Renal Fibrosis:

What? How Much? Why?

Diagnostic/Pathogenetic Features, Quantification, and

Clinicopathologic Implications

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Introduction

Accumulation of interstitial extracellular matrix (ECM) is a common feature of chronic kidney diseases, and believed to contribute to the loss of function. Kidney interstitial fibrosis (IF) can be defined as the accumulation of abnormal amounts of collagen and related molecules in the interstitium of the cortex, which serve as structural scaffolding.

What? Pathogenetic Features: Cellular and Molecular Mediators

Dr. Alison Eddy has divided the process of interstitial fibrosis and tubular atrophy development into four phases in rat models: (1) cellular activation and injury phase; (2) fibrogenic signaling phase; (3) fibrogenic phase; and (4) renal destruction, with obliteration of tubules and capillaries. The first 3 phases are active, involving inflammatory and parenchymal cells, matrix proteins, soluble factors including growth factors, cytokines. Eventually, a dense organized scar develops. Data suggests that fibrosis may be reversible in the early phases, opening the door for possible intervention.[1-3]

Cellular mediators of fibrosis include fibroblasts[4, 5] and myofibroblasts.[5-10] Fibroblasts may acquire a myofibroblastic phenotype, likely a crucial event in IF.[4, 9, 11-15] Fibrocytes are another cell type thought to be important to IF. These are thought to be distinct from fibroblasts.[10, 16-24] The ultimate origin of interstitial myofibroblasts is uncertain with candidates being fibroblasts, pericytes,[10, 25-27], endothelium,[28, 29] and perivascular fibrocytes.[10, 25, 30-32] Some have been mixed with regard to endothelial-mesenchymal transition.[10, 25, 30-32] Inflammatory cells are important in the genesis of

IFTA, including lymphocytes,[33-36] monocyte/macrophages,[24, 37-43] dendritic cells,[5, 35, 44, 45] and mast cells.[46-57]

Tubular cells are postulated to contribute to the increased ECM through the process of epithelial to mesenchymal transition (EMT), defined as the stepwise loss of epithelial markers, such as E-cadherin, and the acquisition of mesenchymal markers, such as vimentin and SMA[4, 58, 59] and the development of increased motility with traversal of the basement membrane into the interstitium.[60] . There is evidence both for and against EMT.[61-63] The Banff Conference on Allograft Pathology in 2011 had a symposium on EMT, concluding that the in situ epithelial response exists but suggesting a name that does not imply emigration of tubular cells to the interstitium, which can be termed the “epithelial mesenchymal phenotype” (EMP).[64]

Tubular epithelial cells undergo marked changes in acute injury[65] and appear to be an important factor in IF.[4, 13] In the process of EMP, there appear to be increased intermediate filaments (vimentin and nestin) in injured tubular epithelium.[66] This may be associated with increased collagen type I and III expression.[67] EMP marker production may be include changes in E-Cadherin production.[68, 69] Transcription factors such as the zinc-finger transcription factor snail homolog 1 (Snai1)[70, 71] appear to be important to EMP. An important component in the regulation of genes in EMP and IF may include micro(mi)RNAs.[72-83] Processes that may be important include endoplasmic reticulum stress[84-90], “lipid nephrotoxicity”,[91-97] and autophagy, the process whereby cells undergo “self digestion”.[4, 98, 99]

The ECM components [other than collagen, the primary molecule in fibrosis] appears to have a crucial role in IF.[100-104] Tissue transglutaminase (tTG) crosslinks

proteins, stabilizing ECM and making it resistant to protease degradation.[105-107] Matrix metalloproteinase (MMP) enzymes are comprised of proteolytic enzymes that can degrade all matrix protein components.[108, 109] Tissue plasminogen activator (tPA) is proteolytic but can worsen IF through its ability to induce MMP-9 gene expression, leading to tubular basement membrane disruption and EMP promotion.[108, 109] ECM production can be affected by the renin/angiotensin system.[110-117] Plasmin, a serine protease, can activate MMPs, leading to matrix protein degradation.[118-120] Laminin-**α**4, a component of glomerular and PTC basement membranes, may be decreased in IFTA.[121, 122]

Vessels altered in IF include peritubular capillaries (PTCs)[123] and lymphatic vessels.[124-129] The importance of the renal vasculature in the development of IF is evidenced by studies on molecules such as vascular endothelial growth factor (VEGF).[130, 131] It has been demonstrated that inhibition of angiogenesis and inflammation with sirolimus can prevent IF.[132] Hypoxia promotes fibrosis through such mechanisms as hypoxia-induced factor-1**α** (HIF-1**α**).[133-136]

There are large number of molecular mediators of IF. Transforming growth factor (TGF) is an important mediator of IF.[4, 105, 137-142] TGF-**β** acts through Smad[143-146] and jagged/notch pathways[147-149] for downstream signaling. TGF-**β** activation blockade is being investigated.[150-153] Other important IF mediators include bone morphogenic protein (BMP)[4, 105, 154-163], toll-like receptors (TLRs),[164, 165] the TGF-β-inducible integrin **α**5**β**6[166], integrin-linked kinases[167], hepatocyte growth factor (HGF),[168] and platelet-derived growth factor (PDGF).[4, 6, 169-175] The complement system appears to be important in the development of IFTA.[176-181]

