Chronic hypoxia as a mechanism of progression of chronic kidney diseases: from hypothesis to novel therapeutics

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In chronic kidney disease, functional impairment correlates with tubulointerstitial fibrosis characterised by inflammation, accumulation of extracellular matrix, tubular atrophy and rarefaction of peritubular capillaries. Loss of the microvasculature implies a hypoxic milieu and suggested an important role for hypoxia when the ''chronic hypoxia hypothesis'' was proposed a decade ago as an explanation for the progressive nature of fibrosis. Recent data in man provide evidence of decreased renal oxygenation in chronic kidney disease while more direct support for a causal role comes from data in rodent models showing that the decline in renal oxygenation precedes matrix accumulation, suggesting hypoxia may both initiate and promote the fibrotic response. Indeed, in vitro studies show that hypoxia can induce pro-fibrotic changes in tubulointerstitial cells. Additional postulated roles for hypoxia in chronic kidney disease are the sustaining of the inflammatory response, the recruitment, retention and differentiation towards a pro-fibrotic phenotype of circulating progenitor cells and the alteration of the function of intrinsic stem cell populations. Given that accumulating data suggests that chronic hypoxia is a final common pathway to end-stage renal disease, therapeutic strategies that target hypoxia may be of benefit in retarding progression. Normalisation of microvascular tone, administration of pro-angiogenic factors to restore microvasculature integrity, activation of hypoxia-inducible transcription factors and hypoxia-mediated targeting and mobilisation of progenitor cells are all potential targets for future therapy. The limited success of existing strategies in retarding chronic kidney disease mandates that these new avenues of treatment be explored.

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CHRONIC KIDNEY DISEASE AND RENAL FIBROSIS

Renal fibrosis is the hallmark of chronic kidney diseases (CKDs) of diverse etiologies in which accumulation of extracellular matrix (ECM) disrupts normal tissue architecture leading to progressive renal dysfunction and organ failure.[1] Regardless of the initiating insult, CKD presents a common pathology of glomerulosclerosis and tubulointerstitial fibrosis and it is well established that tubulointerstitial fibrosis provides the best predictive indicator of progression to end-stage disease. Tubulointerstitial fibrosis presents a number of characteristic features[1] including an inflammatory cell infiltrate, which results from both activation of resident inflammatory cells and recruitment of circulating inflammatory cells; an increase in interstitial fibroblasts due to increased proliferation and decreased apoptosis of resident interstitial cells as well as recruitment of cells to the tubulointerstitium; the appearance of myofibroblasts expressing the cytoskeletal protein α-smooth muscle actin, which arise by differentiation of resident interstitial fibroblasts and infiltrating cells and via transdifferentiation;[2] accumulation of ECM as the net result of increased production and decreased turnover of matrix proteins; tubular atrophy as a consequence of apoptosis and epithelial–mesenchymal transdifferentiation (EMT); and, rarefaction of peritubular capillaries.[1] The development of fibrosis is associated with an increase in expression of proinflammatory, vasoconstrictive, and profibrotic factors (including transforming growth factor (TGF)-β1 connective tissue growth factor, platelet-derived growth factor, fibroblast growth factor-2, endothelin (ET)-1, and angiotensin II), which is paralleled by a decrease in antifibrotic factors (such as hepatocyte growth factor, and bone morphogenetic protein-7).[1,2]

HYPOXIA AND FIBROSIS

Detailed examination of biopsies of patients with CKD showed marked rarefaction of peritubular capillaries.[3] Loss of the microvasculature implies a hypoxic milieu and suggested an important role for hypoxia when the 'chronic hypoxia hypothesis' was proposed a decade ago as a mechanism by which glomerular injury is transmitted to the tubulointerstitium and sets in train the progressive scarring and decline in renal

