate inducible nitric oxide synthase (iNOS) gene expression by increasing NO synthesis, whereas HIF-2\textsubscript{a} induces the expression of arginase-1 (Arg-1) to suppress NO synthesis. Of note, NO, as a vasodilator, plays a vital role in regulating vascular tone by regulating cGMP in smooth muscle cells.\textsuperscript{54} S-nitrosylation of target proteins, activation of sarco/endoplasmic reticulum calcium ATPase and production of cyclic inosine monophosphate.\textsuperscript{55} It is therefore well recognized that hypoxia is a key instigator in CKD-CVD, and targeting hypoxia is likely to be a promising therapeutic strategy for CVD.

**Sarcopenia**

Sarcopenia is the physiological reduction of muscle mass and strength and is one of the complications of CKD patients. Multiple factors participate in the pathogenesis of sarcopenia. Skeletal muscle hypoxia is believed to be the reason for muscle wasting and contractility reduction.\textsuperscript{66} Hypoxia induces oxygen delivery reduction to the muscles. This reduction is more obvious in anaemia with CKD. Oxygen delivery abnormalities lead to a reduction in energy storage and protein synthesis to impair muscle contraction. Mechanistically, HIF-1\textsubscript{a} deficiency can stimulate the secretion of glucagon-like peptide 1 (GLP-1) in human adipocytes,\textsuperscript{57} contributing to sarcopenia in hypoxia. In addition, hypoxia induces muscle disuse by activating the HIF-1\textsubscript{a} and NF-\kappaB catabolic pathways and inhibiting the anabolic mammalian target of rapamycin pathway.\textsuperscript{58,59} Finally, local protein degradation and proteolytic pathways could also be activated by cytokines to affect muscles.

**Therapeutic approaches that target chronic hypoxia in CKD**

**HIF stabilizers**

Oxygen-dependent prolyl-hydroxylases (PHDs) are key regulators of HIF-dependent erythropoiesis, providing an essential theoretical basis for applying HIF-PHD inhibitors (HIF-PHIs) to treat renal anaemia. Roxadustat, as a first-in-class orally administered small molecule HIF-PHI, has received approval in China, Japan, South Korea, Chile, the United Kingdom, and the European Union, although it was not approved by the USA Food and Drug Administration.\textsuperscript{60-63} In addition, several other HIF-PHIs were also approved for marketing in Japan, including daprodustat,\textsuperscript{64} vadadustat,\textsuperscript{65} and enarodustat.\textsuperscript{66} In a recently published meta-analysis including 2045 patients, HIF-PHIs exerted a medium to large positive effect on the haemoglobin rate for dialysis-dependent (DD) and nondialysis-dependent (NDD) CKD patients. Moreover, HIF-PHIs improved the bioavailability of iron and decreased hepcidin levels, contributing to the improvement of renal anaemia.\textsuperscript{67}

Although HIF-PHI treatment appears to be beneficial,\textsuperscript{68} there are many debates on the role of HIF activation in CKD progression. Preclinical studies demonstrated that HIF signalling activation in the kidney appears to be detrimental, as evidenced by the stabilization of HIF-1\textsubscript{a} by genetic deletion of vHL in a 5/6 renal ablation model and the administration of an anti-HIF-1\textsubscript{a} agent in a unilateral ureteral obstruction model.\textsuperscript{69} In addition, HIF activation seemed to play a role in accelerating cyst growth in the polycystic kidney disease mouse model.\textsuperscript{70} In contrast to these detrimental effects, Schley et al. found that HIF-PHIs could shift renal mononuclear phagocytes towards a regulatory, anti-inflammatory phenotype and reduce mononuclear phagocyte-driven renal inflammation in adenine- and crystal-induced tubulointerstitial nephritis models.\textsuperscript{71} Moreover, using a model of ob/ob mice, Sugahara et al. found that enarodustat protected against glucose and lipid metabolic disorders and had renoprotective effects on reducing albuminuria and ameliorating glomerular damage.\textsuperscript{72} In fact, the effect of HIF activation is likely to depend on the pathological context, specific cell types, and timing, which has been summarized and discussed comprehensively elsewhere.\textsuperscript{73,74} Future research is warranted to further elucidate the effects and mechanisms of HIF-PHIs on CKD progression.