How Much? Diagnostic and Quantitative Evaluation of Interstitial Fibrosis

Evaluation of interstitial fibrosis can be conducted in both a qualitative and quantitative manner. Qualitatively, the patterns of IF differ and probably do not have identical causes or consequences. For example, broad scars with loss of tubules as the sequelae of severe focal injury and destruction of parenchyma, such as in pyelonephritis and infarcts.[182] The patchy, "striped" pattern of interstitial fibrosis with corresponding tubular atrophy has been described with calcineurin inhibitor use. This is due to the apparent preferential involvement of the medullary rays. However, this pattern may also be seen with hypertensive kidney disease. This "striped" fibrosis occurs in addition to the other changes of chronic calcineurin-induced nephrotoxicity, including hyaline arteriopathy and nonspecific glomerulosclerosis.[183] Chronic obstruction extrinsic to the ureter can lead to IF/TA with relative glomerular sparing, atubular glomeruli, dilated tubules, and intratubular Tamm-Horsfall protein casts will extravasation into the interstitium.[184, 185] In contrast, another pattern, which is likely far more common in renal biopsies, is diffuse or patchy fine interstitial fibrosis which surrounds tubules which are either atrophic or normal. This is associated with either diffuse or focal disease of glomeruli, tubules or vessels.[3, 185] Many studies have shown a reciprocal correlation between kidney function and the extent of fibrosis.[186]

Tubular atrophy (TA) is defined as loss of specialized transport and metabolic capacity and typically manifested by small tubules with cells with pale cytoplasm or dilated, thin tubules. TA is usually associated with IF (often abbreviated IFTA). However, IF and TA can be separated as distinct processes, as shown by the profound

tubular atrophy in renal artery stenosis, which characteristically has little or no fibrosis.[186]

Quantitatively, the extent of IF is predictive of renal function and renal allograft outcome and may be considered a surrogate marker.[187-191] Several applications require accurate IF measurement including research focused on therapeutic inhibition of IF and comparison of protocol biopsies in studies of renal allografts.[105, 153, 192, 193] Visual assessment of trichrome-stained slides is often standard practice in many institutions,[194] but studies have shown that this approach may have poor reproducibility.[195, 196] According to Banff criteria[197], trichrome is typically used since the recommendation for slide preparation is seven slides containing multiple sequential sections, 3 with H and E, 3 with PAS or silver stains, and 1 with a trichrome stain. Under Banff working classification of renal allograft pathology, fibrosis is scored as follows:

Table 1: Banff Quantitative Criteria for Interstitial Fibrosis ('ci')[197]

ci0	Interstitial fibrosis in up to 5% of the cortical area
ci1	Mild – interstitial fibrosis in 6-25% of the cortical area
ci2	Moderate – interstitial fibrosis in 26-50% of the cortical area
ci3	Severe – interstitial fibrosis in > 50% of the cortical area

Several morphometry techniques are used to assess IF, including morphometry of slides stained with trichrome;[198, 199] Sirius Red, specific for collagen types I and III under polarized light;[7, 200, 201] and collagen immunohistochemistry, particularly type III collagen.[202-204] Sirius Red imparts a pink stain to most tissues when observed under white light. The dye molecule intercalates into the tertiary groove in both types I and III collagen molecules. When observed under polarized light, collagen types I and III are strongly birefringent.[7] Computer-assisted morphometry has shown utility in the analysis of studies employing trichrome, Sirius Red, and collagen III immunohistochemistry; and

analysis in some of these studies have shown correlation with glomerular filtration rate (GFR).[7, 186, 199-211]

The measurement of IF has intrinsic limitations, some of which are due to sampling. For example, one study estimated that repeat biopsies show a decrease in the measured level of fibrosis, presumably due to sampling, in 12% of cases.[212] Overall, there is no consensus regarding the best way to assess IF. Efforts to reach a consensus or at least provide recommendations are currently underway under the auspices of the Banff Conference of Allograft Pathology.[213]

Why? Clinicopathologic Implications

Not all fibrosis is "equal" or the "same" in quality and thus aggregate quantity. For example, "active" or "young" IF may have greater potential for remodeling. Broad scars may have different consequences than diffuse, fine IF. Inflammation in areas of IF has also been noted in several studies to be an adverse risk factor for progression of renal disease.[9, 191, 212, 214-217] For this and other reasons, quantitative criteria for mononuclear cell interstitial inflammation ('ti') in total parenchyma (scarred and unscarred) is sometimes assessed according to the criteria below. This designation has not been officially incorporated into the Banff criteria yet.

Table 2: Quantitative criteria for mononuclear interstitial inflammation ('ti') in total parenchyma (scarred and unscarred)

ti0	No or trivial interstitial inflammation (<10% of parenchyma)
ti1	10–25% of parenchyma inflamed
ti2	26–50% of parenchyma inflamed
ti3	>50% of parenchyma inflamed

It is desirable to find the etiology/pathogenesis so that the problem can be alleviated for the patient. At the 8th Banff Conference on Allograft Pathology held in Edmonton, Canada from July 15-21 2005, major developments included the elimination of the non-specific term 'chronic allograft nephropathy' (CAN) from the Banff classification for kidney allograft pathology and the recognition of the entity of chronic antibody-mediated rejection. [185]

Fibrosis can be important to assess in renal donor biopsies to predict subsequent allograft behavior,[190] as shown below:

Table 3: Fibrosis Assessment in Donor Biopsies

Measure	Predictive Value of Feature	N / Reference
Banff ci > 0	↑ Risk of adverse outcome @ 6 months, 1.9x beyond prediction from age alone	78 [218]
Morphometric interstitial volume	Graft function @ 1 year	43 [219]
Banff ci	No predictive value, not reproducible	199 [220]

In the Oxford Classification of IgA nephropathy, interstitial fibrosis and tubular atrophy was found to be an important parameter. Six pathologic variables that were used to interrogate prognostic significance independent of the clinical data in IgA nephropathy: (1) mesangial cellularity score [percentage of glomeruli showing] (2) segmental sclerosis, (3) endocapillary hypercellularity, or (4) cellular/fibrocellular crescents; (5) percentage of interstitial fibrosis/tubular atrophy; and finally (6) arteriosclerosis score. Of these, mesangial-cell cellularity, segmental sclerosis or

adhesion, endocapillary hypercellularity, interstitial fibrosis and tubular atrophy were independent histopathological features with reproducibility and predictive power in survival analyses using the rate of eGFR decline and a 50% decrease in eGFR/ESRD as outcomes.[221, 222]

