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# Peritubular endothelium: The Achilles heel of the kidney?

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**The development of renal ischemia has been postulated to be a main cause of the progressive nature of kidney diseases. In recent years, it has become clear that inappropriate and sustained activation of the endothelium could mediate this phenomenon. Endothelial activation will result in leucostasis and can compromise peritubular flow. The associated sustained redox signaling will also accelerate the development of endothelial senescence. In addition, risk factors for renal disease progression can reduce endothelial repair. In the course of these events, loss of capillary structure and rarefaction develops, which drives the further development of nephron loss. In this mini review, the evidence for this pathophysiological concept as well as the possibility to detect such endothelial activation in the clinical arena is summarized.**

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In recent years, we have come to understand that the progressive nature of many renal diseases in a way is a remarkable phenomenon as the kidney is capable of substantial repair. These repair processes are not limited to the tubular epithelium but also include complex structures such as glomeruli. For example, in the anti-Thy1 glomerulonephritis model, glomerular capillaries, mesangium, and podocytes are all being regenerated.[1] Also in human renal disease such as type I diabetes and anti-neutrophil cytoplasmic antibody associated glomerulonephritis, glomerular regeneration could be observed.[2,3] This implies that in progressive renal disease, the natural recovery processes of the kidney somehow must have become compromised. In this mini review, we discuss the hypothesis that recurrent endothelial activation and loss of renal endothelial repair could be the Achilles heel in this natural recovery potential of the kidney. In particular, activation of the peritubular endothelium in the renal medulla may compromise renal perfusion and result in loss of tubular function.

## ENDOTHELIAL CELL ACTIVATION AND RENAL PERFUSION

The close and countercurrent apposition of the medullar peritubular plexus in descending and ascending vasa recta allows oxygen and nutrient diffusion to be shunted back to the cortex.[4] Clearly, this is the basis for maintenance of a corticomedullary osmotic gradient and regulation of water transport, but it also renders the medulla relatively hypoxic (10–15 mm Hg). This phenomenon in combination with oxygen consumption by tubular epithelium, in particular the medullary thick ascending limb (mTAL), sensitizes the renal (outer) medullary epithelium to ischemia. Epithelial survival in such an environment thus depends upon an intricate interplay between the peritubular endothelium, interstitial cells and the tubular cell itself. Although these cells are equipped with adaptive mechanisms to function in this milieu (see below), the hypoxic environment reduces the margin for survival. Experimental data have shown that hypoxia may drive renal fibrosis as well, by inducing epithelial-mesenchymal transformation and fibroblast activation.[5]

In general, tubular epithelium can tolerate hypoxia relatively well. Induction of a hypoxia-inducible factor-1 (HIF-1) response is probably a central mechanism. Tubular

epithelium also has a very strong potential to regenerate. Per hour about 70 000 tubular epithelial cells are shed in the urine and replaced.[6] Complete resolution of massive tubular cell necrosis in clinical acute renal failure can be observed. This high capacity for tubular repair is thought to primarily reflect the intrinsic capacity of surviving epithelial cells to dedifferentiate and proliferate. More recently, the renal papilla has been recognized as a stem cell niche and it has been proposed that these cells may play a role in repair as well.[7] Although in older age, signs of tubular senescence can be noticed,[8] there is little clinical or experimental evidence of failure of tubular regenerative potential in the presence of adequate perfusion.

