

*Editorial Reviews*

## Capillary rarefaction, hypoxia, VEGF and angiogenesis in chronic renal disease

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### Abstract

Tubulointerstitial hypoxia and peritubular capillary rarefaction are typical features of chronic progressive renal disease. In response to low oxygen supply, hypoxia-inducible factors (HIFs) are activated but until now, it is unclear if this increased expression leads to a stabilization of the disease process and thus is nephroprotective or contributes to interstitial fibrosis and/or tubular atrophy. This duality has also been described as far as vascular endothelial growth factor (VEGF), one of the major target genes of HIFs, is concerned. On the one hand, neoangiogenesis driven by VEGF, if intact, ameliorates hypoxia, on the other, VEGF is a potent pro-inflammatory mediator and neoangiogenesis, if defective because interference by other pathologies exaggerates injury. In summary, experimental data support the idea that dependent on timing and predominant pathology, hypoxia counter-regulatory factors exert beneficial or undesirable effects. Thus, before their therapeutic potential can be fully explored, a better way to characterize the clinical and pathophysiological situation in an individual patient is mandatory.

**Keywords:** capillary rarefaction; chronic kidney disease; hypoxia-inducible factor; neoangiogenesis; tubulointerstitial hypoxia

### Introduction

To study the transcriptional response that separates patients with a stable proteinuric renal disease from those with a progressive loss of glomerular filtration rate, we isolated proximal tubular epithelial cells by laser capture microdissection from cryocut biopsy tissue. Genome-wide gene expression profiling followed by a systems biology analysis identified an activation of hypoxia response and vascular endothelial growth factor (VEGF) signalling pathways in progressive patients associated with an up-regulation of hypoxia-inducible factor (HIF)-1 $\alpha$ . Interestingly, however, one of the most important HIF target genes, VEGF-A, was down-regulated on messenger RNA and protein level. The expression levels of HIF-1 $\alpha$  and VEGF-A were significantly superior in predicting clinical outcome to proteinuria, renal function

and degree of tubular atrophy and interstitial fibrosis at the time of biopsy [1]. A reduction of VEGF expression was also shown by Lindenmeyer *et al.* [2] in diabetic nephropathy. These studies thus provided evidence for tubular hypoxia in progressive renal disease, which, however, does not lead to the expression of VEGF-A, a potent pro-angiogenic factor (see Table 1).

### Chronic progressive renal disease—a tubulointerstitial process

By convention, chronic progressive renal disease is defined by a decrease of filtration function at the glomerular level. However, on a histopathological level, tubulointerstitial rather than glomerular injury predicts the clinical outcome. Tubular atrophy and interstitial fibrosis are hallmarks of advanced and (probably) irreversible kidney diseases of diverse aetiology. Fibrosis presents a number of characteristic features including an inflammatory cell infiltrate, an increase in interstitial fibroblasts and matrix, while tubular atrophy is linked to epithelial cell apoptosis and epithelial to mesenchymal transdifferentiation. Another characteristic feature of progressive renal disease is a loss of postglomerular peritubular capillaries leading to a reduction in oxygen supply and it has been proposed that hypoxia is one of the common pathways of chronic renal disease progression [3].

### Capillary rarefaction

Several years ago, Bohle *et al.* [4] noted a negative correlation between the area occupied by peritubular capillaries in biopsies and the serum creatinine concentration and prognosis in patients with diverse renal disorders, such as diabetic glomerulosclerosis, amyloidosis, benign nephrosclerosis or chronic interstitial nephritis. This observation has been reproduced in various animal models including experimental glomerulonephritis [5], the remnant kidney [6], ureteral obstruction [7], renal artery stenosis (RAS) [8] and ageing [9].

**Table 1.** Members of the hypoxia response and VEGF signalling pathways showing a significant difference in expression between biopsies of patients with post-biologically stable and progressive chronic kidney disease (positive number: increased expression in patients with a progressive loss of renal function after biopsy when compared to stable subjects and negative number: decreased expression) (adapted from reference [1]); eNOS, endothelial nitric oxide synthase; VEGFR, vascular endothelial growth factor receptor; ERK, extracellular regulated kinase