Assessment of interstitial fibrosis can be important in predicting renal function. Multiple studies have been conducted to assess fibrosis, some of which have used computerized morphometry, as shown below:

Table 4: Studies to Assess Interstitial Fibrosis

Method	Description	Measure	Ref.(s)
SR CA	SR image analysis predicted long-term renal allograft function	Cortical IF correlated with time to graft failure ($r = 0.64$, $P < 0.001$) at 6 months post transplant	[186]
SR CA	SR image analysis predicted long-term renal allograft function	Positive correlation ($r = 0.62$, $P<0.001$) between SR fibrosis and decreases in GFR	[223]
SR CA	SR image analysis quantitation corresponded to light microscopic semiquantitative measurements ($r = 0.439$, $P = 0.0003$ overall and $r = 0.704$, $P < 0.0001$ for just baseline specimens) in kidney allografts	Semiquantitative methods correlated best with long-term graft function (serum creatinine at 8 – 10 years ($P = 0.010$) and late graft loss ($P = 0.0445$)	[201]
SR CA	IF in non-heart-beating donor kidneys and conventional heart-beating donor kidneys	No significant difference in IF between the two groups	[224]
SR CA	IF scoring predicts survival in lupus nephritis	Fibrillary collagen was predictive of creatinine doubling ($P = 0.01$) and relapse ($P = 0.06$)	[225]
SR CA	Development of an image analysis-based application (Fibrosis HR) for interstitial fibrosis and glomerular morphometry	Intra- and interoperator variability was present in manual segmentation of IF, mesangial matrix, and glomerular areas but interactive identification didn't have this variability	[226]
SR CA	IF measurements using digital imaging coupled with point counting correlated with GFR	Direct relationship between interstitial volume fraction and renal function ($r^2 = 0.54$)	[205]

Method	Description	Measure	Ref.(s)
SR CA	Sirius red measurement of fibrosis combined with ultrasound measurements of renal artery resistance index helped predict “chronic allograft nephropathy”	Positive correlation (r = 0.62, P <0.001) between picrosirius red-stained cortical fractional if volume and decreased GFR	[227]
SR and collagen CA	Renal IF correlates with the presence of TGF-β, decorin, smooth muscle actin, and interstitial collagens	In all samples with IF, up-regulation of TGF-beta was observed in combination with reduced decorin expression	[206, 228-230]
Masson TC CA	IF morphometry correlates with serum creatinine in IgA nephropathy and membranoproliferative glomerulonephritis	IF occupied > 10% of the interstitium in all 10 cases and > 20% in 6 and IF morphometry correlated with serum creatinine	[231]
Masson TC	Cyclosporine (CsA) therapy effects on quantitative fibrosis morphometry	IF measured by morphometry was significantly higher in the CsA group only in renal allografts 6 months posttransplant (P < 0.04)	[232, 233]
Masson TC	Quantification of IF in patients receiving cyclosporine	Quantitative IF grade correlated with worsened creatinine clearance between 1 and 3 years	[198]
Light green TC	Quantitative IF morphometry in patients randomized to cyclosporine or conversion to sirolimus	No difference in groups with respect to fibrosis but GFR improved significantly in the conversion group	[199]
Light green TC	Quantitative IF in sequential renal biopsies	IF evolution correlated with eGFR	[207]
CIII IHC CA	IF measurements by a semiautomatic system correlate with GFR in protocol renal transplant biopsy specimens	Area fraction of immunostained collagen III of > 40% @ 6 months associated with decreased GFR @ 24 months compared with ≤ 40% (r=-0.32, P=0.03)	[204]
CIII IHC CA	Fibrosis measurements by a semiautomatic system correlate with GFR in protocol renal transplant biopsy specimens	GFR correlated negatively with interstitial volume fraction @ 6 months (P = 0.05)	[234]
Masson TC, SR, and SMA IHC CA	Quantitative (CA) and semi-quantitative assessments (VA) were performed on late allograft biopsies	IF by VA predicted Banff ’97 ci scores (p < 0.0001) and correlated with GFR, creatinine, and urine total protein (r = - 0.48, p = 0.0007; r = 0.46, p = 0.0009; r= 0.51, 0.0009, respectively). Of the CA methods, Only the SR-nonpolarized score correlated with GFR and urine total protein (r = -0.29, p = 0.05; r = 0.29, p = 0.05, respectively)	[235]
VA	IF, inflammation, and glomerulopathy correlated with poor allograft outcome	By multivariate Cox analysis, IF and inflammation lead to poorer survival (HR = 8.5, p < 0.0001); fibrosis alone had less effect (HR = 4.8, p = not significant)	[191]

CIII: Collagen III, CA: computerized analysis, eGFR: estimated GFR, GFR: glomerular filtration rate, IHC: immunohistochemistry, IF: interstitial fibrosis, SMA: smooth muscle actin, SR: Sirius red, Ref(s): References, TC: Trichrome, TGF-β: transforming growth factor, VA: visual analysis.

Summary

The molecular mechanisms leading to IFTA are complex and typically interrelated with the primary processes leading to renal injury. Further elucidation of these mechanisms could lead to targeted inhibitors to alleviate the terminal scarring that may occur. Furthermore, many methods are available for assessing fibrosis; and efforts are underway to improve the way pathologists assess fibrosis.