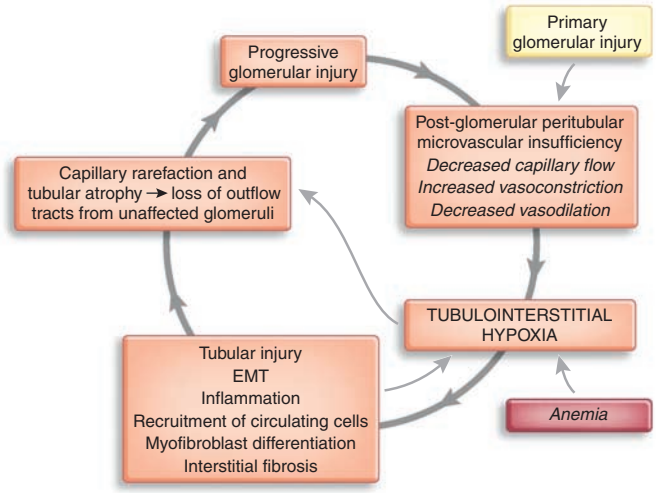

Figure 1 | Hypoxia and progression of CKD. Primary glomerular injury leads to changes in postglomerular peritubular hemodynamics inducing endothelial injury and microvascular insufficiency, creating a hypoxic tissue environment and triggering tubular injury, EMT, inflammation, recruitment of circulating precursors, myofibroblast differentiation, and fibrosis. The evolving inflammatory response and accumulation of ECM impact on adjacent unaffected capillaries and tubules compromising the outflow tracts of previously intact glomeruli, exacerbating injury and leading to progressive scarring and a loss of organ function. In addition to rarefaction of the microvasculature, concomitant anemia, pathological vasoconstriction, increased oxygen diffusion distances due to ECM accumulation, and increased metabolic demand of tubular cells may all contribute to localized tubulointerstitial hypoxia.

function.[4] The hypothesis proposed that primary glomerular disease leads to restricted postglomerular flow from affected glomeruli and downstream injury to the peritubular capillary network (Figure 1). Microvasculature dysfunction creates a hypoxic environment that triggers a fibrotic response in tubulointerstitial cells. This, in turn, impacts on adjacent previously unaffected capillaries, nephrons and glomeruli, exacerbating injury, extending the regions of hypoxia, and setting up an inexorable cycle of destruction to organ failure. Data from animal models have established a direct correlation between rarefaction of the peritubular capillaries and development of glomerular and tubulointerstitial scarring.[5] However, before dropout of capillaries, other mechanisms may contribute to decreased tissue oxygenation including anemia, increased vasoconstriction driven by an increase in vasoconstrictors and/or loss of vasodilators, increased metabolic demand as a result of hyperfiltration and hypertrophy of uninjured nephrons, and increased oxygen diffusion distances as ECM accumulates between blood vessels and adjacent cells.[6–8]

EFFECTS OF HYPOXIA ON TUBULOINTERSTITIAL CELLS

Support for a causal role of hypoxia in fibrosis comes from *in vitro* studies of the effect of hypoxia on tubular epithelial cells and interstitial fibroblasts that, as described above, undergo a series of characteristic changes in fibrosis.

Studies on tubular epithelial cells have largely focused on proximal tubular epithelial cells (PTE) as the predominant epithelial cell type in the cortex and susceptible to hypoxic injury. In PTE, hypoxia induces a complex transcriptional response with changes in expression of a large number of genes involved in cell survival and adaptation.[9] Although a number of transcription factors have been shown to be activated by hypoxia,[10] the hypoxia-inducible factor (HIF) family are considered master regulators of the adaptive response controlling expression of hundreds of genes.[7,10,11] HIFs are heterodimeric transcription factors comprising a constitutively expressed β-subunit and an oxygen-regulated α-subunit. In normoxia, stability, and activity of the α-subunit is regulated by oxygen-dependent hydroxylation of proline and asparagine residues by prolyl hydroxylase 2 and factor inhibiting HIF-1, respectively. In hypoxia, HIFα proteins are stabilized, dimerize with HIFβ and bind to hypoxia-response elements in the regulatory regions of target genes.[7,10,11] Three mammalian α-subunits have been identified: HIF-1α, HIF-2α (EPAS), and HIF-3α (IPAS). Study has largely concentrated on HIF-1α and HIF-2α, HIF-3α is less well characterized but a splice variant has been suggested to act as an intrinsic repressor of the HIF pathway. HIF-1α and HIF-2α appear to have both unique and overlapping functions and to act in a cell type- and stimulus-specific manner.[7,11] In the hypoxic kidney, HIF-1α accumulates in tubules and in papillary interstitial cells whereas HIF-2α is induced in peritubular endothelial cells and fibroblasts (reviewed in Nangaku and Ekardt[7]).