Gene name	Pathway component	Fold change
CREB-binding protein (Rubinstein–Taybi syndrome)	CREBBP/p300	2.877
PTK2B protein tyrosine kinase 2 beta	FAK	2.539
Rho GTPase-activating protein 1	Rac	2.475
NO synthase-1 (neuronal)	ENOS	2.191
Phosphoinositide-3 kinase, regulatory subunit 2 (p85 beta)	PI3K	2.059
Mitogen-activated protein kinase 1	MEK/Erk	1.913
Protein kinase C, epsilon	PKC	1.866
Phosphoinositide-3 kinase, regulatory subunit 2 (p85 beta)	PI3K	1.814
HIF-1, alpha subunit (basic helix-loop-helix transcription factor)	HIF-1 $\alpha$	1.792
Phosphoinositide-3 kinase, catalytic, beta polypeptide	PI3K	1.765
Aryl hydrocarbon receptor nuclear translocator	HIF-1- $\beta$	1.748
Phospholipase C, gamma 2 (phosphatidylinositol specific)	PLC- $\gamma$	-1.750
FK506-binding protein 12-rapamycin-associated protein 1	MTOR	-1.764
PTK2 protein tyrosine kinase 2	FAK	-1.798
VEGF-A	VEGFR-2	-2.516

Damage to the postglomerular microvasculature determines also the long-term outcome after ischaemia-reperfusion injury [10]. After bilateral renal artery clamping for 45–60 min, glomerular filtration rate in Sprague–Dawley rats returns to normal after 1–2 weeks. However, after 40 weeks, the animals develop proteinuria and prominent interstitial scarring, which is associated with a 30–50% reduction in microvascular density. Similar results were obtained in mice [11]. These experimental data resemble the observation made in humans that acute kidney injury (AKI) dramatically increases the risk of end-stage renal disease (ESRD) [12]. When compared to matched controls, the hazard ratio for developing ESRD after AKI is 41.2 (95% confidence interval 34.6–49.1) for patients with pre-existing chronic kidney disease and 13 (10.6–16.0) for those with normal renal function prior to AKI. Similar associations were found by Wald *et al.* In their study, the incidence of ESRD was 2.63 per 100 person-years among 3769 individuals with a history of AKI requiring dialysis and 0.91 among 13598 matched controls [13]. In line with this hypothesis, delayed renal allograft function is an important predictor of long-term transplant outcome and injury to and progressive loss of peritubular capillaries is a characteristic feature of chronic allograft nephropathy [14].

### Hypoxia in renal disease

Hypoxia-induced alterations in gene expression profiles have been detected at the glomerular as well as tubulointerstitial level [15]. One of the most obvious sequels of capillary rarefaction in the tubulointerstitial compartment of the kidney is a reduction in oxygen supply. Ischaemia can also be induced without a loss in peritubular capillaries via excess postglomerular vasoconstriction induced by angiotensin II, endothelin or a reduction in the availability of vasodilators like nitric oxide (NO). Interestingly in this context, angiotensin II receptor blockade, usually associated with renal protection on a glomerular level, also improves microvascular oxygen tension in the interstitium [16].

Paradoxically, the renal medulla even under normal conditions has the lowest oxygen tension compared with any other organ despite the fact that the kidney receives the largest fraction of the cardiac output. Hypoxia is the inevitable consequence of the unique medullary vasculature that serves a counter-current function to prevent the medullary solute gradient from being dissipated. However, the anti-parallel arrangement of blood vessels also allows diffusional shunting of oxygen from the descending arterial vasa recta to the ascending venous vessels. As a consequence, the oxygen tension in the renal outer medulla is between 10 and 20 mm Hg. At the same time, tubular epithelial cells have a large oxygen demand because of high transporter activity. However, only the cells in the loop of Henle, but not the proximal tubular epithelial cells of the S3 segment, can switch to anaerobic glycolysis in case of oxygen deprivation [17].

Tubulointerstitial hypoxia was demonstrated immunohistochemically by the presence of pimonidazole protein adducts, by blood oxygen-dependent magnetic resonance imaging detecting deoxygenated haemoglobin or in hypoxia-responsive element-driven luciferase vector animals with experimental glomerulonephritis, remnant kidney and diabetic nephropathy as well as during ageing and polycystic renal disease [18].

In the kidney, a reduction in oxygen delivery is a potent stimulus for inflammation providing a homing signal for inflammatory cells [19] and pro-fibrotic-circulating progenitor cells [20] via interactions between the chemokine receptor CXCR4 and its ligand, stromal cell-derived factor-1 [21]. In biopsies from patients with chronic renal disease, Eardley *et al.* [22] found a significant association between peritubular capillary density and interstitial macrophage accumulation as well as urinary monocyte chemoattractant protein 1 levels.

