

Chronic Hypoxia and Tubulointerstitial Injury: A Final Common Pathway to End-Stage Renal Failure

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Recent studies emphasize the role of chronic hypoxia in the tubulointerstitium as a final common pathway to end-stage renal failure. When advanced, tubulointerstitial damage is associated with the loss of peritubular capillaries. Associated interstitial fibrosis impairs oxygen diffusion and supply to tubular and interstitial cells. Hypoxia of tubular cells leads to apoptosis or epithelial-mesenchymal transdifferentiation. This in turn exacerbates fibrosis of the kidney and subsequent chronic hypoxia, setting in train a vicious cycle whose end point is ESRD. A number of mechanisms that induce tubulointerstitial hypoxia at an early stage have been identified. Glomerular injury and vasoconstriction of efferent arterioles as a result of imbalances in vasoactive substances decrease postglomerular peritubular capillary blood flow. Angiotensin II not only constricts efferent arterioles but, *via* its induction of oxidative stress, also hampers the efficient utilization of oxygen in tubular cells. Relative hypoxia in the kidney also results from increased metabolic demand in tubular cells. Furthermore, renal anemia hinders oxygen delivery. These factors can affect the kidney before the appearance of significant pathologic changes in the vasculature and predispose the kidney to tubulointerstitial injury. Therapeutic approaches that target the chronic hypoxia should prove effective against a broad range of renal diseases. Current modalities include the improvement of anemia with erythropoietin, the preservation of peritubular capillary blood flow by blockade of the renin-angiotensin system, and the use of antioxidants. Recent studies have elucidated the mechanism of hypoxia-induced transcription, namely that prolyl hydroxylase regulates hypoxia-inducible factor. This has given hope for the development of novel therapeutic approaches against this final common pathway.

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Once renal damage reaches a certain threshold, the progression of renal disease is consistent, irreversible, and largely independent of the initial insult. The final common pathway in this process has been studied closely. The hyperfiltration theory of Brenner *et al.* (1), which suggests that the progression of renal disease results from glomerular hemodynamic changes, has emerged as a popular concept. However, close pathologic analysis shows that functional impairment of the kidney is better correlated with the degree of tubulointerstitial damage than with that of glomerular injury (2–5), and this finding in turn has led to the broad recognition that the final common pathway of kidney failure operates principally in the tubulointerstitium (6–8).

The tubulointerstitial damage induced by the final common pathway leads to a decrease in GFR *via* several mechanisms. Tubular atrophy increases fluid delivery to the macula densa and triggers a decrease in GFR *via* tubuloglomerular feedback. Tubular damage also leads to the development of atubular glomeruli and decreases the number of functional nephrons. Finally, tubulointerstitial fibrosis impairs blood flow in the corresponding region and induces ischemic injury of nephrons.

One common mechanism that leads to renal failure *via* tubu-

linterstitial injury is massive proteinuria (9,10). Large-scale prospective studies, including the Modification of Diet in Renal Disease and Ramipril Efficacy in Nephropathy, have established the relationship between proteinuria and progressive renal disease (11,12). Systematic analyses of these reveal that greater urinary protein excretion predicts a faster decline in GFR (13,14). Accumulating evidence suggests that filtered macromolecules exert a number of critical effects on tubular cells, including the more general effects of lysosomal rupture and energy depletion, as well as more particular effects involving direct tubular injury by specific substances such as complement components (15,16).

In some diseases, however, including hypertensive nephrosclerosis, tubulointerstitial injury progresses to end-stage kidney failure in the absence of massive proteinuria. Furthermore, analysis of previous clinical studies shows that decreasing systemic BP and proteinuria only partially explain the beneficial effects of blockade of the renin-angiotensin system (RAS) on reducing the risk for progression of kidney disease (17,18). It thus is crucial to identify an alternative or additional mechanism—and, hopefully, a more unifying one—that is common to many forms of glomerular disease.

Chronic Hypoxia at the Center of Tubulointerstitial Injury and ESRD

In the kidney, most afferent glomerular arterioles arise from the interlobular arteries. The afferent arterioles divide dichotomously and gives rise to glomerular capillaries, which merge

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together again at the vascular pole to form the efferent arterioles. Efferent arterioles enter the peritubular capillary plexus, which surrounds tubules and offers oxygen and nutrients to tubular and interstitial cells (Figure 1).

Although blood flow to the kidney is high, accounting for 20% of cardiac output, the presence of oxygen shunt diffusion between arterial and venous vessels that run in close parallel contact means that renal tissue oxygen tensions are in fact comparatively low (19,20). Oxygen tension in the renal medulla, for example, does not rise above 10 mmHg. That in the renal cortex is more variable, however, with an average pO_2 of approximately 30 mmHg, but decreases dramatically in accordance with changes in renal perfusion. As a consequence, the kidney is somewhat sensitive to changes in oxygen delivery. Although this sensitivity has the merit of facilitating the kidneys in their adjustment of erythropoietin (EPO) production to changes in oxygen supply, it also renders them prone to hypoxic injury.

The chronic hypoxia hypothesis, proposed by Fine *et al.* (21), emphasizes chronic ischemic damage in the tubulointerstitium as a final common pathway in end-stage kidney injury. Since its introduction, this fascinating hypothesis has been investigated intensively and subsequently validated by Eckardt, Johnson, and many other investigators (22–24).