Hypoxia alters PTE matrix metabolism, promoting ECM accumulation with a switch to production of interstitial collagen and suppression of matrix degradation.[8] EMT is increasingly implicated in fibrosis;[2] exposure of PTE to hypoxia induces a myofibroblastic phenotype whereas more prolonged exposure leads to mitochondrial injury and apoptosis consistent with the loss of tubular cells *in vivo*.[6–8] In PTE, hypoxia also induces expression of fibrogenic factors including TGFβ and ET-1 as well as angiogenic factors, vascular endothelial growth factor and angiopoietin-4, capable of acting as autocrine or paracrine mediators.[6–8]

Notably, although TGFβ is induced by hypoxia in PTE, the changes in ECM metabolism appear to occur via TGFβ1-independent mechanisms.[8] In addition to directly inducing fibrogenic changes, hypoxia can synergize with other known fibrogenic stimuli to amplify the pathological response (reviewed in Norman and Fine[8]).

Fibroblasts are the major ECM-producing cells in the tubulointerstitium.[1] *In vitro*, hypoxia promotes a fibrogenic phenotype in these cells with increased proliferation, enhanced myofibroblast differentiation and contraction, and altered ECM metabolism; changes that are associated with sustained activation of focal adhesion kinase and downstream signaling pathways including those mediated by the Ras guanosine triphosphatases and mitogen-activated protein kinases.[8] As in PTE, exposure to low oxygen increases fibroblast ECM production, upregulating a variety of matrix proteins.[8] In addition, hypoxia upregulates enzymes involved in post-translational modification of collagen, potentially producing a matrix with altered mechanical properties and resistant to degradation (reviewed in Norman and Fine[8]). Some aspects of the fibrogenic phenotype induced by hypoxia are likely due to the autocrine actions of hypoxia-induced growth factors, however, transcriptional induction of collagen type I by hypoxia is independent of TGFβ. Furthermore, this transcriptional activation is HIF-independent and regulated by the transcription factor, Sp1.[8]

In parallel with increased ECM production, hypoxia also suppresses matrix degradation via decreased expression and activity of matrix metalloproteinases, in particular interstitial collagenase matrix metalloproteinase-1, and increased HIF-dependent expression of the endogenous inhibitor tissue inhibitor of metalloproteinase (TIMP)-1.[8] Another family of metalloproteases, the adamalysins, are involved in processing of ECM proteins and shedding of cell-surface molecules.[12] These proteases have not been widely studied in renal pathophysiology, however members of adamalysin family are regulated by hypoxia in renal fibroblasts (unpublished observations) and may have as yet unexplored roles in hypoxia-induced fibrosis.

Data from nonrenal fibroblasts suggest additional profibrotic effects of hypoxia in renal fibroblasts in suppressing apoptosis,[13] inducing production of proinflammatory factors[14,15] and upregulating components of the renin–angiotensin system.[16] Taken together, the *in vitro* data indicate that hypoxia can, directly or indirectly, induce functional and phenotypic changes in PTE and interstitial fibroblasts consistent with changes seen in these cells in CKD.

Although endothelial cells of the peritubular capillaries are a primary target for hypoxic injury in the kidney and numerous studies have examined the response of a variety of endothelial cell types to hypoxia, the hypoxic response in renal microvascular endothelial cells is largely unexplored. As hypoxia is classically a potent angiogenic stimulus the lack of vascular repair in the hypoxic kidney may suggest that these endothelial cells differ in their response to a low oxygen milieu. Loss of endothelial cells in fibrotic kidneys[5] implies that the predominant response of renal endothelial cells to hypoxia may be apoptosis. There is increasing evidence the endothelial cells possess the capacity to transdifferentiate to (myo)fibroblasts[17] and it is interesting to speculate that in the kidney, hypoxia might drive this process leading to disruption of peritubular capillaries, simultaneously exacerbating tissue hypoxia and increasing the number of ECM-producing fibroblasts. Another hypoxia-sensitive component of the peritubular microvasculature, which may play an as yet unappreciated role in renal fibrosis, are the pericytes, contractile cells which surround the endothelial cells and stabilize the vessels.[18] These cells have the potential to contribute to pathological vasoconstriction and also to ECM accumulation as they too possess the capacity to differentiate into synthetic (myo)fibroblast-like cells.[19]