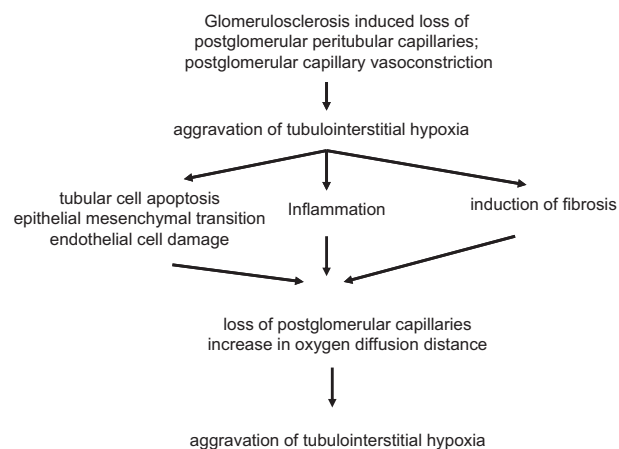
Hypoxia may also lead to the loss of tubular epithelial cells resulting in atubular glomeruli [23] or trigger transition of tubular cells towards a myofibroblastic phenotype [24–26]. Oxygen-deprived fibroblasts enter a fibrogenic pheno-

type and matrix degradation is reduced by promoting expression of endogenous tissue inhibitors of metalloproteinase [26]. The resulting interstitial accumulation of extracellular matrix reduces peritubular capillary blood flow even further and impairs oxygen diffusion thus aggravating regional hypoxia. Hypoxia also causes endothelial cell apoptosis, endothelial to mesenchymal transition as well as damage to the vascular pericytes [27, 28] further promoting loss of peritubular capillaries and creating a vicious cycle typical for chronic progressive renal disease (Figure 1).

## The two faces of HIFs

In the kidney, just like in other organs, various response pathways are activated in the presence of hypoxia. On a molecular level, the most important steps of adaptation are mediated by HIFs. They belong to a broader group of transcription factors comprising the basic helix-loop-helix-per-arnt-sim (PAS) family and consist of a labile  $\alpha$ -subunit (1 or 2 $\alpha$ , which both have identical but also distinct activities, and 3 $\alpha$ , which contains no transactivation domain and antagonizes HIF-1 $\alpha$  and -2 $\alpha$ -driven gene expression) and a stable  $\beta$ -subunit (ARNT). Once heterodimerized, they form a transcriptional complex which translocates to the nucleus and binds to its consensus enhancer (hypoxia-responsive element) transactivating, in collaboration with a number of cofactors, 100–200 target genes involved in angiogenesis, erythropoiesis and energy metabolism [28].

The primary mechanism of regulation of HIF activity is oxygen-dependent proteasomal degradation of the  $\alpha$ -subunit. At low oxygen tension or in the absence of the von Hippel Lindau E3 ubiquitin ligase, which is composed of von Hippel-Lindau tumour suppressor protein, Cullin2, Elongin C and Rbx1, HIF- $\alpha$  escapes degradation, heterodimerizes with HIF- $\beta$  and binds the transcriptional coactivator CBP/p300. Besides hypoxia, several other coregulators of HIF- $\alpha$  have been described recently. They include reactive oxygen species, ascorbate, succinate, fumarate or NO, the acetyltransferase ARD1 and under hypoxic conditions, the small ubiquitin modifiers group (SUMO) [29]. In view of this tight regulation, obviously HIF- $\alpha$  overactivity as well as underactivity likely has drastic consequences on cell growth, differentiation and metabolism.

