Role of Microcirculation in the Pathogenesis of Kidney Fibrosis

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Renal microcirculation:

While large arteries are essential for delivering blood flow into the kidney, renal microcirculation, namely glomerular capillaries and peritubular capillaries (PTC) are responsible for delivering oxygen and nutrients to the renal parenchymal cells, thus also required to sustain sufficient glomerular filtration rate for clearing waste products and production of urine. Postglomerular capillary blood flow exits the glomerulus via efferent arterioles and divide to form PTC plexuses interacting with the tubules of the nephron and interstitium.1

Renal glomerular and PTC endothelial cells are highly specialized cells with a flattened cell shape and transcytoplasmic perforating fenestrations. Glomerular endothelial cells line the inner aspect of the glomerular basement membrane and an essential part of the glomerular filtration barrier. Therefore, primary or secondary injuries targeting glomerular or PTC endothelium can lead to direct detriment to the nephrons and renal function.1

Paradigms of nephron loss and kidney fibrogenesis

Kidney fibrosis is the final common pathway of chronic progressive kidney diseases and therefore a surrogate marker for end-stage renal failure, both in native and transplanted kidneys.2,3 Kidney fibrosis (=interstitial fibrosis and tubular atrophy), rather than structural glomerular damage (i.e. glomerulosclerosis), is the best histological correlate of declined function of native or transplanted kidneys, regardless of the underlying disease type.4-6 Several paradigms were generated to explain how nephron loss and fibrosis progress after sustained and/or non-recoverable injuries. Despite intense interest, the mechanisms of nephron loss and kidney fibrogenesis are not fully understood. The postulated mechanisms include hemodynamic alterations and resultant increased intraglomerular pressure, toxic effects of proteinuria on tubular epithelium, transition of epithelium, endothelium or pericytes to fibroblasts, migration of
bone-marrow derived fibroblasts, accelerated senescence, and chronic tubular hypoxia secondary to loss of PTCs.\textsuperscript{2,7-14}

Advanced chronic kidney disease is characterized with a common pathology phenotype shared by almost all progressive renal diseases, which includes extensive interstitial fibrosis with chronic nonspecific inflammation, tubular atrophy and loss, glomerulosclerosis, and PTC loss.\textsuperscript{2} These histopathological features are closely correlated with each other, but their interdependence and causal priorities are unknown. Recently, lineage-tracing studies determined pericytes as the major source of interstitial myofibroblasts in the fibrotic kidney in a rodent model, soliciting that research on renal fibrosis should be refocused on vascular injury mechanisms.\textsuperscript{10}

Evidence for contribution of microvascular loss to progressive kidney fibrosis: animal models

The degree of glomerulosclerosis and interstitial fibrosis is correlated with glomerular and PTC loss in progressive models of renal disease.\textsuperscript{14} It has been postulated that mechanisms underlying the loss of renal microvasculature are mediated by impaired angiogenic responses by either incomplete endothelial proliferation and/or local alterations of angiogenic (i.e. VEGF) and anti-angiogenic (i.e. thrombospondin 1) factors in the kidney.\textsuperscript{15}

Numerous animal models of glomerular or non-glomerular progressive kidney diseases and aging, showed that interstitial fibrosis is correlated with reduced PTC density, but none of these studies demonstrated that PTC structural loss precedes interstitial fibrosis.\textsuperscript{15-24} Furthermore, some of the data stemming from animal models contradict with each other. For example, in contrast to other rodent remnant kidney models, Pillebout et al showed proliferation of PTCs with increased PTC density accompanied to severe tubulointerstitial fibrosis in mice after 75\% surgical nepron reduction.\textsuperscript{25} On the other hand, there is convincing growing evidence that tubular hypoxia precedes development of tubulointerstitial fibrosis.\textsuperscript{19,17}

Thus, functional PTC changes may be preceding nephron loss and fibrosis via inducing a chronic ischemic milieu at least in some models, but structural PTC loss appear as a late feature, possibly occurs after nephron loss as a drop-out of disuse mechanism, and correlates with advanced kidney fibrosis.
Evidence for contribution of microvascular loss to progressive kidney fibrosis: human studies

In contrast to animal models, a few clinical studies yet examined PTCs in human kidneys, which generated contradicting data. The difficulty is that most of these human studies (5 out of 9) were conducted in end-stage kidneys with severe scarring affecting all renal compartments, thus making the interpretation quite difficult (in the end, all renal parenchymal structures (glomeruli, tubules, larger vessels, PTC) become scarred and eventually disappear). Bohle et al. were the first to describe reduced PTC density in correlation with interstitial fibrosis in chronic renal failure secondary to diabetic nephropathy, amyloidosis, hypertensive nephropathy, and chronic interstitial nephritis. Studies of explants of native or transplant kidneys well documented reduced microvascular density. A study by Ishii et al. also reported PTC loss in late kidney allografts biopsied after 6.8 years post-transplant with extensive scarring and chronic graft dysfunction. More recently, Steegh et al. reported reduced PTC in 3-month protocol biopsies after kidney transplantation, which negatively correlated with inflammation and predicted higher fibrosis/atrophy and lower renal function at 12-month. In contrast, Ozdemir et al. reported angiogenesis and increased PTC density in kidney allograft biopsies with acute rejection, and interestingly this angiogenic response was related with more fibrosis in follow-up biopsies.