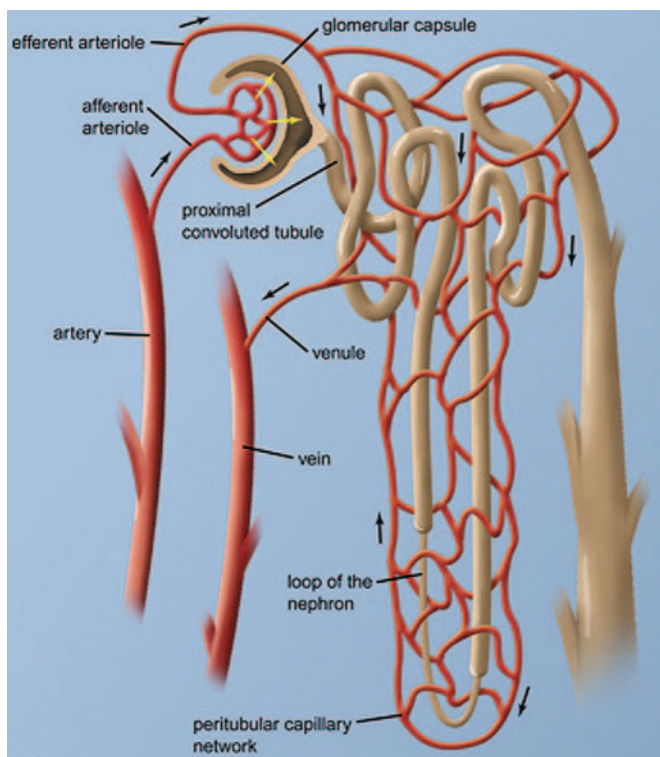


Figure 1. The microvasculature of the nephron. The peritubular capillary plexus is fed by glomerular efferent arterioles and supplies nutrients and oxygen to tubular and interstitial cells. Illustration by Josh Gramling—Gramling Medical Illustrations.

Chronic Hypoxia in the Kidney Is Multifactorial

Loss of Peritubular Capillaries and Fibrosis in Chronic Renal Disease

Chronic ischemia in the tubulointerstitium occurs *via* several mechanisms acting in concert. Histologic studies of human kidneys and animal models have shown that extensive tubulointerstitial injury is associated with damage to renal arterioles and arteries as well as with distortion and loss of peritubular capillaries (25–29). It therefore is of little wonder that fibrotic kidneys with advanced renal disease are devoid of peritubular capillary blood supply and oxygenation to the corresponding region (Figure 2A).

Even when the peritubular capillaries are essentially intact, however, interstitial fibrosis still impairs tubular oxygen supply. This is because the extended distance between the capillaries and tubular cells reduces the efficiency of oxygen diffusion (Figure 2B). In this regard, it is notable that hypoxia *per se* is a profibrogenic stimulus for tubular cells, interstitial fibro-

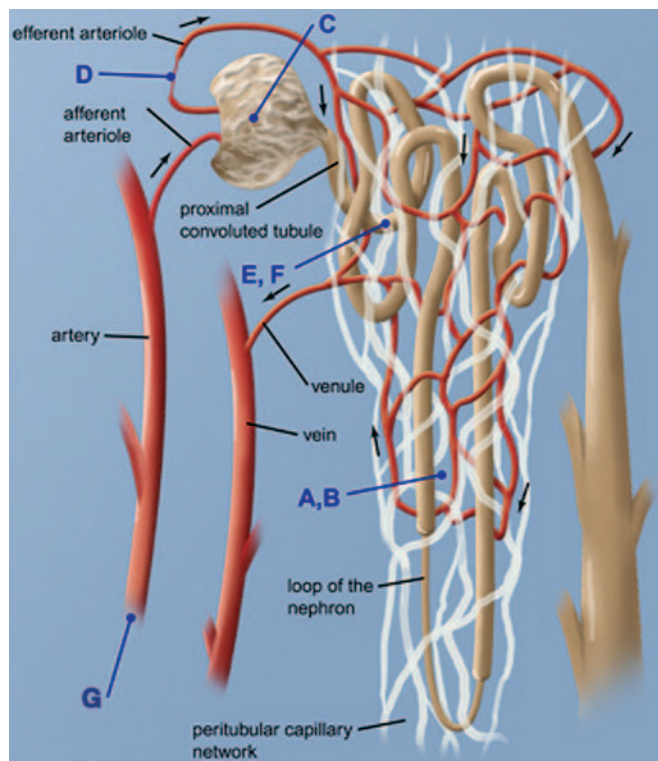


Figure 2. Multiple mechanisms of chronic hypoxia in the kidney. Mechanisms of hypoxia in the kidney of chronic kidney disease include loss of peritubular capillaries (A), decreased oxygen diffusion from peritubular capillaries to tubular and interstitial cells as a result of fibrosis of the kidney (B), stagnation of peritubular capillary blood flow induced by sclerosis of “parent” glomeruli (C), decreased peritubular capillary blood flow as a result of imbalance of vasoactive substances (D), inappropriate energy usage as a result of uncoupling of mitochondrial respiration induced by oxidative stress (E), increased metabolic demands of tubular cells (F), and decreased oxygen delivery as a result of anemia (G). Illustration by Josh Gramling—Gramling Medical Illustrations.

blasts, and renal microvascular endothelial cells. Tubular cells under hypoxic conditions undergo epithelial-mesenchymal transdifferentiation to become myofibroblasts (30). Hypoxia can also activate fibroblasts and change the extracellular matrix metabolism of resident renal cells (31,32). A fibrogenic response leads in turn to the obliteration of peritubular capillaries. Furthermore, renal tubular cells that are subjected to severe or prolonged hypoxia develop in their mitochondria functional deficits that lead to persistent energy deficits, subsequently causing them to undergo apoptosis (33). Together, chronic hypoxia in this compartment can lead to transdifferentiation or apoptosis (or both) of tubular cells, activation of resident fibroblasts, and further obliteration and loss of peritubular capillaries with progression of fibrosis. These changes may combine to institute a vicious cycle of regional hypoxia and progressive kidney failure in the late stages of disease.

Glomerular Damage and Hypoxia of the Tubulointerstitium

Hypoxia also plays a pathogenic role in the relatively early stages of kidney disease, well before the development of structural tubulointerstitial injury. Peritubular capillaries occur downstream of the glomerular efferent arterioles. Impairment of the “parent” glomerular capillary bed, as occurs in glomerulosclerosis, for example, thus automatically results in a decrease in peritubular perfusion and tubular oxygen supply (Figure 2C). In a model of accelerated glomerulosclerosis induced by repeated injection of anti-Thy1 antibody in uninephrectomized rats, we observed a decrease in blood flow in peritubular capillaries using intravital microscopy and physiologic lectin perfusion (34). Stagnation of peritubular capillary blood flow was associated with hypoxia in the corresponding tubulointerstitium, and both preceded the development of histologic tubulointerstitial injury and peritubular capillary loss.