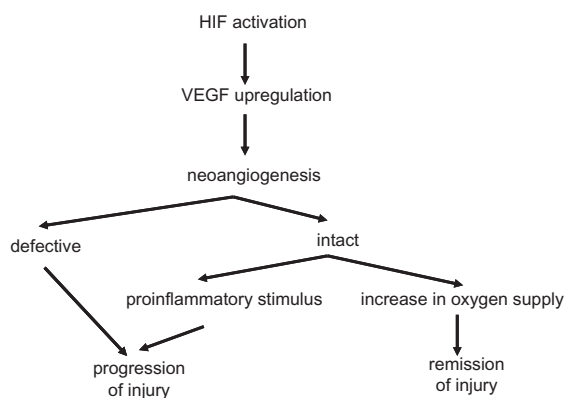


**Fig. 1.** Pathogenesis and consequences of tubulointerstitial hypoxia.

Under hypoxic conditions typical for renal injury, the HIF system is activated and in the remnant kidney model as well as after ureteral obstruction, this occurs even before any histological evidence of tubulointerstitial damage [30, 31]. Increased HIF expression has also been shown in biopsies from patients with diabetic nephropathy, IgA glomerulonephritis and chronic allograft nephropathy [18]. In many of these diseases, the degree of HIF expression correlated with the extent of tubular injury. However, whether this increased activity is beneficial or harmful is unclear and may well depend on the context, the cell type-affected and/or the duration of HIF expression. One target gene for HIF is the pro-fibrotic connective tissue growth factor. Accordingly, tissue-specific deletion of HIF-1 $\alpha$  inhibited the development of tubulointerstitial disease in mice [31]. In the von Hippel-Lindau tumour suppressor knockout mouse, HIF-1 $\alpha$  is also a critical contributor to the progression of fibrosis [32]. Interestingly, however, in kidney disease, HIF activation remains suboptimal and thus, it is still possible that augmentation of HIF activity might be nephroprotective [33] as at least in some forms of experimental renal disease (ischaemia reperfusion, progressive uninephrectomized anti-Thy-1 nephritis, cisplatin nephrotoxicity or Habu snake venom nephritis) activation of HIFs improves outcome [34]. Also, in experimental acute ischaemic renal disease, therapeutic interventions to increase HIF activity are beneficial [35]. Even though these encouraging results are not consistent with a recent study in humans [36] inducing prolonged HIF activation, a beneficial effect of short-term HIF up-regulation in AKI cannot be excluded.

## The two faces of VEGF and angiogenesis

HIFs also up-regulate pro-angiogenic genes like placental growth factor, angiopoietin-1 and -2 and platelet derived growth factor BB (Figure 2) [37]. However, the induction of VEGF is perhaps the most remarkable effect—up to 30-fold within minutes. VEGF stimulates angiogenesis in a dose-dependent manner [38] and is an absolutely critical mediator of vasculogenesis. Knockout mice die with major vascular defects several days after coitus [39] and VEGF also has a role in glomerular development [40]. So the results of our study [1], where an impaired expression of VEGF in the kidney-characterized patients with a loss of glomerular filtration rate could provide evidence that progressive renal disease is due to an impaired angiogenic response to hypoxia, which may be further dampened by the additional induction of anti-angiogenic molecules, such as thrombospondin-1 [25]. An important protective role for VEGF in the maintenance of an intact tubulointerstitial compartment was suggested by the study of Choi *et al.* [41]. In human kidney biopsies, they observed that peritubular capillary density was positively correlated to proximal tubular size and negatively with interstitial volume. Compared with normal control kidneys, where only podocytes consistently expressed VEGF, an increased expression was found in the tubules that maintained their structural integrity. A nephroprotective role of VEGF can also be deduced from the fact that proteinuric renal disease usually progress



**Fig. 2.** Possible consequences of HIF activation in chronic renal disease-associated tubulointerstitial pathology.

faster to ESRD than pathologies characterized by a low urinary albumin excretion as it has been shown that albumin reduces the production of VEGF by tubular epithelial cells [42]. The beneficial effect of VEGF, which is normally expressed by podocytes, tubular epithelial cells as well as endothelial cells [43, 44] on the kidney, is supported by the observation that a loss of VEGF expression in knockout mice leads to endothelial cell swelling, capillary collapse and proteinuria [45], a picture that resembles pre-eclampsia in the human setting. Treatment with VEGF antagonists can lead to proteinuria and thrombotic microangiopathy in humans [46]. In experimental ischaemia-reperfusion injury, VEGF expression is reduced [47] and in a study by Leonard *et al.* [48], early, but not late, administration of VEGF prevented the long-term damage described in placebo-treated rats after renal artery clamping.