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REFERENCES

1. Eddy AA. Molecular basis of renal fibrosis. *Pediatric nephrology* 2000; 15(3-4):290-301.

2. Fogo AB. Progression and potential regression of glomerulosclerosis. *Kidney international* 2001; 59(2):804-19.

3. Racusen LC, Solez K, Colvin R. Fibrosis and atrophy in the renal allograft: interim report and new directions. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2002; 2(3):203-6.

4. Boor P, Ostendorf T, Floege J. Renal fibrosis: novel insights into mechanisms and therapeutic targets. *Nat Rev Nephrol* 2010; 6(11):643-56.

5. Kaissling B, Le Hir M. The renal cortical interstitium: morphological and functional aspects. *Histochemistry and cell biology* 2008; 130(2):247-62.

6. Floege J, Eitner F, Alpers CE. A new look at platelet-derived growth factor in renal disease. *Journal of the American Society of Nephrology : JASN* 2008; 19(1):12-23.

7. Grimm PC, Nickerson P, Gough J*, et al.* Computerized image analysis of Sirius Red-stained renal allograft biopsies as a surrogate marker to predict long-term allograft function. *J Am Soc Nephrol* 2003; 14(6):1662-8.

8. Eyden B. The myofibroblast: an assessment of controversial issues and a definition useful in diagnosis and research. *Ultrastructural pathology* 2001; 25(1):39-50.

9. Hinz B. The myofibroblast: paradigm for a mechanically active cell. *J Biomech* 2010; 43(1):146-55.

10. Lin SL, Kisseleva T, Brenner DA, Duffield JS. Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. *Am J Pathol* 2008; 173(6):1617-27.

11. Dussaule JC, Guerrot D, Huby AC*, et al.* The role of cell plasticity in progression and reversal of renal fibrosis. *Int J Exp Pathol* 2011; 92(3):151-7.

12. Meran S, Steadman R. Fibroblasts and myofibroblasts in renal fibrosis. *Int J Exp Pathol* 2011; 92(3):158-67.

13. Rohatgi R, Flores D. Intratubular hydrodynamic forces influence tubulointerstitial fibrosis in the kidney. *Current opinion in nephrology and hypertension* 2010; 19(1):65-71.

14. Li L, Zepeda-Orozco D, Patel V*, et al.* Aberrant planar cell polarity induced by urinary tract obstruction. *American journal of physiology Renal physiology* 2009; 297(6):F1526-33.

15. Fujigaki Y, Muranaka Y, Sun D*, et al.* Transient myofibroblast differentiation of interstitial fibroblastic cells relevant to tubular dilatation in uranyl acetate-induced acute renal failure in rats. *Virchows Archiv : an international journal of pathology* 2005; 446(2):164-76.

16. Wada T, Sakai N, Sakai Y*, et al.* Involvement of bone-marrow-derived cells in kidney fibrosis. *Clin Exp Nephrol* 2011; 15(1):8-13.

17. Sakai N, Wada T, Yokoyama H*, et al.* Secondary lymphoid tissue chemokine (SLC/CCL21)/CCR7 signaling regulates fibrocytes in renal fibrosis. *Proceedings of the National Academy of Sciences of the United States of America* 2006; 103(38):14098-103.

18. Niedermeier M, Reich B, Rodriguez Gomez M*, et al.* CD4+ T cells control the differentiation of Gr1+ monocytes into fibrocytes. *Proceedings of the National Academy of Sciences of the United States of America* 2009; 106(42):17892-7.

19. Sakai N, Furuichi K, Shinozaki Y*, et al.* Fibrocytes are involved in the pathogenesis of human chronic kidney disease. *Human pathology* 2010; 41(5):672-8.

20. Pilling D, Fan T, Huang D, Kaul B, Gomer RH. Identification of markers that distinguish monocyte-derived fibrocytes from monocytes, macrophages, and fibroblasts. *PLoS One* 2009; 4(10):e7475.

21. Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. *Nat Rev Immunol* 2004; 4(8):583-94.

22. Shao DD, Suresh R, Vakil V, Gomer RH, Pilling D. Pivotal Advance: Th-1 cytokines inhibit, and Th-2 cytokines promote fibrocyte differentiation. *J Leukoc Biol* 2008; 83(6):1323-33.

23. Broekema M, Harmsen MC, van Luyn MJ*, et al.* Bone marrow-derived myofibroblasts contribute to the renal interstitial myofibroblast population and produce procollagen I after ischemia/reperfusion in rats. *Journal of the American Society of Nephrology : JASN* 2007; 18(1):165-75.

24. Lin SL, Castano AP, Nowlin BT, Lupher ML, Jr., Duffield JS. Bone marrow Ly6Chigh monocytes are selectively recruited to injured kidney and differentiate into functionally distinct populations. *Journal of immunology* 2009; 183(10):6733-43.

25. Humphreys BD, Lin SL, Kobayashi A*, et al.* Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol* 2010; 176(1):85-97.

26. Picard N, Baum O, Vogetseder A, Kaissling B, Le Hir M. Origin of renal myofibroblasts in the model of unilateral ureter obstruction in the rat. *Histochemistry and cell biology* 2008; 130(1):141-55.

27. Crisan M, Yap S, Casteilla L*, et al.* A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 2008; 3(3):301-13.

28. Zeisberg EM, Potenta SE, Sugimoto H, Zeisberg M, Kalluri R. Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. *J Am Soc Nephrol* 2008; 19(12):2282-7.

29. Li J, Qu X, Bertram JF. Endothelial-myofibroblast transition contributes to the early development of diabetic renal interstitial fibrosis in streptozotocin-induced diabetic mice. *The American journal of pathology* 2009; 175(4):1380-8.