Hemodynamic Maladjustment in the Tubulointerstitium: Imbalance of Vasoactive Substances

Even in the presence of structurally intact glomeruli, imbalances in vasoactive substances and associated intrarenal vasoconstriction can cause chronic hypoxia in the kidney in the early stage of kidney disease, before the development of histologic changes in the tubulointerstitium (Figure 2D). Futrakul *et al.* (35) performed intrarenal hemodynamic studies in patients with severe glomerulonephritis using radioisotope techniques and showed that elevated efferent arteriolar resistance and decreased peritubular capillary flow were associated with reversible renal functional impairment. This reversible change in peritubular capillary flow may have reflected an improvement in the imbalance of vasoactive substances in the kidney. They recently extended these observations to report a correlation between a decrease in peritubular capillary flow and tubular dysfunction in patients with type 2 diabetes and normoalbuminuria (36). These results support the concept that chronic hypoxia may/can induce tubulointerstitial injury, which eventually leads to ESRD in patients with a variety of kidney diseases.

Among various vasoactive substances, local activation of RAS is especially important because it can lead to constriction

of efferent arterioles, hypoperfusion of postglomerular peritubular capillaries, and subsequent hypoxia of the tubulointerstitium in the downstream compartment. To clarify the mechanism of these effects, we used a remnant kidney model in rats induced by ligation of renal artery branches, in which RAS is markedly activated. Our computer-assisted morphologic analysis demonstrated narrowing and distortion of peritubular capillaries with decreased blood flow and hypoxia in a very early phase in this model, before the development of structural kidney damage (37). In addition, angiotensin II damages endothelial cells directly: Administration of angiotensin II to rats causes the loss of peritubular capillaries, an effect that is ameliorated by receptor blockade (38,39). A second important mechanism of angiotensin II-induced ischemia is inefficient cellular respiration and hypoxia *via* oxidative stress, which is detailed below. Thus, angiotensin II induces tubulointerstitial hypoxia *via* both hemodynamic and nonhemodynamic mechanisms. Intrarenal vasoconstriction may also occur secondary to increased local endothelin or a local loss of vasodilating nitric oxide (NO).

Role of Anemia in Hypoxia of the Kidney

The amount of O₂ delivered, either to the whole body or to specific organs, is the product of blood flow and arterial O₂ content. Under most circumstances, oxygen delivery (DO₂) is determined using the equation $DO_2 = CO \times (\%Sat \times 1.39 \times [Hb])$, where CO is cardiac output in liters per minute, %Sat is percentage of hemoglobin O₂ saturation, [Hb] is hemoglobin concentration in grams per liter, and 1.39 is the hemoglobin binding constant. From the equation, anemia in kidney disease may accelerate the decline in renal function by inducing tubulointerstitial hypoxia (Figure 2G). The important role of anemia is emphasized by the fact that anemia is observed at a relatively early stage of renal dysfunction. Both the Third National Health and Nutrition Examination Survey and the National Kidney Foundation Kidney Early Evaluation Program showed that the risk for anemia significantly increases when GFR falls below 60 ml/min per 1.73 m² (40,41). Studies that have confirmed anemia as an independent risk factor for ESRD include a retrospective multivariate logistic analysis of 71,802 subjects that was performed by Iseki *et al.* (42) and an analysis of the data of the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan study of patients with type 2 diabetic nephropathy (43). The average increase in adjusted relative risk in the latter study was 11% for each 1-g/dl decrease in hemoglobin concentration.

Oxidative Stress and Inefficient Cellular Respiration

Chronic kidney disease is associated with oxidative stress. Angiotensin II, which is often upregulated in renal diseases, also promotes renal oxidative stress by stimulating NADPH oxidase. Furthermore, renal anemia contributes to oxidative stress as erythrocytes represent a major antioxidant component of the blood.

Superoxide leads to decreased NO bioavailability through ONOO⁻ formation. Adler *et al.* (44) showed that, because NO is a suppressor of mitochondrial respiration, depletion of NO by oxidative stress may stimulate mitochondrial respiration and

uncouple it from chemical energy consumption, resulting in tissue hypoxia (Figure 2E).

Kidneys of the spontaneously hypertensive rat (SHR), which characteristically undergo oxidative stress, revealed enhanced oxygen usage relative to tubular sodium transport and lower intrarenal pO_2 (45). Amelioration of oxidative stress improved renal oxygenation in a model of diabetic nephropathy (46) and in the angiotensin II continuous infusion model (47). The same oxidative stress-related mechanism may cause tubulointerstitial hypoxia in the aging kidney (48). It is likely that the renal hypoxia in these models results from a decrease in NO bioavailability and subsequent uncoupling of mitochondrial respiration as a result of oxidative stress.

Relative Hypoxia as a Result of Increased Metabolic Demand

When metabolic demand is increased, cells may suffer from relative hypoxia even under the maintenance of otherwise normal blood flow. Studies that have used the blood oxygen level-dependent (BOLD)–magnetic resonance imaging (MRI) technique (see Detection of Hypoxia in the Kidney section) have demonstrated that streptozotocin-induced diabetic kidneys suffer from tissue hypoxia at an early stage, before the development of structural changes (49). A possible explanation is that the hyperfiltration that occurs early in diabetic nephropathy leads to the increased delivery of sodium to tubular cells, imposing an excessive tubular sodium reabsorption workload relative to oxygen supply and subsequently resulting in tubular hypoxia (Figure 2F). Whether proteinuria causes functional hypoxia as a result of increased metabolic demand for reabsorption is an important question for future study.

Detection of Hypoxia in the Kidney

Despite an ever-increasing need for methods to identify and quantify hypoxic cells *in vivo*, suitable tools for detecting low oxygenation within tissues remain in short supply. Among those with potential diagnostic and research use are chemical tools such as pimonidazole, which is reduced under conditions of low oxygen availability. Visualization of this reaction allows us to detect hypoxic cells. These chemical methods are subject to a number of limitations, however: Their sensitivity is relatively low, detecting hypoxic cells at oxygen levels of <10 mmHg only, and they are not quantitative. Moreover, the hypoxia probe is metabolized and bound to cells over a 1- to 3-h period, requiring the assumption that oxygen content as well as delivery of the chemical compound, in terms of blood flow to the tissue, remain constant over the observation period. An additional limitation is that ischemia might impair the delivery of the compound to hypoxic tissues.

Polarographic oxygen sensors serve as true oxygen monitors, but the method is invasive and functional in only a limited range of tissues. In addition, because their signal is proportional to the measured quantity, they can become noisy and inaccurate, especially at low oxygen levels over relatively large tissue volumes.