Additionally, the effects of HIF-PHIs were also investigated in CKD-associated conditions. Preclinical
studies found that HIF-1 was essential for enhancing vascular smooth muscle cell (VSMC) calcification. Moreover, HIF-PHI, which stabilizes HIF-α, also augmented the phosphate-induced osteochondrogenic phenotypic switch and led to VSMC calcification. For human studies, a recent clinical trial reported that daprodustat was noninferior to EPO regarding cardiovascular outcomes in DD CKD patients, while in NDD CKD patients, vadadustat did not meet the prespecified non-inferiority criterion for cardiovascular safety. In the CKD-associated myopathy model, our group demonstrated that MK-8617, a novel orally active HIF-PHI, could ameliorate muscle impairment due to the promoted angiogenesis in the skeletal muscle of CKD mice. More randomized controlled trials will be needed to ensure the exact safety and effectiveness of HIF-PHIs on CKD-related complications in the future.

Sodium-glucose cotransporter 2 (SGLT2) inhibitors
SGLT2 is a high-capacity, low-affinity transporter almost exclusively located in the initial proximal renal tubules, which plays a vital role in the reabsorption of > 90% of the glucose filtered at the glomerulus. SGLT2 inhibitors (SGLT2is), a novel class of oral antihyperglycaemic agents leading to substantial loss of glucose and solutes in the urine, are currently indicated for type 2 diabetes mellitus (T2DM). More importantly, a recently published meta-analysis reported that in patients with CKD, the use of SGLT2i significantly decreased the risk of hospitalization for heart failure, myocardial infarction, and composite kidney endpoints, regardless of the T2DM status. Meanwhile, a prespecified analysis from the DAPA-CKD trial further demonstrated that the effect of dapagliflozin on reducing major adverse kidney and cardiovascular events and all-cause mortality was consistent among patients with diabetic and nondiabetic CKD, which may strongly expand their potential clinical applications. Currently, the potential mechanisms of these benefits are being extensively investigated because they cannot be fully explained by the improved levels of blood glucose, blood pressure, or glomerular filtration pressures.

The characterization of SGLT2 inhibition application is the rapid eGFR decline during the first few weeks of treatment, after which it gradually returns to the baseline and delays the progression of eGFR decline. The reason for this effect is not clear, possibly due to attenuation of the proximal tubular reabsorption workload and subsequent normalization of tubuloglomerular feedback. However, studies have also suggested that the reduction in cortical oxygen consumption by using SGLT2i may also play a role. The shift of glucose, sodium, and fluid transport to S3 segments, thick ascending limbs, and collecting ducts in the deep cortex and outer medulla may reduce the oxygen partial pressure in this region. Consequently, the reduced oxygen pressure may mimic systemic hypoxia and become a stimulus for activating the HIF signalling pathway, which may augment erythropoiesis. In addition to these beneficial effects, SGLT2i was also found to prevent renal capillary rarefaction and subsequent hypoxia and fibrosis in a murine model of renal ischaemia–reperfusion injury. Because many renoprotective benefits of SGLT2i may be associated with their role in hypoxia signalling, future studies are needed to examine the exact effects and mechanisms of SGLT2 inhibition on renal physiology and tissue oxygenation.