HYPOXIA AS AN INFLAMMATORY STIMULUS

Persistent inflammation is considered to be an intrinsic component of the fibrotic response.[1] Hypoxia provides a homing signal for inflammatory cells[20] that accumulate at sites of injury. It may also activate resident immune cells[21] and as such may be an important inflammatory stimulus in the setting of CKD particularly where, in the absence of vascular regeneration, chronic hypoxia may potentiate an ongoing proinflammatory response or impede resolution and stimulate fibrosis. In addition, it has been suggested that some inflammatory cells have the potential to differentiate into fibroblasts and to contribute to pathological ECM accumulation,[19] whether hypoxia can drive this process is an intriguing possibility which remains to be tested.

HYPOXIA AS A STIMULUS FOR PROGENITOR CELL RECRUITMENT

In the fibrotic kidney a proportion of ECM-producing cells are derived from circulating precursor cells.[22] Although the mechanisms of cell recruitment and retention within the kidney have not been defined, a role for hypoxia seems plausible as progenitor cells preferentially home to ischemic sites through interactions between the CXCR4 chemokine receptor and its ligand, stromal cell-derived factor-1, both of which are HIF target genes.[23] It is interesting to speculate that once progenitor cells are recruited the hypoxic milieu within the kidney may affect cell differentiation, potentially inducing a profibrotic phenotype. Similarly, hypoxia may alter the differentiation of intrinsic progenitor/stem cells such that these cells contribute to the fibrotic process either directly or indirectly by failure to repair injury.

IN VIVO EVIDENCE FOR HYPOXIA IN CKD

Since the introduction of the 'chronic hypoxia hypothesis',[4] an increasing number of studies in animal models have shown an association between hypoxia and CKD (Table 1) (reviewed in Nangaku,[6] Nangaku and Eckardt,[7] and Norman and Fine,[8] see also Rosenberger *et al.*[24] and Bernhardt *et al.*[25]) with the early decline in tissue oxygenation suggesting a causal relationship. A variety of different techniques have

Table 1 | *In vivo* evidence of hypoxia in CKD

Method for detecting hypoxia	Species	Disease model
Porphyrin phosphorescence	Rat	Antiglomerular basement membrane antibody
Pimonidazole protein adduct histochemistry	Rat	Uninephrectomy+anti-Thy-1 antibody
		Remnant kidney
		Diabetic nephropathy
		Cyclosporin A-induced nephropathy
		Aging kidney
		Polycystic kidney disease
	Mouse	Folic acid nephropathy
		Adriamycin nephrosis
		Unilateral ureteral obstruction
Hypoxia-sensing transgenic rat	Rat	Remnant kidney
		Puromycin aminonucleoside nephrosis
		Aging kidney
Blood oxygen-dependent magnetic resonance imaging	Rat	Diabetic nephropathy
Clarke-type microelectrode	Rat	Diabetic nephropathy

been used to measure tissue oxygenation (Table 1). Historically oxygen microelectrodes have been widely used in studies of renal oxygenation and have demonstrated the presence of hypoxia in diabetic nephropathy.[6–8] However, in the context of animal models of CKD, the most commonly used method to evaluate hypoxia is immunohistochemical detection of hypoxia-dependent pimonidazole proteins adducts. Pimonidazole staining has revealed the early presence of hypoxia in uninephrectomy and anti-Thy1 glomerulonephritis, the remnant kidney, diabetic nephropathy,[24] cyclosporine nephropathy, the aging kidney and polycystic kidney disease (PKD) in the rat,[25] and in murine models including folic acid nephropathy, adriamycin nephrosis, and unilateral ureteral obstruction.[6–8] The limitation of this approach is that pimonidazole forms adducts at $PO_2 < 10$ mm Hg and thus is nonquantitative. Imaging techniques such as blood oxygen-dependent magnetic resonance imaging may provide quantitative information on renal oxygenation and have been used to show hypoxia in the diabetic kidney.[8] The hypoxia-sensing transgenic rat expressing a hypoxia-response element-driven luciferase vector provides a novel means to examine the patterns of renal hypoxia and has been used to demonstrate the presence of hypoxia in puromycin aminonucleoside nephrosis and in the aging kidney,[7] which displays many of the histological features of CKD including loss of peritubular capillaries. Further, hypoxia was detected in aging kidneys with only mild tubulointerstitial disease indicating that similar to the findings in CKD hypoxia occurs early in the development of the fibrosis and correlates with the degree of tubulointerstitial injury, suggesting a causal role for hypoxia in age-related fibrotic renal disease.