Therefore, whether PTC structural loss is a cause or a consequence of nephron loss remains an open question.

Is microcirculation loss a cause or consequence of nephron loss?

End-stage kidney fibrosis is associated with PTC loss, but their interdependence is unknown. We hypothesized that kidney fibrosis is dependent on PTC loss. We studied PTC density in 100 kidney transplant indication biopsies from 83 recipients (42% presenting with chronic renal dysfunction; median time post-transplant: 15-months), and compared to 40 normal control biopsies taken at time of transplantation. We labeled PTCs with CD31 immunostaining and quantified density using two methods: 1. PTC number per unit area (0.25 mm²); 2. PTC-to-tubule ratio. We also measured PTC surface area by image analysis. PTC number per unit area was lower in transplant biopsies with edema and tubulointerstitial inflammation than in controls. Surprisingly, PTC number per unit area was not reduced in biopsies with kidney scarring (interstitial fibrosis, tubular atrophy, transplant glomerulopathy, PTC multilayering) or late post-transplant time. In multivariate-analysis, interstitial edema was the only determinant of reduced
PTC density. PTC-to-tubule ratio was higher in biopsies with interstitial fibrosis, indicating remained PTCs despite loss of nephrons. PTCs were larger in biopsies with capillaritis, but not smaller in biopsies with fibrosis/atrophy compared to controls. PTC density by both methods did not relate to renal function or survival. Contrary to our predictions, the histologic feature that correlated with reduced PTC density in biopsies was edema, not fibrosis. Edema expands the interstitium, giving a false impression of reduced PTC density in biopsies. Although postglomerular PTC hypoperfusion might still be a potential contributor, kidney transplant fibrosis is not dependent on structural PTC loss.\(^{35}\) (Osasan et al. manuscript in submission)

Why do our results not confirm previous experimental and clinical studies, which clearly documented that reduced PTC density correlates with advanced renal tubulointerstitial fibrosis? The answer lies within the differences between the current and previous study populations. First, most (5 of 9) human studies on PTC density were done in end-stage kidneys (failed native or transplant nephrectomies or kidneys with extensive fibrosis >50%).\(^{26-31}\) Analyzing end-stage kidneys reveals nonspecific results because all renal compartments become atrophic and eventually disappear within the scar tissue over time. Previous studies in the end-stage kidneys of patients maintained in chronic hemodialysis, showed nonspecific fibrous obliteration of large renal vessels, most likely representing a disuse type of change secondary to parenchymal atrophy.\(^{36}\)

Thus previous studies documenting PTC loss in advanced chronic kidney diseases were right and their findings and the current study suggest that PTC loss occurs as a consequence of nephron loss i.e., PTCs drop-out possibly due to lack of use. For example, Ishii et al. observed that PTC density was reduced in transplant biopsies with chronic allograft nephropathy taken after a mean of 6.8 years post transplant and this was associated with the severity of fibrosis/atrophy.\(^{31}\) In contrast, the biopsy time in the current study was much earlier with a median 15 months post transplant. Although we studied earlier kidney transplants, majority (74\%) of the cohort included fibrotic kidneys with different severity of interstitial fibrosis. We conclude that studying very late time points naturally show that PTCs are lost in addition to other renal structures, thus studies at earlier time points are crucial to analyze whether PTC loss becomes evident before later stages of chronic renal failure.

Edema expands the interstitium, giving a false impression of reduced PTC density in biopsies. Therefore, edema should be taken into account when PTC density is being analyzed in biopsy samples and requires normalization strategies when investigators compare PTC
density in biopsies with edema and without edema. Recently, Steegh et al. reported that PTCs were lost and inversely correlated with the severity of interstitial inflammation in early protocol biopsies, and predicted higher interstitial fibrosis and tubular atrophy, and reduced renal allograft function at 12-month post-transplant. We believe that this data were misinterpreted because reduced PTC density can be caused by the dilution effect of interstitial edema/inflammation in biopsy tissues. Regarding how come PTC loss could predict reduced renal function and fibrosis at one-year, it is well documented that subclinical inflammation in protocol biopsies predict worse renal outcomes and more chronic damage because subclinical inflammation causes ongoing kidney transplant injury. 6,37,38

Animal models suggest that functional PTC changes, not structural PTC loss, precede development of kidney fibrosis