To overcome these problems, Tanaka from our group recently established a novel transgenic rat (50). These animals, which were highlighted in a recent issue of the *JASN* (51),

express luciferase tagged with FLAG under a promoter composed of a tandem repeat of hypoxia-inducible factor (HIF) binding sites, providing a wide dynamic detection range of quantitative oxygen concentration with resolution down to the individual cell level. These animals enabled us to demonstrate different patterns of hypoxia at the early stage in various kidney disease models. An impressive regional correlation was noted between areas of hypoxia and areas of macrophage accumulation, apoptosis, and cell proliferation.

With regard to future clinical applications, BOLD-MRI is a promising tool for the estimation of tissue oxygenation *in vivo*. Whereas oxyhemoglobin is diamagnetic, deoxyhemoglobin is paramagnetic. Thus, when red blood cells that contain deoxyhemoglobin are placed in the magnetic field of an MRI, they cause field distortion, which appears as BOLD contrast in the resulting images. Limitations at this time include difficulty in obtaining reproducible and reliable information in this mobile organ, *i.e.*, the kidney.

Therapeutic Approaches to Chronic Hypoxia

Because chronic hypoxia in the tubulointerstitium is a final common pathway to ESRD, therapeutic approaches that target the chronic hypoxia should prove effective against a broad range of renal diseases. Potential treatment modalities that target chronic hypoxia in the kidney are summarized in Table 1. Details of each are discussed in the following sections.

Treatment Targeting Hypoxic Tubulointerstitial Damage: EPO

Because anemia is a risk factor for renal failure, correction of anemia by EPO and the subsequent improvement in oxygen delivery to the kidney may delay the progression of renal failure. This expectation was supported by several studies that suggested that progression might be delayed by an improvement in anemia by treatment with EPO. Gouva *et al.* (52) recently conducted a randomized, controlled trial of early *versus* deferred initiation of EPO in nondiabetic predialysis patients. The early treatment arm was started immediately on EPO titrated to produce a target hemoglobin level of >13 g/dl, whereas the deferred treatment arm started EPO only when

Table 1. Treatment modalities that target chronic hypoxia in the kidney^a

Improvement of anemia by EPO
Preservation of peritubular capillary blood flow by blockade of the renin-angiotensin system
Protection of the vascular endothelium
VEGF
dextran sulfate
Antioxidants to improve the efficiency of cellular respiration
HIF-based therapy
prolyl hydroxylase inhibitors
gene transfer of constitutively active HIF

^aEPO, erythropoietin; VEGF, vascular endothelial growth factor; HIF, hypoxia-inducible factor.

hemoglobin decreased below 9 g/dl. The results clearly showed that early initiation of EPO in predialysis patients with anemia significantly slows the progression of renal disease. However, some other trials, including the much larger Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin β trial, could not confirm beneficial effects of intensive treatment with EPO, and renoprotective effects of EPO requires further investigation.

Blockade of RAS to Ameliorate Tubulointerstitial Hypoxia

Norman *et al.* (53) were the first to show that blockade of RAS preserved peritubular capillary perfusion and tissue oxygenation in healthy anesthetized rats. In a remnant kidney model, we demonstrated that treatment with the angiotensin receptor blocker olmesartan restored blood flow in peritubular capillaries and improved oxygenation of the kidney (37). Although these improvements in kidney oxygenation by RAS inhibition are multifactorial, one important mechanism is the dilation of efferent glomerular arterioles and consequent increase in blood supply to the downstream tubulointerstitium. Inhibitors of RAS also serve as antioxidants and should ameliorate uncoupling of mitochondrial respiration, leading to more efficient use of oxygen. Supporting the latter mechanism, administration of an angiotensin receptor blocker corrected the reduced pO_2 in the cortices of the SHR and reversed the inefficient use of O_2 for Na^+ transport (54).

Protection of the Tubulointerstitial Vasculature

Protection of the tubulointerstitial vasculature theoretically should preserve blood supply and guarantee oxygenation to the corresponding compartment. Physiologically, endothelial cells are covered with a layer of heparan sulfate proteoglycans, which are crucial to the anticoagulant and anti-inflammatory properties of the endothelium. Endothelial cell injury is associated with the loss of these proteoglycans on the cell surface and thrombus formation, followed by subsequent ischemic tubulointerstitial damage. On this basis, we hypothesized that administration of dextran sulfate may protect the kidney from endothelial damage by re-establishing the intact endothelial surface. To investigate this, we used a model of thrombotic microangiopathy induced by renal artery perfusion of an antiglomerular endothelial antibody. Results showed that the administration of dextran sulfate protected the kidney against endothelial damage, probably by acting as a “repair coat” (55) to re-establish the intact anticoagulant and anti-inflammatory surface of the injured endothelium.

Kang *et al.* (56) treated rats with remnant kidneys with vascular endothelial growth factor (VEGF). This treatment improved renal function and lowered mortality rates compared with the vehicle control, and histology confirmed an increase in peritubular capillary endothelial cell proliferation and a decrease in peritubular capillary rarefaction. These results showed that treatment with VEGF protected the kidney by both the preservation of the capillary endothelium and the partial reversal of the impaired angiogenesis.

HIF as a Target for Drug Development

Although VEGF is a promising therapeutic modality, a potential pitfall of the induction of vessels by overexpression of a single gene such as VEGF is that the resulting vessels may be leaky, immature, or irregular. This is because the formation of a functionally intact microvasculature requires the coordinated activation of various genes. Rather, a more promising approach to protecting tissues against hypoxia is the activation of a “master gene” switch that results in a broad and coordinated downstream reaction.

At the center of the cellular response to hypoxia is HIF (57,58). HIF is composed of two subunits, an oxygen-sensitive HIF- α subunit and a constitutively expressed HIF- β subunit (also known as aryl hydrocarbon receptor nuclear translocator [ARNT]). The first isoform of HIF- α , HIF-1 α , was originally identified and cloned as a high-affinity DNA binding protein localized to the 3' hypoxia-responsive element of the EPO gene (59,60). Both HIF-1 α and HIF-1 β are members of the basic helix-loop-helix PER/ARNT/SIM (HLH-PAS) family of transcription factors. HIF binds to the hypoxia-responsive element in the cis-regulatory regions of its target genes and transcriptionally activates various genes encoding proteins that mediate adaptive responses to reduced oxygen availability.