By contrast, there is considerable evidence that the capillary perfusion and integrity of the renal endothelium is a locus minoris resistentiae. One of the key characteristics of the endothelial cell (EC) is their ability to respond to metabolic and immunological signals. This is normal endothelial physiology as it allows leukocytes to target to sites of infection and to adapt tissue perfusion. The EC does so by switching from the quiescent NO signaling dominated phenotype, to redox signaling resulting in endothelial activation.[9] In many renal diseases, this (physiological) endothelial redox signaling is inappropriately activated by factors such as angiotensin II, modified (lipo)proteins, insulin resistance, and elevated uric acid. Sustained endothelial activation may impact on the renal microcirculation. Rolling and sticking of leukocytes will reduce blood flow in the capillaries. As in the kidney, oxygen consumption directly depends on blood flow, it is likely that such endothelium–leukocyte interactions will thus reduce oxygen tensions (Figure 1).[10] Although renal ECs themselves express HIF-2$\alpha$, which induces strong prosurvival responses to hypoxia, such reductions in renal tissue oxygenation may jeopardize tubular survival. Secondly, sustained production of reactive oxygen species will exceed the capacity of the endothelial enzymatic and non-enzymatic anti-oxidants and results in protein modifications and stress-induced senescence. Activation of the tumor suppressor P53 and subsequent lysosomal destabilization is probably a main mechanism driving the development of senescence.[11] Careful labeling studies with proliferation markers such as Ki67 have demonstrated, even in healthy subjects, the presence of a very high glomerular and peritubular EC turnover. The proliferation index doubles that of the tubular epithelial cells.[6] This likely is a reflection of ongoing microvascular repair. This high cell turnover may have further implications for regenerative capacity of the ECs. DNA synthesis during cell division results in the progressive shortening of telomeric DNA and replicative senescence, in particular, in the presence of low activity of the telomere synthesizing enzyme telomerase. The importance of loss of telomerase activity for endothelial repair is illustrated by experiments where forced expression of the catalytic unit of telomerase, Telomerase Reverse Transcriptase (TERT) can expand endothelial lifespan and its proliferative capacity.[12] Redox stress has been identified as the main cause of loss of telomerase activity[13] adding another mechanism that reduces endothelial renewal upon its activation. With the development of endothelial senescence in the kidney, capillary attrition will develop as well resulting in nephron drop-out (see below).

## ENDOTHELIAL BACK-UP SYSTEMS

In recent years, attention has been drawn to circulating progenitor cells, as well as to resident mural cells that can participate in re-endothelialization and could act as a salvage mechanism to restore the renal microcirculation. In the capillary circulation, it appears to be primarily the circulating

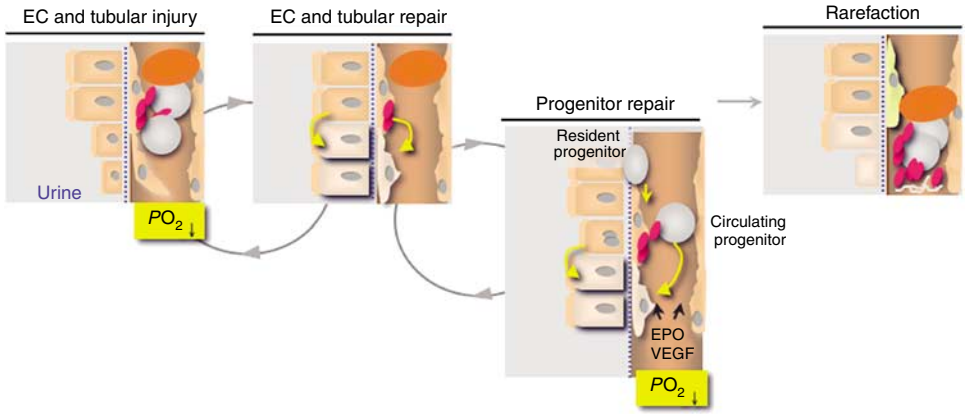


**Figure 1 | Working hypothesis on the role of peritubular capillary function as a determinant of renal function.** Endothelial activation in the microcirculation allows rolling and stickiness of leukocyte thus reducing peritubular perfusion in the peritubular capillaries and oxygen and nutrient delivery to the tubular epithelium. Sustained endothelial activation will finally lead to EC detachment. Local endothelial and tubular cells can regenerate and restore endothelial capillary integrity. When senescence of resident ECs occurs the capillary architecture may become dependent upon re-endothelialization by resident or circulating progenitor populations that can differentiate into endothelial-like cells under the influence of hypoxia inducible cytokines such as VEGF and erythropoietin. Maintenance of the peritubular network and thus epithelial function is then determined by the balance between endothelial injury and repair. When these back-up systems fail thrombotic occlusion occurs and nephron drop-out develops.