Antioxidant drugs
In the context of CKD, oxidative stress is enhanced, leading to increased oxygen consumption, resulting in renal hypoxia. In turn, renal hypoxia magnifies oxidative stress, forming a vicious cycle. Amelioration of oxidative stress contributes to the protection of the renal vasculature and the normalization of oxygen utilization, thus improving oxygenation of the kidney. Importantly, antioxidants have become an effective strategy to treat CKD and CKD-related complications. Recently, Wu et al. investigated the role of a HIF stabilizer (Roxadustat) in the AKI-to-CKD transition induced by unilateral kidney ischaemia–reperfusion. Interestingly, they found that roxadustat markedly alleviated kidney fibrosis and enhanced renal vascular regeneration and redox balance, possibly by activating the HIF-1α/VEGF-A/VEGF receptor 1 signalling pathway and driving the expression of the endogenous antioxidant superoxide dismutase 2. Meanwhile, melatonin, which has antioxidant and anti-inflammatory properties, optimized the therapeutic effects of mesenchymal stem cells in CKD models, including unilateral ureteral obstruction rats and rats with diabetic nephropathy. In addition, as a gaseous signalling molecule, hydrogen sulphide (H2S) plays an important role in maintaining the redox status at safe levels by promoting the scavenging of reactive oxygen species and modifying cysteine residues on key signalling molecules. Increasing evidence has demonstrated the potential for H2S-based therapies in the renal system. Of note, one donor of H2S, sodium thiosulfate, is currently used in the clinical treatment of calciphylaxis in dialysis patients and cisplatin toxicities in cancer therapy. The safety and tolerability of sodium thiosulfate have also been demonstrated in patients with acute coronary syndrome (ClinicalTrials.gov identifier NCT03017963), representing a promising subject for further translational studies. Although the role of vitamin E (a traditional antioxidant) for kidney diseases is unlikely to help much, a recent phase IIb randomized controlled trial found that 400 mg tocotrienol-rich vitamin E supplementation for 12 months could ameliorate the progression of diabetic kidney disease (assessed by serum creatinine and eGFR). Therefore, these results indicated that oxidative stress is a promising therapeutic target in the context of CKD.
Schema of the effects and mechanisms of drugs targeting hypoxia in CKD are shown in Figure 4. Clearly, their clinical efficacy, especially the long-term outcome on patient survival, urgently needs to be determined via large-scale clinical trials.

Assessment of hypoxia in CKD
To date, no gold standard or feasible method is available to accurately assess hypoxia in CKD. The techniques for the assessment of hypoxia in tissues clinically include pimonidazole staining, microelectrode-dependent measurements, analyses of the HIF pathway, and two-photon phosphorescence lifetime microscopy of oxygen in living animals and blood oxygenation level-dependent magnetic resonance imaging and positron emission tomography computed tomography. However, the above available methods to assess hypoxia in tissues have several limitations, including low accuracy and specificity, high discrepancies between different analyses of hypoxia, high operating cost and difficult implementation. Currently, an analysis of HIF stabilization/HIF transcriptional activity is widely used to represent hypoxia, although it is an indirect method. Obviously, the effects of hypoxia on cells and tissues should be distinguished from HIF activation, as HIF transcriptional activity may be potentially biased by nonhypoxic regulation during kidney disease.

Outstanding questions
Accumulating data have suggested that hypoxia is not only a key instigator of AKI but also a critical mediator of the progression of CKD. The severity and length of hypoxia determine the prognosis of kidney disease. Therefore, further elucidating the exact responses of kidney to hypoxia is of great significance to understand the pathophysiology of renal disease. Fortunately, the...
mechanisms of cell sensing and responding to oxygen change has been largely recognized in the last three decades, which had stimulated the development of novel therapy for renal anemia.

However, numerous challenging questions remain to be addressed in future studies. First, what are the exact molecular and metabolic responses of the kidney to hypoxia and its relationship with kidney inflammation? Second, why different cell types undergo the different response to hypoxia in kidney? Third, are there measurable biomarkers or methods to monitor the response in the earlier stage of hypoxia, which could predict the outcome of kidney injury responding to hypoxia? Forth, given the beneficial efficacy of HIF-PHIs in correcting anemia, what is the long-term effect of this new class of drugs on the development of AKI and progression of CKD? Last but the least, due to the potential off target effects of HIF-PHIs, concerns should also be given to the long-term side effects beyond their beneficial erythropoiesis.

Search strategy and selection criteria
Data for this Review were identified by searches of PubMed and references from relevant articles using the search terms “Hypoxia”, “Hypoxia-inducible factor”, “Fibrosis”, “Inflammation”, “Anaemia”, “CKD”, and “Chronic kidney disease”. Only articles published in English and up to February 22th, 2022 were included.

Contributors
LBC generated the initial concepts of this review, gave insightful comments and approved the final version of the manuscript. WB, LZL, ZYL, WY, and GYM wrote the manuscript. WB, LZL, ZYL, WY, and GYM wrote and approved the final version of the manuscript.

Declaration of interests
The authors confirm that there are no conflicts of interest.

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