Under normoxic conditions, two conserved proline residues within the central oxygen-dependent degradation domains of the HIF proteins are hydroxylated by the protein products prolyl hydroxylase domain containing (PHD) (61). This promotes binding of the von Hippel Lindau tumor suppressor protein, part of a ubiquitin ligase complex, resulting in polyubiquitylation and rapid degradation. Similarly, a conserved asparagine residue in the carboxyl-terminal transactivation domain of the HIF proteins is hydroxylated in normoxia by factor inhibiting HIF (FIH), preventing recruitment of the p300/CREB-binding protein transcriptional co-activators and thus leading to transcriptional repression. Under hypoxia, oxygen is lacking as an essential substrate for the hydroxylation reaction, and the unmodified HIF proteins avoid degradation but rather heterodimerize with HIF- β and upregulate the transcription of target genes. The biologic significance of HIF in the kidney under physiologic and pathologic conditions was demonstrated recently by Manotham from our group, who used *in vivo* gene transfer of DNA expressing negative dominant HIF and constitutively active fusion protein of HIF (62).

Owing to its ability to induce the expression of a variety of oxygen-regulated and renoprotective genes in a coordinated and physiologic manner, stimulation of HIF-1 signaling may be more effective in ischemic states. For emphasizing the efficacy of this “master gene” switch, transgenic mice expressing constitutively active HIF-1 α in the epidermis displayed an increase in dermal capillaries with a 13-fold elevation of VEGF (63). Despite a marked induction of hypervascularity, HIF-1 α did not induce edema, inflammation, or vascular leakage, phenotypes that develop in transgenic mice that overexpress VEGF in skin.

A recently discovered isoform of HIF-1 α , HIF-2 α , has been shown to possess both structural and functional similarity to HIF-1 α . HIF-1 α and HIF-1 β are expressed in most cell types,

whereas HIF-2 α shows a more restricted pattern of expression (57,64). To study the expression of HIF-1 α and HIF-2 α in the kidney, Eckardt's group used high-amplification immunohistochemical analyses (65,66) and showed that HIF-2 α was induced by hypoxia in peritubular endothelial cells and fibroblasts as well as glomerular endothelial cells, whereas HIF-1 was localized predominantly in the tubular cells (65). These results are consistent with those of studies that have used a surrogate marker for HIF-2 α in genetically engineered mice (67). In these mice, disruption of the murine *HIF-2a* gene was accomplished by homologous recombination in embryonic stem cells using a targeting plasmid in which a modified form of β -galactosidase (β -gal) was substituted for exon 2 of the *HIF-2a* gene. Activity staining for nuclear-localized β -gal revealed strong expression predominantly in vascular endothelial cells but also in the renal interstitial cell compartment, whereas β -gal staining was not evident in renal tubular cells.

Upregulation of the two HIF- α isoforms in the kidney by hypoxia was demonstrated in models of segmental renal infarction and radiocontrast nephropathy (68,69). Although cell-type specificity of HIF isoforms in these models was consistent with previous findings, temporal and spatial profiles of HIF activation were relatively complex, suggesting an important but complicated role of HIF in tissue preservation as a response to regional renal hypoxia. Our recent *in vitro* experiments showed that HIF-1 in tubular epithelial cells promotes proliferation of endothelial cells and that HIF-2 that is overexpressed in renal endothelial cells mediates migration and network formation; these results suggest a specific role of each isoform in certain cell types (70), although a clear differentiation of their roles independent of localization remains controversial.

Prolyl Hydroxylase

Three HIF prolyl hydroxylases with the potential to catalyze this reaction have been identified, and these proteins, termed PHD1, PHD2, and PHD3, seem to have arisen by gene duplication. The contribution of each to the physiologic regulation of HIF remains uncertain. These respective isoforms each have unique but overlapping patterns of tissue expression. Recent experiments using suppression by small interference RNA showed that each contributes in a nonredundant manner to the regulation of both HIF-1 α and HIF-2 α subunits and that the contribution of each PHD is strongly dependent on the abundance of the enzyme (71). In most cells, PHD2 has the most dominant effect because it is substantially the most abundant. Whereas both PHD2 and PHD3 proteins are induced by hypoxia, induction of PHD3 is particularly striking in certain cells, and under these conditions, the contribution of PHD3 is greater than that of PHD2. PHD3 seems to contribute more substantially to the regulation of HIF-2 α .

Prolyl hydroxylase inhibitors have been the focus of recent studies on novel strategies to stabilize HIF. More than half a century ago, oral administration of cobaltous chloride was used to treat anemia associated with chronic renal disease (72). Cobalt therapy led to a significant erythropoietic response in association with improved appetite and greater tolerance for medications that are necessary to correct electrolyte abnormal-

ities. However, blood values promptly declined to pretreatment levels when cobalt therapy was discontinued. Although the mechanism of erythropoiesis was unknown at that time, cobalt is now recognized as an inhibitor of PHD and thereby serves as a stimulator of HIF. We demonstrated the renoprotective effects of chemical preconditioning with cobaltous chloride in an ischemic model of renal injury (73). Administration induced upregulation of HIF-regulated genes, such as VEGF and EPO, and subsequently protected the kidney against the tubulointerstitial damage induced by hypoxia. Cobalt treatment was also effective when given after the initial insult in a chronic progressive glomerulonephritis model, a model of cyclosporin nephrotoxicity, and a model of chronic renal failure with glomerular hypertension, demonstrating not only its preventive but also its therapeutic potential (70,74,75).

Although cobalt administration has been somewhat effective in experimental animals, long-term administration to humans is hindered by various side effects. Less toxic and more potent PHD inhibitors have been sought, and a variety of new candidates are now under development (76). Whereas the mammalian genome encodes three closely related proteins with HIF prolyl hydroxylase activity, only a single HIF asparaginyl hydroxylase, FIH, has been identified to date. A therapeutic potential of FIH inhibitors is also an interesting subject to be pursued.