30. Kida Y, Duffield JS. Pivotal role of pericytes in kidney fibrosis. *Clin Exp Pharmacol Physiol* 2011; 38(7):417-23.

31. Piera-Velazquez S, Li Z, Jimenez SA. Role of endothelial-mesenchymal transition (EndoMT) in the pathogenesis of fibrotic disorders. *Am J Pathol* 2011; 179(3):1074-80.

32. Schrimpf C, Duffield JS. Mechanisms of fibrosis: the role of the pericyte. *Curr Opin Nephrol Hypertens* 2011; 20(3):297-305.

33. Tapmeier TT, Fearn A, Brown K*, et al.* Pivotal role of CD4+ T cells in renal fibrosis following ureteric obstruction. *Kidney international* 2010; 78(4):351-62.

34. Nikolic-Paterson DJ. CD4+ T cells: a potential player in renal fibrosis. *Kidney international* 2010; 78(4):333-5.

35. Snelgrove SL, Kausman JY, Lo C*, et al.* Renal Dendritic Cells Adopt a Pro-Inflammatory Phenotype in Obstructive Uropathy to Activate T Cells, but Do Not Directly Contribute to Fibrosis. *Am J Pathol* 2011.

36. Scian MJ, Maluf DG, Archer KJ*, et al.* Gene expression changes are associated with loss of kidney graft function and interstitial fibrosis and tubular atrophy: diagnosis versus prediction. *Transplantation* 2011; 91(6):657-65.

37. Anders HJ, Ryu M. Renal microenvironments and macrophage phenotypes determine progression or resolution of renal inflammation and fibrosis. *Kidney international* 2011; 80(9):915-25.

38. Vernon MA, Mylonas KJ, Hughes J. Macrophages and renal fibrosis. *Semin Nephrol* 2010; 30(3):302-17.

39. Henderson NC, Mackinnon AC, Farnworth SL*, et al.* Galectin-3 expression and secretion links macrophages to the promotion of renal fibrosis. *The American journal of pathology* 2008; 172(2):288-98.

40. Okamura DM, Pasichnyk K, Lopez-Guisa JM*, et al.* Galectin-3 preserves renal tubules and modulates extracellular matrix remodeling in progressive fibrosis. *Am J Physiol Renal Physiol* 2011; 300(1):F245-53.

41. Wang Y, Cao Q, Zheng G*, et al.* By homing to the kidney, activated macrophages potently exacerbate renal injury. *The American journal of pathology* 2008; 172(6):1491-9.

42. Nishida M, Okumura Y, Fujimoto S*, et al.* Adoptive transfer of macrophages ameliorates renal fibrosis in mice. *Biochem Biophys Res Commun* 2005; 332(1):11-6.

43. Semedo P, Donizetti-Oliveira C, Burgos-Silva M*, et al.* Bone marrow mononuclear cells attenuate fibrosis development after severe acute kidney injury. *Lab Invest* 2010; 90(5):685-95.

44. Macconi D, Chiabrando C, Schiarea S*, et al.* Proteasomal processing of albumin by renal dendritic cells generates antigenic peptides. *Journal of the American Society of Nephrology : JASN* 2009; 20(1):123-30.

45. Heymann F, Meyer-Schwesinger C, Hamilton-Williams EE*, et al.* Kidney dendritic cell activation is required for progression of renal disease in a mouse model of glomerular injury. *The Journal of clinical investigation* 2009; 119(5):1286-97.

46. Timoshanko JR, Kitching AR, Semple TJ, Tipping PG, Holdsworth SR. A pathogenetic role for mast cells in experimental crescentic glomerulonephritis. *Journal of the American Society of Nephrology : JASN* 2006; 17(1):150-9.

47. El-Koraie AF, Baddour NM, Adam AG, El Kashef EH, El Nahas AM. Role of stem cell factor and mast cells in the progression of chronic glomerulonephritides. *Kidney international* 2001; 60(1):167-72.

48. Roberts IS, Brenchley PE. Mast cells: the forgotten cells of renal fibrosis. *Journal of clinical pathology* 2000; 53(11):858-62.

49. Kondo S, Kagami S, Kido H*, et al.* Role of mast cell tryptase in renal interstitial fibrosis. *Journal of the American Society of Nephrology : JASN* 2001; 12(8):1668-76.

50. Li Y, Zhou L, Liu F*, et al.* Mast Cell Infiltration Is Involved in Renal Interstitial Fibrosis in a Rat Model of Protein-Overload Nephropathy. *Kidney Blood Press Res* 2010; 33(3):240-8.

51. Veerappan A, Reid AC, O'Connor N*, et al.* Mast Cells are Required for the Development of Renal Fibrosis in the Rodent Unilateral Ureteral Obstruction Model. *Am J Physiol Renal Physiol* 2011.

52. Kim DH, Moon SO, Jung YJ*, et al.* Mast cells decrease renal fibrosis in unilateral ureteral obstruction. *Kidney international* 2009; 75(10):1031-8.

53. Kanamaru Y, Scandiuzzi L, Essig M*, et al.* Mast cell-mediated remodeling and fibrinolytic activity protect against fatal glomerulonephritis. *Journal of immunology* 2006; 176(9):5607-15.

54. Miyazawa S, Hotta O, Doi N, Natori Y, Nishikawa K. Role of mast cells in the development of renal fibrosis: use of mast cell-deficient rats. *Kidney international* 2004; 65(6):2228-37.

55. Hochegger K, Siebenhaar F, Vielhauer V*, et al.* Role of mast cells in experimental anti-glomerular basement membrane glomerulonephritis. *Eur J Immunol* 2005; 35(10):3074-82.

56. Fan YY, Nishiyama A, Fujisawa Y*, et al.* Contribution of chymase-dependent angiotensin II formation to the progression of tubulointerstitial fibrosis in obstructed kidneys in hamsters. *J Pharmacol Sci* 2009; 111(1):82-90.