However, the role of VEGF in renal disease is much more complex as it also mediates glomerular hypertrophy and sclerosis in diabetes [49] and overexpression can lead to a form of collapsing glomerulopathy [45]. Treatment with anti-VEGF antibodies was beneficial in an animal model of early diabetes [50]. An interesting insight into the dual role of VEGF in renal disease is provided by studies in the remnant kidney model. In early stages, glomerular hypertrophy is accompanied by an increase in glomerular VEGF expression and VEGF is also found at increased levels in the tubulointerstitial compartment. In later stages, however, VEGF expression decreases in parallel with the progression of histological damage and VEGF administration results in the preservation of peritubular capillaries, with a partial reduction of tubulointerstitial injury but cannot prevent glomerulosclerosis [51, 52].

Even as far as the role of VEGF-driven angiogenesis in renal disease is concerned, the situation is complex. Microvascular plasticity is the ability of the microvascular networks to adapt to the metabolic local conditions by up- or down-regulating vascular proliferation [53]. Even though neovascularization is closely linked to ischaemia, excessive generation of new blood vessels is also observed in situations like chronic inflammatory diseases, malignancies and diabetic retinopathy and nephropathy [54].

In inflammation, leucocytes and platelets induce and deliver pro-angiogenic factors to mediate the proliferation of local endothelial cells and/or facilitate the recruitment of

endothelial progenitor cells (EPCs) [49]. This response resolves with the resolution of the acute inflammation; however, excessive neovascularization can be found in chronic disease [53]. As pro-inflammatory cytokines have pro-angiogenic properties vice versa VEGF and NO activate inflammation [55, 56]. In line with this concept is the fact that neovessels are usually associated with leucocyte infiltration; these vessels are 'sticky' [57] and VEGF plays a central role in this process as has been shown in chronic allograft nephropathy [58]. Hence, inflammation and angiogenesis induce overlapping and interactive processes [49] that could be self-perpetuating. The complexity of the regulatory networks in renal disease was nicely demonstrated recently by Mu *et al.* [59]. Angiostatin, a potent anti-angiogenic peptide, that also exerts massive anti-inflammatory activity, was genetically induced in animals with remnant kidneys. Treatment reduced renal peritubular capillary number but despite so ameliorated interstitial fibrosis. As this was associated with a reduced influx of macrophage and T-cell infiltration, the authors concluded that the protective role of angiostatin in this animal model is due to its anti-inflammatory properties despite a negative effect on neoangiogenesis.

Finally for angiogenesis to function properly, an orchestrated interplay of several key players like the recruitment of EPCs, endothelial cell proliferation and an adequate amount of VEGF, NO production and angiopoietin-1 to induce vessel maturation is required [60]. In chronic renal disease, many of these mechanisms are disturbed. NO availability is reduced due to high asymmetric dimethylarginine levels [61] leading to a reduced release of EPC from the bone marrow [62]. Accordingly, the number of circulating EPCs is reduced in renal failure [63]. Accumulation of extracellular matrix may also physically interfere with EPC and local endothelial cell migration and extracellular matrix molecules such as thrombospondin-1 can exert anti-angiogenic effects by inhibiting endothelial cell proliferation, migration and tube formation [64].

Immature and distorted microvessels may present functional abnormalities, increased permeability or blind endings [65, 66] and new and fragile vessels might even exacerbate tissue injury [53]. These defects have not only been described in the kidney but also act systemically [67]. Simple administration of VEGF, therefore, is unlikely to be beneficial and in obstructive nephropathy, administration of VEGF actually worsened injury [68]. Intriguingly in some animal models of renal disease, anti-angiogenic drugs like thalidomide restore renovascular function [65, 69].

Recent studies have shown the capability of endothelial or mesenchymal progenitor cells as a targeted intervention to promote correct vasculogenesis [53]. They have been shown to promote vascular proliferation and maturation, which may in part be explained by their paracrine secretion of a variety of angiogenic factors. Chade *et al.* [70] infused EPCs in a pig model of RAS. Autologous EPCs increased the renal expression of angiogenic factors like VEGF, stimulated proliferation and maturation of new vessels and attenuated renal microvascular remodelling and fibrosis in RAS. Furthermore, they normalized renal microvascular and filtration function.

It thus can finally be speculated that the reduced activity of VEGF observed by us and Lindenmeyer [1, 2] represents an intrinsic protective mechanism to avoid inflammation even at the cost of hypoxia.

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## Iron and vascular calcification. Is there a link?

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### Abstract

Iron deficiency is frequently seen in patients with end-stage renal disease, particularly in those treated by dialysis, this is

because of an impairment in gastrointestinal absorption and ongoing blood losses or alternatively, due to an impaired capacity to mobilize iron from its stores, called functional