populations that can contribute to re-endothelialization, whereas in larger vessels, resident progenitors are probably more important.[14] This is exemplified by studies in human kidney and bone marrow transplantation patients where (bone marrow) derived circulating cells were found to have incorporated in peritubular capillaries.[15] The origin and functional importance of this repair mechanism is still under debate. *In vitro* studies have mostly focused on the endothelial differentiation of a hemangioblast-like bone marrow progenitor cell.[16] These are, however, rare cells and their clinical repair potential is relatively unexplored. The *in vivo* evidence of progenitor-mediated repair is mostly based on attaching mononuclear cells that have committed to an endothelial-like phenotype as illustrated by expression of, for example, von Willebrand factor and endothelial nitric oxide synthase. These cells are present in large numbers in the circulation. Infusion of these cells has been shown to restore damaged endothelial surface and stimulate angiogenesis.[17] At close examination, these cells bear characteristics of myeloid cells with the potential to become immune competent cells as well when exposed to an inflammatory milieu.[18] Nevertheless, in the model of adriamycin glomerulonephritis in the mouse, integrity of the capillaries was shown to depend upon recruitment of bone marrow-derived progenitor cells. By preventing apoptosis of these progenitor cells, the investigators could not only preserve the capillary structures, but also prevent the development of tubular atrophy.[19]

It may well be that interstitial cells also provide signals for maintenance of the peritubular capillary network, particularly during decrements in oxygen delivery. Interstitial cells have been shown to be a main source of HIF-2-mediated transcriptional defense against hypoxia. These include the production of the cytoprotective erythropoietin, progenitor cell recruiting cytokines such as vascular endothelial growth factor (VEGF) and stromal cell-derived factor 1 (SDF-1), and modulation of the extracellular matrix.[20]

Finally, one can hypothesize that the tubular epithelium itself may play a role in maintaining peritubular endothelium. In recent years, it has become clear that vascular structures require continuous exposure to growth factors such as VEGF and angiopoietin I not to disintegrate. Seminal work of the Quaggin group has shown that at the glomerular level, VEGF release from epithelial cells is essential for maintenance and survival of the glomerular endothelium.[21] Also the tubular epithelium has been recognized as a site of VEGF production, whereas overexpression of HIF-1 in tubular cells could promote proliferation of ECs.[22]

## PERITUBULAR RAREFACTION: CAUSE OR EFFECT?

Many clinical biopsy studies have shown an association between loss of tubular function and structure on the one hand and loss of glomerular and peritubular endothelium on the other. These conditions vary from glomerulonephritis, to diabetes as well as to loss of allograft function.[5,23–25] Experimental studies, which allow sequential follow-up, revealed that the loss of proximal tubular cell (PTC) (rarefaction) in fact precedes the development of tubulointerstitial fibrosis. Micro fill analysis in rats subjected to bilateral ischemia followed by reperfusion revealed a 30–50% reduction in PTC density as early as 4–8 weeks, whereas tubulointerstitial fibrosis and proteinuria started to develop at 16 weeks.[26] Similar observations were carried out in a rat model with aristolochic acid nephropathy, where there was a progressive reduction in PTCs from the 8th week onwards, whereas tubular atrophy developed from week 16 onwards.[25] In a model of accelerated glomerulosclerosis using repeated anti-Thy1 injections, stagnation of peritubular capillary flow was observed, preceding the development of tubulointerstitial injury and loss of peritubular capillaries.[27] Finally, in animal models with nephrosis, any histological evidence of tubulointerstitial fibrosis was antedated by hypoxia (confirmed by a dramatic and significant increase in nuclear localization of HIF-1$\alpha$) following loss of PTCs and decreased PTC blood flow.[5,24,25]