There is convincing growing evidence that tubular hypoxia precedes development of tubulointerstitial fibrosis. At least two well-designed studies in rodent models of progressive kidney diseases documented that postglomerular PTC blood flow decreased after the initial injury (assessed by red blood cell velocity or marker diffusion techniques) and that PTC hypoperfusion correlated with tubular hypoxia, and preceded subsequent development of interstitial fibrosis, tubular atrophy, and PTC loss. 17,19 Elegant studies by Manotham et al. in the remnant kidney model suggested that tubular hypoxia, which was evident in the early days of injury, emerged secondary to PTC hypoperfusion before the development of interstitial fibrosis and tubular atrophy, and that functional PTC changes and hypoxia were dependent on activation of the renin-angiotensin system (vasoconstriction of efferent arteriole and glomerular hyperfiltration). As a proof of concept, angiotensin II receptor blocker treatment rescued both PTC hypoperfusion and tubular hypoxia, whereas untreated rats developed kidney fibrosis and PTC loss.19 Moreover, studies by Wong et al in a mouse anti-glomerular basement membrane glomerulonephritis model documented that wild-type mice with reduced postglomerular PTC flow and proteinuria developed worse renal function and more kidney fibrosis when compared to Fc receptor knockout mice with less severe disease but comparable degrees of proteinuria without reduced PTC blood flow. 17

Because PTCs are the only vessels that carry O2 and nutrients to the tubular epithelium, it is reasonable to think that PTC hypoperfusion would trigger and/or contribute to the progressive tubular cell death, nephron loss and interstitial fibrosis. Thus, functional PTC
changes may be preceding nephron loss and fibrosis via inducing a chronic ischemic milieu at least in some models, but structural PTC loss appear as a late feature, possibly occurs after nephron loss as a drop-out of disuse mechanism, and correlates with advanced kidney fibrosis.

Summary

Human studies and animal models show that endstage kidney fibrosis is associated with PTC loss. Our studies in kidney transplant biopsies from earlier time points than in previous human kidney studies showed that contrary to our predictions, the histologic feature that correlated with reduced PTC density in kidney transplant biopsies was edema, not fibrosis. Edema gives a false impression of reduced PTC density in biopsies, thus should be corrected for in PTC quantification studies. We conclude that although postglomerular PTC hypoperfusion might still be a potential contributor, structural PTC loss is not associated with interstitial fibrosis, thus kidney transplant fibrosis is not dependent on structural PTC loss. Animal models suggest that functional postglomerular PTC hypoperfusion precedes development of nephron loss and interstitial fibrosis, which is yet to be confirmed in human diseased kidneys.
SAM Questions

1. Reduced peritubular capillary is associated with interstitial fibrosis and tubular atrophy, thus suggested to contribute progressive nephron loss in diseased kidneys. All of the following statements are correct, except:

   a. Rodent models of non-glomerular progressive kidney diseases reported peritubular capillary loss in areas of interstitial fibrosis.

   b. Clinical studies in chronic kidney diseases tissues showed that capillary loss correlates with the severity of interstitial fibrosis and tubular atrophy.

   c. Peritubular capillary loss was assumed to be related with the location of initial injury, because no reduction in capillary density was observed in experimental or clinical glomerulonephritis.

   d. Both animal models and clinical studies that analyzed peritubular capillary density were done in kidneys with advanced fibrosis.

   Correct answer is C. Studies showed that PTC loss was evident in several different categories of chronic kidney diseases, both glomerular or non-glomerular with no disease-specificity.

2. The quantification of peritubular capillary density is problematic and this is due to multiple reasons, except:

   a. Peritubular capillary density measurements require that absolute capillary numbers to be divided by a constant denominator (unit microscopic area, number of tubules)

   b. The denominator is often not constant among specimens, e.g., number of tubules decrease in chronic kidney diseases.
c. Capillary density per unit area measurements are vulnerable to be affected by expansion of the interstitial space by edema and inflammation, which may cause a false impression of reduced capillary density in biopsies.

d. Peritubular capillary rarefaction has been observed as a diffuse feature, thus probably not affected by area selection bias.

Correct answer is D. PTC loss was especially reported around atrophic tubules and within fibrotic areas, therefore, area selection biases may introduce errors in quantitative data.

3. It has been suggested that PTC hypoperfusion, rather than structural loss of PTCs, precedes development of interstitial fibrosis. This is because:

   a. Postglomerular PTC blood flow decreased after the initial injury in rodent models of remnant kidney and anti-GBM glomerulonephritis.

   b. Tubular hypoxia, which was evident in the early days of injury, emerged secondary to PTC hypoperfusion before the development of interstitial fibrosis and PTC loss.

   c. PTC loss was reported in advanced or end-stage human kidneys, thus giving no clues about the priorities.

   d. All of the above.

Correct answer is D.
Reference List