Conclusion

Chronic hypoxia is the final common pathway to end-stage renal failure. Ischemia of the kidney is induced by the loss of peritubular capillaries in the tubulointerstitium in the late stage of renal disease. Accumulating evidence also suggests a crucial role for hypoxia in the tubulointerstitium before structural microvasculature damage in the corresponding region, emphasizing the pathogenic role of this condition from an early stage of kidney disease. Given this background, therapeutic approaches against this final common pathway should be effective in a broad range of renal diseases. Presently, administration of EPO to correct anemia and blockade of RAS to preserve peritubular capillary flow and reduce oxidative stress are key to the improvement of kidney oxygenation. In the future, the HIF transcription factor at the center of many cellular hypoxic response pathways will be an attractive target for therapeutic manipulation.

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References

- Brenner BM, Meyer TW, Hostetter TH: Dietary protein intake and the progressive nature of kidney disease: The role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Engl J Med* 307: 652–659, 1982
- Risdon RA, Sloper JC, de Wardener HE: Relationship between renal function and histological changes found in renal biopsy specimens from patients with persistent glomerular nephritis. *Lancet* 2: 363–366, 1968
- Schainuck LI, Striker GE, Cutler RE, Benditt EP: Structural-functional correlations in renal disease. II. The correlations. *Hum Pathol* 1: 631–641, 1970
- Striker GE, Schainuck LI, Cutler RE, Benditt EP: Structural-functional correlations in renal disease. I. A method for assaying and classifying histopathologic changes in renal disease. *Hum Pathol* 1: 615–630, 1970
- Mackensen-Haen S, Bader R, Grund KE, Bohle A: Correlations between renal cortical interstitial fibrosis, atrophy of proximal tubules and impairment of glomerular filtration rate. *Clin Nephrol* 15: 167–171, 1981
- Nangaku M: Mechanisms of tubulointerstitial injury in the kidney: Final common pathways to end-stage renal failure. *Intern Med* 43: 9–17, 2004
- Eddy AA: Experimental insights into the tubulointerstitial disease accompanying primary glomerular lesions. *J Am Soc Nephrol* 5: 1273–1287, 1994
- Nath KA: Tubulointerstitial changes as a major determinant in the progression of renal damage. *Am J Kidney Dis* 20: 1–17, 1992
- Remuzzi G, Bertani T: Pathophysiology of progressive nephropathies. *N Engl J Med* 39: 1448–1456, 1998
- Zoja C, Benigni A, Remuzzi G: Cellular responses to protein overload: Key event in renal disease progression. *Curr Opin Nephrol Hypertens* 13: 31–37, 2004
- Ruggenenti P, Perna A, Gherardi G, Gaspari F, Benini R, Remuzzi G: Renal function and requirement for dialysis in chronic nephropathy patients on long-term ramipril: REIN follow-up trial. Gruppo Italiano di Studi Epidemiologici in Nefrologia (GISEN). Ramipril Efficacy in Nephropathy. *Lancet* 352: 1252–1256, 1998
- Klahr S, Levey AS, Beck GJ, Caggiula AW, Hunsicker L, Kusek JW, Striker G: The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of Diet in Renal Disease Study Group. *N Engl J Med* 330: 877–884, 1994
- Ruggenenti P, Perna A, Mosconi L, Pisoni R, Remuzzi G: Urinary protein excretion rate is the best independent predictor of ESRF in non-diabetic proteinuric chronic nephropathies. “Gruppo Italiano di Studi Epidemiologici in Nefrologia” (GISEN). *Kidney Int* 53: 1209–1216, 1998
- Hunsicker LG, Adler S, Caggiula A, England BK, Greene T, Kusek JW, Rogers NL, Teschan PE: Predictors of the progression of renal disease in the Modification of Diet in Renal Disease Study. *Kidney Int* 51: 1908–1919, 1997
- Nangaku M, Pippin J, Couser WG: Complement membrane attack complex (C5b-9) mediates interstitial disease in experimental nephrotic syndrome. *J Am Soc Nephrol* 10: 2323–2331, 1999
- Nangaku M, Pippin J, Couser WG: C6 mediates chronic progression of tubulointerstitial damage in rats with remnant kidneys. *J Am Soc Nephrol* 13: 928–936, 2002
- Jafar TH, Stark PC, Schmid CH, Landa M, Maschio G, de Jong PE, de Zeeuw D, Shahinfar S, Toto R, Levey AS; AIPRD Study Group: Progression of chronic kidney disease: The role of blood pressure control, proteinuria, and angiotensin-converting enzyme inhibition: A patient-level meta-analysis. *Ann Intern Med* 139: 244–252, 2003
- Atkins RC, Briganti EM, Lewis JB, Hunsicker LG, Braden G, Champion de Crespigny PJ, DeFerrari G, Drury P, Locatelli F, Wiegmann TB, Lewis EJ: Proteinuria reduction and progression to renal failure in patients with type 2 diabetes mellitus and overt nephropathy. *Am J Kidney Dis* 45: 281–287, 2005
- Brezis M, Rosen S, Silva P, Epstein FH: Renal ischemia: A new perspective. *Kidney Int* 26: 375–383, 1984
- Schurek HJ, Jost U, Baumgartl H, Bertram H, Heckmann U: Evidence for a preglomerular oxygen diffusion shunt in rat renal cortex. *Am J Physiol* 259: F910–F915, 1990
- Fine LG, Bandyopadhyay D, Norman JT: Is there a common mechanism for the progression of different types of renal diseases other than proteinuria? Towards the unifying theme of chronic hypoxia. *Kidney Int* 75: S22–S26, 2000
- Kang DH, Kanellis J, Hugo C, Truong L, Anderson S, Kerjaschki D, Schreiner GF, Johnson RJ: Role of the microvascular endothelium in progressive renal disease. *J Am Soc Nephrol* 13: 806–816, 2002
- Eckardt KU, Rosenberger C, Jurgensen JS, Wiesener MS: Role of hypoxia in the pathogenesis of renal disease. *Blood Purif* 21: 253–257, 2003
- Nangaku M: Hypoxia and tubulointerstitial injury: A final common pathway to end-stage renal failure. *Nephron Exp Nephrol* 98: e8–e12, 2004
- Bohle A, von Gise H, Mackensen-Haen S, Stark-Jakob B: The obliteration of the postglomerular capillaries and its influence upon the function of both glomeruli and tubuli. Functional interpretation of morphologic findings. *Klin Wochenschr* 59: 1043–1051, 1981
- Choi YJ, Chakraborty S, Nguyen V, Nguyen C, Kim BK, Shim SI, Suki WN, Truong LD: Peritubular capillary loss is associated with chronic tubulointerstitial injury in human kidney: Altered expression of vascular endothelial growth factor. *Hum Pathol* 31: 1491–1497, 2000
- Yuan H-T, Li X-Z, Pitera JE, Long DA, Woolf AS: Peritubular capillary loss after mouse acute nephrotoxicity correlates with down-regulation of vascular endothelial growth factor-A and hypoxia-inducible factor-1alpha. *Am J Pathol* 163: 2289–2301, 2003
- Kairaitis LK, Wang Y, Gassmann M, Tay YC, Harris DC: HIF-1alpha expression follows microvascular loss in advanced murine adriamycin nephrosis. *Am J Physiol Renal Physiol* 288: F198–F206, 2005
- Ohashi R, Kitamura H, Yamanaka N: Peritubular capillary injury during the progression of experimental glomerulonephritis in rats. *J Am Soc Nephrol* 11: 47–56, 2000
- Manotham K, Tanaka T, Matsumoto M, Ohse T, Inagi R, Miyata T, Kurokawa K, Fujita T, Ingelfinger JR, Nangaku M: Transdifferentiation of cultured tubular cells induced by hypoxia. *Kidney Int* 65: 871–880, 2004
- Norman JT, Orphanides C, Garcia P, Fine LG: Hypoxia-induced changes in extracellular matrix metabolism in renal cells. *Exp Nephrol* 7: 463–469, 1999