57. Scandiuzzi L, Beghdadi W, Daugas E*, et al.* Mouse mast cell protease-4 deteriorates renal function by contributing to inflammation and fibrosis in immune complex-mediated glomerulonephritis. *J Immunol* 2010; 185(1):624-33.

58. Tang R, Yang C, Tao JL*, et al.* Epithelial-mesenchymal transdifferentiation of renal tubular epithelial cells induced by urinary proteins requires the activation of PKC-alpha and betaI isozymes. *Cell Biol Int* 2011; 35(9):953-9.

59. Wiwanitkit V. Fibrosis and evidence for epithelial-mesenchymal transition in the kidneys of patients with staghorn calculi. *BJU Int* 2011; 107(11):1847.

60. Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. *The Journal of clinical investigation* 2009; 119(6):1429-37.

61. Kriz W, Kaissling B, Le Hir M. Epithelial-mesenchymal transition (EMT) in kidney fibrosis: fact or fantasy? *J Clin Invest* 2011; 121(2):468-74.

62. Fragiadaki M, Mason RM. Epithelial-mesenchymal transition in renal fibrosis - evidence for and against. *Int J Exp Pathol* 2011; 92(3):143-50.

63. Quaggin SE, Kapus A. Scar wars: mapping the fate of epithelial-mesenchymal-myofibroblast transition. *Kidney international* 2011; 80(1):41-50.

64. Mengel M, Sis B, Haas M*, et al.* Banff 2011 Meeting Report: New Concepts in Antibody-Mediated Rejection. *Am J Transplant* In press 2012.

65. Sharfuddin AA, Molitoris BA. Pathophysiology of ischemic acute kidney injury. *Nature reviews Nephrology* 2011; 7(4):189-200.

66. Sakairi T, Hiromura K, Yamashita S*, et al.* Nestin expression in the kidney with an obstructed ureter. *Kidney international* 2007; 72(3):307-18.

67. Rastaldi MP, Ferrario F, Giardino L*, et al.* Epithelial-mesenchymal transition of tubular epithelial cells in human renal biopsies. *Kidney international* 2002; 62(1):137-46.

68. Docherty NG, Calvo IF, Quinlan MR*, et al.* Increased E-cadherin expression in the ligated kidney following unilateral ureteric obstruction. *Kidney international* 2009; 75(2):205-13.

69. Hertig A, Anglicheau D, Verine J*, et al.* Early epithelial phenotypic changes predict graft fibrosis. *Journal of the American Society of Nephrology : JASN* 2008; 19(8):1584-91.

70. Ohnuki K, Umezono T, Abe M*, et al.* Expression of transcription factor Snai1 and tubulointerstitial fibrosis in progressive nephropathy. *J Nephrol* 2011:0.

71. Saad S, Stanners SR, Yong R, Tang O, Pollock CA. Notch mediated epithelial to mesenchymal transformation is associated with increased expression of the Snail transcription factor. *Int J Biochem Cell Biol* 2010; 42(7):1115-22.

72. Saal S, Harvey SJ. MicroRNAs and the kidney: coming of age. *Current opinion in nephrology and hypertension* 2009; 18(4):317-23.

73. Scian MJ, Maluf DG, David KG*, et al.* MicroRNA profiles in allograft tissues and paired urines associate with chronic allograft dysfunction with IF/TA. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2011; 11(10):2110-22.

74. Wang B, Herman-Edelstein M, Koh P*, et al.* E-cadherin expression is regulated by miR-192/215 by a mechanism that is independent of the profibrotic effects of transforming growth factor-beta. *Diabetes* 2010; 59(7):1794-802.

75. Xiong M, Jiang L, Zhou Y*, et al.* MiR-200 family regulates TGF-{beta}1-induced renal tubular epithelial to mesenchymal transition through Smad pathway by targeting ZEB1 and ZEB2 expression. *Am J Physiol Renal Physiol* 2011.

76. Kato M, Zhang J, Wang M*, et al.* MicroRNA-192 in diabetic kidney glomeruli and its function in TGF-beta-induced collagen expression via inhibition of E-box repressors. *Proceedings of the National Academy of Sciences of the United States of America* 2007; 104(9):3432-7.

77. Zarjou A, Yang S, Abraham E, Agarwal A, Liu G. Identification of a microRNA signature in renal fibrosis: role of miR-21. *Am J Physiol Renal Physiol* 2011; 301(4):F793-801.

78. Martin J, Jenkins RH, Bennagi R*, et al.* Post-transcriptional regulation of Transforming Growth Factor Beta-1 by microRNA-744. *PLoS One* 2011; 6(10):e25044.

79. Qin W, Chung AC, Huang XR*, et al.* TGF-beta/Smad3 signaling promotes renal fibrosis by inhibiting miR-29. *Journal of the American Society of Nephrology : JASN* 2011; 22(8):1462-74.

80. Zhong X, Chung AC, Chen HY, Meng XM, Lan HY. Smad3-mediated upregulation of miR-21 promotes renal fibrosis. *Journal of the American Society of Nephrology : JASN* 2011; 22(9):1668-81.

81. Wang Q, Wang Y, Minto AW*, et al.* MicroRNA-377 is up-regulated and can lead to increased fibronectin production in diabetic nephropathy. *Faseb J* 2008; 22(12):4126-35.

82. Oba S, Kumano S, Suzuki E*, et al.* miR-200b precursor can ameliorate renal tubulointerstitial fibrosis. *PLoS One* 2010; 5(10):e13614.

83. Lorenzen JM, Haller H, Thum T. MicroRNAs as mediators and therapeutic targets in chronic kidney disease. *Nature reviews Nephrology* 2011; 7(5):286-94.