A direct causal relationship between endothelial injury and the development of tubulointerstitial disease is further supported by studies that have specifically modulated endothelial function. For example, endothelial overexpression of the receptor for advanced glycation end products (RAGE) induces proteinuria and progressive kidney disease.[28] Endothelial activation may start at the glomerular level with endothelial–leukocyte interaction and development of fibrin and platelet thrombi and subsequently may also compromise efferent flow to the peritubular capillaries. Such changes at the glomerular endothelium could, for example, be observed when diabetes was induced in the absence of a functional endothelial NOS.[29] Conversely, restoration of endothelial function prevents the development of tubular atrophy. For example, EC infusion in ischemia-reperfusion injury of the kidney has been shown to restore areas where ECs have detached and reduced tubular injury.[30] The importance for maintenance of endothelial integrity is further illustrated by the aforementioned study by Li *et al.*[19] where reduction of apoptosis of progenitor-derived peritubular EC could reduce the development of tubulointerstitial disease.

## CAN WE CLINICALLY ASSESS RENAL ENDOTHELIAL ACTIVATION?

Obviously, the kidney itself is not easy to access and to assess the presence of endothelial activation or its anatomical consequence rarefaction. However, there is substantial evidence that EC activation is a systemic process that occurs in a similar manner in multiple tissue beds throughout the body. Endothelial function is most frequently measured by its capacity to induce vasodilatation in response to specific agonists or increased shear stress.[31] Brachial flow-mediated vasodilatation is most widely used and has also been studied in patients with renal disease.[32] Unfortunately, this methodology has thus far not been used to predict progression of renal disease. In experimental conditions, however, endothelium-dependent vasodilatation of renal arteries was a very strong predictor of development of injury and proteinuria in

models of 5/6 nephrectomy and adriamycin nephrosis,[33] thus providing a rationale for such studies.

More relevant to the process of endothelial activation in the kidney probably would be to assess the interaction of the endothelium with leukocytes. Recently, it was shown that orthogonal polarization spectroscopy of the sublingual circulation can be used to measure endothelial glycocalyx thickness and leukocyte–endothelial interaction.[34] In a pilot study, it was suggested that microalbuminuria could be related to decrements in glycocalix. Such intravital microscopic techniques also allow direct visualization of the microvasculature and measurements of capillary density. Although associations between peripheral capillary density and the occurrence of diabetic retinopathy have been found, no such relationships have been explored with respect to the potential to predict of renal disease progression.

As acquired rarefaction can be considered failure of endothelial repair mechanisms, one may also consider enumerating circulating progenitor cells as a proxy of regenerative capacity. Indeed, in the field of cardiology, measuring circulating CD34/kdr-positive cells has been shown to predict the occurrence of cardiovascular events.[35] As discussed before, the clinical relevance of these cells for repair is still uncertain and the fact that they predict cardiovascular events may well be related to the fact that release from the bone marrow is a NO-dependent process.[36] Characterization of myeloid precursor cells for surface markers that indicate endothelial commitment is clinically feasible and could be investigated as an alternative approach to assess repair capacity.

## CONCLUSION

The recent appreciation of the potential to repair damaged renal tissue also changes our working hypothesis on the progressive nature of renal disease. Loss of renal function can thus be considered as a dysbalance between injury and repair. Whereas the capacity of epithelial regeneration has long been recognized, and more recently even extended to glomerular epithelium, we propose that renal endothelium may be more limited in its repair capacity. This drives a vicious circle of rarefaction and renal ischemia leading to loss of nephrons. It would implicate that repair and maintenance of the peritubular capillaries could be a therapeutic target for prevention of loss of renal function.

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