32. Norman JT, Clark IM, Garcia PL: Hypoxia promotes fibrogenesis in human renal fibroblasts. *Kidney Int* 58: 2351–2366, 2000
33. Tanaka T, Hanafusa N, Ingelfinger JR, Ohse T, Fujita T, Nangaku M: Hypoxia induces apoptosis in SV40-immortalized rat proximal tubular cells through the mitochondrial pathways, devoid of HIF-1-mediated upregulation of Bax. *Biochem Biophys Res Commun* 309: 222–231, 2003
34. Matsumoto M, Tanaka T, Yamamoto T, Noiri E, Miyata T, Inagi R, Fujita T, Nangaku M: Hypoperfusion of peritubular capillaries induced chronic hypoxia prior to progression of tubulointerstitial injury in a progressive model of rat glomerulonephritis. *J Am Soc Nephrol* 15: 1574–1581, 2004
35. Futrakul N, Tohsukhowong P, Patumraj S, Siriviriyakul P, Tippukmas N, Futrakul P: Treatments of hemodynamic maladjustment and oxidative stress prevent renal disease progression in chronically severe glomerulonephritides. *Ren Fail* 25: 839–844, 2003
36. Futrakul N, Vongthavarawat V, Sirisalipot S, Chairatanarat T, Futrakul P, Suwanwalaikorn S: Tubular dysfunction and hemodynamic alteration in normoalbuminuric type 2 diabetes. *Clin Hemorheol Microcirc* 32: 59–65, 2005
37. Manotham K, Tanaka T, Matsumoto M, Ohse T, Miyata T, Inagi R, Kurokawa K, Fujita T, Nangaku M: Evidence of tubular hypoxia in the early phase in the remnant kidney model. *J Am Soc Nephrol* 15: 1277–1288, 2004
38. Lombardi D, Gordon KL, Polinsky P, Suga S, Schwartz SM, Johnson RJ: Salt-sensitive hypertension develops after short-term exposure to angiotensin II. *Hypertension* 33: 1013–1019, 1999
39. Shao J, Nangaku M, Miyata T, Inagi R, Yamada K, Kurokawa K, Fujita T: Imbalance of T-cell subsets in angiotensin II-infused hypertensive rats with kidney injury. *Hypertension* 42: 31–38, 2003
40. Astor BC, Muntner P, Levin A, Eustace JA, Coresh J: Association of kidney function with anemia: The Third National Health and Nutrition Examination Survey (1988–1994). *Arch Intern Med* 162: 1401–1408, 2002
41. NKF Kidney Early Evaluation Program: Anemia and chronic kidney disease. *Am J Kidney Dis* 45[Suppl 2]: S71–S80, 2005
42. Iseki K, Ikemiya Y, Iseki C, Takishita S: Haematocrit and the risk of developing end-stage renal disease. *Nephrol Dial Transplant* 18: 899–905, 2003
43. Mohanram A, Zhang Z, Shahinfar S, Keane WF, Brenner BM, Toto RD: Anemia and end-stage renal disease in patients with type 2 diabetes and nephropathy. *Kidney Int* 66: 1131–1138, 2004
44. Adler S, Huang H: Impaired regulation of renal oxygen consumption in spontaneously hypertensive rats. *J Am Soc Nephrol* 13: 1788–1794, 2002
45. Welch WJ, Baumgartl H, Lubbers D, Wilcox CS: Nephron pO₂ and renal oxygen usage in the hypertensive rat kidney. *Kidney Int* 59: 230–237, 2001
46. Palm F, Cederberg J, Hansell P, Liss P, Carlsson PO: Reactive oxygen species cause diabetes-induced decrease in renal oxygen tension. *Diabetologia* 46: 1153–1160, 2003
47. Welch WJ, Blau J, Xie H, Chabrashvili T, Wilcox CS: Angiotensin-induced defects in renal oxygenation: Role of oxidative stress. *Am J Physiol Heart Circ Physiol* 288: H22–H28, 2005
48. Adler S, Huang H, Wolin MS, Kaminski PM: Oxidant stress leads to impaired regulation of renal cortical oxygen consumption by nitric oxide in the aging kidney. *J Am Soc Nephrol* 15: 52–60, 2004
49. Ries M, Basseau F, Tyndal B, Jones R, Deminiere C, Catargi B, Combe C, Moonen CW, Grenier N: Renal diffusion and BOLD MRI in experimental diabetic nephropathy. Blood oxygen level-dependent. *J Magn Reson Imaging* 17: 104–113, 2003
50. Tanaka T, Miyata T, Inagi R, Fujita T, Nangaku M: Hypoxia in renal disease with proteinuria and/or glomerular hypertension. *Am J Pathol* 165: 1979–1992, 2004
51. Ritz E: Starvation in the midst of plenty? The role of hypoxia in progression. *J Am Soc Nephrol* 16: 831–833, 2005
52. Gouva C, Nikolopoulos P, Ioannidis JP, Siamopoulos KC: Treating anemia early in renal failure patients slows the decline of renal function: A randomized controlled trial. *Kidney Int* 66: 753–760, 2004
53. Norman JT, Stidwill R, Singer M, Fine LG: Angiotensin II blockade augments renal cortical microvascular pO₂ indicating a novel potential renoprotective action. *Nephron Physiol* 94: 39–46, 2003
54. Welch WJ, Baumgartl H, Lubbers D, Wilcox CS: Renal oxygenation defects in the spontaneously hypertensive rat: Role of AT₁ receptors. *Kidney Int* 63: 202–208, 2003
55. Eto N, Kojima I, Inagi R, Miyata T, Fujita T, Johnson RJ, Shankland SJ, Nangaku M: Protection of endothelial cells by dextran sulfate in rats of thrombotic microangiopathy. *J Am Soc Nephrol* 16: 2997–3005, 2005
56. Kang DH, Hughes J, Mazzali M, Schreiner GF, Johnson RJ: Impaired angiogenesis in the remnant kidney model: II. Vascular endothelial growth factor administration reduces renal fibrosis and stabilizes renal function. *J Am Soc Nephrol* 12: 1448–1457, 2001
57. Maxwell P: HIF-1: An oxygen response system with special relevance to the kidney. *J Am Soc Nephrol* 14: 2712–2722, 2003
58. Marx J: Cell biology. How cells endure low oxygen. *Science* 303: 1454–1456, 2004
59. Semenza GL, Wang GL: A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol* 12: 5447–5454, 1992
60. Wang GL, Semenza GL: General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. *Proc Natl Acad Sci U S A* 90: 4304–4308, 1993
61. Epstein ACR, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian Y-M, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ, Ratcliffe PJ: *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* 107: 43–54, 2001
62. Manotham K, Tanaka T, Ohse T, Kojima I, Miyata T, Inagi R, Tanaka H, Sassa R, Fujita T, Nangaku M: A biological role of HIF-1 in the renal medulla. *Kidney Int* 67: 1428–1439, 2005
63. Elson DA, Thurston G, Huang LE, Ginzinger DG, McDonald DM, Johnson RS, Arbeit JM: Induction of hypervascularity without leakage or inflammation in transgenic mice overexpressing hypoxia-inducible factor-1 α . *Genes Dev* 15: 2520–2532, 2001