The accumulating data from animal models provide a compelling argument for hypoxia as a primary mediator of progressive scarring in the kidney, however the key question is whether this also applies in humans. Although there are currently only limited clinical data, increased expression of HIF has been reported in biopsies of patients with diabetic nephropathy,[26] immunoglobulin A nephropathy, PKD,[25] and chronic allograft nephropathy[27] suggesting the presence of hypoxia in these disease settings and supporting the idea that hypoxia is an important contributory factor in the pathogenesis of CKD in humans. Furthermore, the changes in HIF expression correlate with the extent of tubulointerstitial injury. The majority of studies in human samples have used immunohistochemical detection of HIFs as a surrogate marker of hypoxia. A potential limitation of this approach is that although HIFs are clearly induced by hypoxia, non-hypoxic stabilization of HIF has also been reported. Moreover in animal models only partial overlap of pimonidazole and HIF signals is observed, thus, immunohistochemical data need to be verified by more direct measurements of tissue oxygenation.

CHRONIC HYPOXIA AS A THERAPEUTIC TARGET IN CKD

The central role for hypoxia in renal fibrosis suggests that therapeutic manipulation of the hypoxic response may be of benefit in preventing or retarding disease.[6–8] Approaches to ameliorate hypoxia-mediated profibrotic changes induced include correction of anemia (not discussed here); normalization of vascular tone and intrarenal microvascular perfusion; preservation, repair, and stabilization of the tubulointerstitial microvasculature; stabilization of HIF in the tubulointerstitium; and, manipulation of hypoxia-induced cell homing.

Normalization of vascular tone and improved intrarenal microvascular perfusion by alleviating vasoconstriction and/or enhancing vasodilation is a logical goal. Activation of the rennin–angiotensin system has long been implicated in the pathogenesis of renal fibrosis and the therapeutic efficacy of angiotensin-converting enzyme inhibition and angiotensin receptor blockade is well established. Importantly, in the rat remnant kidney, administration of an angiotensin-converting enzyme inhibitor and an angiotensin receptor blockade restored cortical perfusion and reduced tissue hypoxia thereby extending the possible range of renoprotective mechanisms exerted by these classes of agents.[6,7] ET, another potent vasoconstrictor, is also overexpressed in fibrosis; selective and nonselective ET receptors antagonists have been developed and show renoprotective effects in animal models. In particular, selective antagonism of ET-A receptors may have maximal benefit to decrease ET-mediated vasoconstriction and block direct fibrogenic effects of ET while leaving intact ET-B receptor-mediated functions. On the other hand, to promote vasodilation it may also be possible to enhance NO signaling which is compromised in the setting of fibrosis.

Loss of the peritubular capillaries is a hallmark of renal fibrosis and was the observation that initiated the investigation of the potential role of hypoxia in renal scarring. In tumors, hypoxia is a potent angiogenic stimulus inducing a range of angiogenic factors and one of the anomalies in

considering hypoxia as a fibrogenic stimulus is the apparent failure of angiogenic repair in the hypoxic kidney. In models of CKD, although there is an early, transient increase in vascular endothelial growth factor, levels of expression are reduced in advanced disease.[5] The mechanism for this reduction is unclear but it appears to correlate with inflammatory cell infiltration and may relate to tubular atrophy as PTEs are an important source of vascular endothelial growth factor.[8] Interestingly, a recent *in vitro* study suggests that under hypoxic conditions albumin suppresses vascular endothelial growth factor production by tubular cells, providing a possible link between proteinuria and hypoxia in CKD and evidence of a potentially important interaction between tubular epithelial cells and microvascular endothelial cells in the setting of hypoxia although it should be noted that the concentrations of albumin used were high and equivalent to plasma concentrations.[28] Expression of another angiogenic factor, angiopoietin-1, is also suppressed in a mouse model of CKD,[29] suggesting an overall reduction in proangiogenic factors.