84. Cybulsky AV. Endoplasmic reticulum stress in proteinuric kidney disease. *Kidney international* 2010; 77(3):187-93.

85. Kawakami T, Inagi R, Takano H*, et al.* Endoplasmic reticulum stress induces autophagy in renal proximal tubular cells. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2009; 24(9):2665-72.

86. Inagi R. Endoplasmic reticulum stress in the kidney as a novel mediator of kidney injury. *Nephron Experimental nephrology* 2009; 112(1):e1-9.

87. Periyasamy-Thandavan S, Jiang M, Schoenlein P, Dong Z. Autophagy: molecular machinery, regulation, and implications for renal pathophysiology. *American journal of physiology Renal physiology* 2009; 297(2):F244-56.

88. Pallet N, Bouvier N, Legendre C*, et al.* Autophagy protects renal tubular cells against cyclosporine toxicity. *Autophagy* 2008; 4(6):783-91.

89. Gozuacik D, Bialik S, Raveh T*, et al.* DAP-kinase is a mediator of endoplasmic reticulum stress-induced caspase activation and autophagic cell death. *Cell Death Differ* 2008; 15(12):1875-86.

90. Dickhout JG, Carlisle RE, Austin RC. Interrelationship between cardiac hypertrophy, heart failure, and chronic kidney disease: endoplasmic reticulum stress as a mediator of pathogenesis. *Circ Res* 2011; 108(5):629-42.

91. Ruan XZ, Varghese Z, Moorhead JF. An update on the lipid nephrotoxicity hypothesis. *Nature reviews Nephrology* 2009; 5(12):713-21.

92. Cho KH, Kim HJ, Kamanna VS, Vaziri ND. Niacin improves renal lipid metabolism and slows progression in chronic kidney disease. *Biochim Biophys Acta* 2010; 1800(1):6-15.

93. Kim HJ, Moradi H, Yuan J, Norris K, Vaziri ND. Renal mass reduction results in accumulation of lipids and dysregulation of lipid regulatory proteins in the remnant kidney. *American journal of physiology Renal physiology* 2009; 296(6):F1297-306.

94. Toblli JE, Ferrini MG, Cao G*, et al.* Antifibrotic effects of pioglitazone on the kidney in a rat model of type 2 diabetes mellitus. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2009; 24(8):2384-91.

95. Kawai T, Masaki T, Doi S*, et al.* PPAR-gamma agonist attenuates renal interstitial fibrosis and inflammation through reduction of TGF-beta. *Laboratory investigation; a journal of technical methods and pathology* 2009; 89(1):47-58.

96. Toblli JE, Cao G, Giani JF*, et al.* Antifibrotic effects of pioglitazone at low doses on the diabetic rat kidney are associated with the improvement of markers of cell turnover, tubular and endothelial integrity, and angiogenesis. *Kidney Blood Press Res* 2011; 34(1):20-33.

97. Tanaka Y, Kume S, Araki S*, et al.* Fenofibrate, a PPARalpha agonist, has renoprotective effects in mice by enhancing renal lipolysis. *Kidney international* 2011; 79(8):871-82.

98. Yuen DA, Connelly KA, Advani A*, et al.* Culture-modified bone marrow cells attenuate cardiac and renal injury in a chronic kidney disease rat model via a novel antifibrotic mechanism. *PLoS One* 2010; 5(3):e9543.

99. Li L, Zepeda-Orozco D, Black R, Lin F. Autophagy is a component of epithelial cell fate in obstructive uropathy. *The American journal of pathology* 2010; 176(4):1767-78.

100. Takakuta K, Fujimori A, Chikanishi T*, et al.* Renoprotective properties of pirfenidone in subtotally nephrectomized rats. *Eur J Pharmacol* 2010; 629(1-3):118-24.

101. RamachandraRao SP, Zhu Y, Ravasi T*, et al.* Pirfenidone is renoprotective in diabetic kidney disease. *Journal of the American Society of Nephrology : JASN* 2009; 20(8):1765-75.

102. Cho ME, Kopp JB. Pirfenidone: an anti-fibrotic therapy for progressive kidney disease. *Expert Opin Investig Drugs* 2010; 19(2):275-83.

103. Cho ME, Smith DC, Branton MH, Penzak SR, Kopp JB. Pirfenidone slows renal function decline in patients with focal segmental glomerulosclerosis. *Clinical journal of the American Society of Nephrology : CJASN* 2007; 2(5):906-13.

104. Macias-Barragan J, Sandoval-Rodriguez A, Navarro-Partida J, Armendariz-Borunda J. The multifaceted role of pirfenidone and its novel targets. *Fibrogenesis Tissue Repair* 2010; 3:16.

105. Boor P, Sebekova K, Ostendorf T, Floege J. Treatment targets in renal fibrosis. *Nephrol Dial Transplant* 2007; 22(12):3391-407.

106. Shweke N, Boulos N, Jouanneau C*, et al.* Tissue transglutaminase contributes to interstitial renal fibrosis by favoring accumulation of fibrillar collagen through TGF-beta activation and cell infiltration. *The American journal of pathology* 2008; 173(3):631-42.

107. Huang L, Haylor JL, Hau Z*, et al.* Transglutaminase inhibition ameliorates experimental diabetic nephropathy. *Kidney international* 2009; 76(4):383-94.

108. Wang X, Zhou Y, Tan R*, et al.* Mice lacking the matrix metalloproteinase-9 gene reduce renal interstitial fibrosis in obstructive nephropathy. *Am J Physiol Renal Physiol* 2010; 299(5):F973-82.