64. Freeburg PB, Abrahamson DR: Hypoxia-inducible factors and kidney vascular development. *J Am Soc Nephrol* 14: 2723–2730, 2003
65. Rosenberger C, Mandriota S, Jurgensen JS, Wiesener MS, Horstrup JH, Frei U, Ratcliffe PJ, Maxwell PH, Bachmann S, Eckardt K-U: Expression of hypoxia-inducible factor-1 and -2 in hypoxic and ischemic rat kidneys. *J Am Soc Nephrol* 13: 1721–1732, 2002
66. Wiesener MS, Jurgensen JS, Rosenberger C, Scholze CK, Horstrup JH, Warnecke C, Mandriota S, Bechmann I, Frei UA, Pugh CW, Ratcliffe PJ, Bachmann S, Maxwell PH, Eckardt KU: Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. *FASEB J* 17: 271–273, 2003
67. Scortegagna M, Ding K, Zhang Q, Oktay Y, Bennett MJ, Bennett M, Shelton JM, Richardson JA, Moe O, Garcia JA: HIF-2alpha regulates murine hematopoietic development in an erythropoietin-dependent manner. *Blood* 105: 3133–3140, 2005
68. Rosenberger C, Griethe W, Gruber G, Wiesener M, Frei U, Bachmann S, Eckardt KU: Cellular responses to hypoxia after renal segmental infarction. *Kidney Int* 64: 874–886, 2003
69. Rosenberger C, Heyman SN, Rosen S, Shina A, Goldfarb M, Griethe W, Frei U, Reinke P, Bachmann S, Eckardt KU: Up-regulation of HIF in experimental acute renal failure: Evidence for a protective transcriptional response to hypoxia. *Kidney Int* 67: 531–542, 2005
70. Tanaka T, Kojima I, Ohse T, Ingelfinger JR, Adler S, Fujita T, Nangaku M: Cobalt promotes angiogenesis via hypoxia-inducible factors and protects ischemic tubulointerstitium in the remnant kidney. *Lab Invest* 85: 1292–1307, 2005
71. Appelhoff RJ, Tian YM, Raval RR, Turley H, Harris AL, Pugh CW, Ratcliffe PJ, Gleadle JM: Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. *J Biol Chem* 279: 38458–38465, 2004
72. Gardner HF: The use of cobaltous chloride in the anemia associated with chronic renal disease. *J Lab Clin Med* 41: 56–64, 1953
73. Matsumoto M, Makino Y, Tanaka T, Tanaka H, Ishizaka N, Noiri E, Fujita T, Nangaku M: Induction of renoprotective gene expression by cobalt ameliorates ischemic injury of the kidney in rats. *J Am Soc Nephrol* 14: 1825–1832, 2003
74. Tanaka T, Matsumoto M, Inagi R, Kojima I, Fujita T, Miyata T, Nangaku M: Induction of renoprotective gene expression by cobalt ameliorates tubulointerstitial injury in the progressive Thy1 nephritis model. *Kidney Int* 2005, in press
75. Tanaka T, Kojima I, Ohse T, Inagi R, Miyata T, Ingelfinger JR, Fujita T, Nangaku M: Hypoxia-inducible factor (HIF) modulates tubular cell survival in cisplatin nephrotoxicity. *Am J Physiol Renal Physiol* 289: F1123–F1133, 2005
76. Warnecke C, Griethe W, Weidemann A, Jurgensen JS, Willem C, Bachmann S, Ivashchenko Y, Wagner I, Frei U, Wiesener M, Eckardt KU: Activation of the hypoxia-inducible factor-pathway and stimulation of angiogenesis by application of prolyl hydroxylase inhibitors. *FASEB J* 17: 1186–1188, 2003