In parallel, an increase in anti-angiogenic factors may also contribute to failure of angiogenic repair as at least two matricellular proteins upregulated in fibrosis have anti-angiogenic activity[30] and, at least in some cell types, expression of an endogenous inhibitor of angiogenesis, endostatin, is increased by hypoxia.[31] Thus preservation, repair, and stabilization of the tubulointerstitial microvasculature by administration of exogenous angiogenic factors[5,29] or inhibition of endogenous inhibitors may represent promising therapeutic strategies although as highlighted by a recent reports that showed variable outcomes of angiopoietin-1 therapy in different models,[29,32] the clinical efficacy of angiogenic factor therapy will depend on a detailed understanding of the role of such factors in the pathogenesis of CKD in humans.

HIF is a major regulator of the adaptive response to hypoxia and several lines of evidence suggest that activation of HIF pathway may be renoprotective. Consistent with a protective role for HIF, activation of HIF by cobalt chloride, carbon monoxide, or inhibition of prolyl hydroxylase has been shown to attenuate injury in a number of different disease models including ischemic injury, cisplatin nephropathy, acute and progressive glomerulonephritis, the remnant kidney (reviewed in Nangaku,[6] Nangaku and Eckardt,[7] and Norman and Fine,[8] see also Weidemann *et al.*[33]). Conversely, genetic deficiency in HIF was found to exacerbate ischemic injury.[34] Collectively, these data suggest that activation of HIF protects the kidney against injury induced by a variety of stimuli and points to this system as a potential therapeutic target. In terms of clinical applications, the toxicity of cobalt chloride precludes its therapeutic use and a more promising approach may be to stabilize HIF by blocking prolyl hydroxylase activity, a number of inhibitors are under development with initially promising results.[35] Blockade of factor inhibiting HIF and gene transfer may provide additional alternative strategies to activate the HIF pathway.

Although the therapeutic potential of activation of the HIF pathway in CKD has elicited considerable interest, a note of caution needs to be sounded with the recent demonstration that cell-specific activation of HIF in podocytes induces rapidly progressive glomerulonephritis and renal failure;[36] that HIF drives EMT;[26] and, that HIF activates profibrotic genes.[6–8] Further, deletion of *Hif1a* in tubular epithelial cells has been shown to reduce fibrosis in the unilateral ureteral obstruction model of CKD.[26] Given that activation of HIF appears to have both cytoprotective and profibrotic effects, potential therapeutic strategies that target the HIF signaling pathway will require careful evaluation. A particular challenge will be to develop drugs that preferentially stabilize HIF in the kidney to avoid the potentially detrimental effects of widespread activation of this system.

A more speculative therapeutic aspect of the hypoxic response is the manipulation of hypoxia-induced cell homing to prevent homing, retention, and/or differentiation of fibrogenic precursor cells within the kidney. Conversely, an intriguing possibility is that hypoxia-mediated recruitment and differentiation mechanisms can be used to selectively direct progenitor cells and genetically-engineered precursors to the kidney and to sites of injury to either inhibit fibrosis or promote regression.

CONCLUSION

The 'chronic hypoxia hypothesis' was first put forward in 1998,[4] in the ensuing decade a substantial body of evidence has accumulated from *in vitro* studies, from *in vivo* models, and, more recently, from studies in humans to place hypoxia at the center of ideas on mechanisms of progression of CKD. A detailed understanding of the role of hypoxia in fibrosis and the interaction of hypoxia with other factors influencing progression opens the door to a variety of novel therapeutic strategies aimed at preventing or retarding a wide range of intractable kidney diseases.

DISCLOSURE

All the authors declared no competing interests.

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