109. Yang J, Shultz RW, Mars WM*, et al.* Disruption of tissue-type plasminogen activator gene in mice reduces renal interstitial fibrosis in obstructive nephropathy. *The Journal of clinical investigation* 2002; 110(10):1525-38.

110. Zhong J, Guo D, Chen CB*, et al.* Prevention of angiotensin II-mediated renal oxidative stress, inflammation, and fibrosis by angiotensin-converting enzyme 2. *Hypertension* 2011; 57(2):314-22.

111. Zhou L, Xue H, Yuan P*, et al.* Angiotensin AT1 receptor activation mediates high glucose-induced epithelial-mesenchymal transition in renal proximal tubular cells. *Clin Exp Pharmacol Physiol* 2010; 37(9):e152-7.

112. Naito T, Ma LJ, Yang H*, et al.* Angiotensin type 2 receptor actions contribute to angiotensin type 1 receptor blocker effects on kidney fibrosis. *Am J Physiol Renal Physiol* 2010; 298(3):F683-91.

113. Takeda Y, Nishikimi T, Akimoto K, Matsuoka H, Ishimitsu T. Beneficial effects of a combination of Rho-kinase inhibitor and ACE inhibitor on tubulointerstitial fibrosis induced by unilateral ureteral obstruction. *Hypertens Res* 2010; 33(9):965-73.

114. Narasimhan KL, Madhu K, Balpinder K*, et al.* Association of angiotensin converting enzyme and angiotensin type 2 receptor gene polymorphisms with renal damage in posterior urethral valves. *J Pediatr Urol* 2010; 6(6):560-6.

115. Schulman IH, Zhou MS, Treuer AV*, et al.* Altered renal expression of angiotensin II receptors, renin receptor, and ACE-2 precede the development of renal fibrosis in aging rats. *Am J Nephrol* 2010; 32(3):249-61.

116. Rodrigues Diez R, Rodrigues-Diez R, Lavoz C*, et al.* Statins inhibit angiotensin II/Smad pathway and related vascular fibrosis, by a TGF-beta-independent process. *PLoS One* 2010; 5(11):e14145.

117. Scruggs BS, Zuo Y, Donnert E*, et al.* Increased capillary branching contributes to angiotensin type 1 receptor blocker (ARB)-induced regression of sclerosis. *Am J Pathol* 2011; 178(4):1891-8.

118. Edgtton KL, Gow RM, Kelly DJ, Carmeliet P, Kitching AR. Plasmin is not protective in experimental renal interstitial fibrosis. *Kidney international* 2004; 66(1):68-76.

119. Cheng S, Lovett DH. Gelatinase A (MMP-2) is necessary and sufficient for renal tubular cell epithelial-mesenchymal transformation. *The American journal of pathology* 2003; 162(6):1937-49.

120. Radisky DC, Levy DD, Littlepage LE*, et al.* Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature* 2005; 436(7047):123-7.

121. Abrass CK, Hansen KM, Patton BL. Laminin alpha4-null mutant mice develop chronic kidney disease with persistent overexpression of platelet-derived growth factor. *The American journal of pathology* 2010; 176(2):839-49.

122. Xie P, Sun L, Nayak B*, et al.* C/EBP-beta modulates transcription of tubulointerstitial nephritis antigen in obstructive uropathy. *Journal of the American Society of Nephrology : JASN* 2009; 20(4):807-19.

123. Steegh FM, Gelens MA, Nieman FH*, et al.* Early loss of peritubular capillaries after kidney transplantation. *Journal of the American Society of Nephrology : JASN* 2011; 22(6):1024-9.

124. Sakamoto I, Ito Y, Mizuno M*, et al.* Lymphatic vessels develop during tubulointerstitial fibrosis. *Kidney international* 2009; 75(8):828-38.

125. Matsui K, Nagy-Bojarsky K, Laakkonen P*, et al.* Lymphatic microvessels in the rat remnant kidney model of renal fibrosis: aminopeptidase p and podoplanin are discriminatory markers for endothelial cells of blood and lymphatic vessels. *Journal of the American Society of Nephrology : JASN* 2003; 14(8):1981-9.

126. Kriz W, LeHir M. Pathways to nephron loss starting from glomerular diseases-insights from animal models. *Kidney international* 2005; 67(2):404-19.

127. Rienstra H, Katta K, Celie JW*, et al.* Differential expression of proteoglycans in tissue remodeling and lymphangiogenesis after experimental renal transplantation in rats. *PLoS One* 2010; 5(2):e9095.

128. Kerjaschki D, Regele HM, Moosberger I*, et al.* Lymphatic neoangiogenesis in human kidney transplants is associated with immunologically active lymphocytic infiltrates. *Journal of the American Society of Nephrology : JASN* 2004; 15(3):603-12.

129. Stuht S, Gwinner W, Franz I*, et al.* Lymphatic neoangiogenesis in human renal allografts: results from sequential protocol biopsies. *American journal of transplantation : official journal of the*

American Society of Transplantation and the American Society of Transplant Surgeons 2007; 7(2):377-84.

130. Hakroush S, Moeller MJ, Theilig F*, et al.* Effects of increased renal tubular vascular endothelial growth factor (VEGF) on fibrosis, cyst formation, and glomerular disease. *The American journal of pathology* 2009; 175(5):1883-95.

131. Lian YG, Zhou QG, Zhang YJ, Zheng FL. VEGF ameliorates tubulointerstitial fibrosis in unilateral ureteral obstruction mice via inhibition of epithelial-mesenchymal transition. *Acta Pharmacol Sin* 2011.

132. Ozdemir BH, Ozdemir AA, Erdal R, Ozdemir FN, Haberal M. Rapamycin prevents interstitial fibrosis in renal allografts through decreasing angiogenesis and inflammation. *Transplant Proc* 2011; 43(2):524-6.

133. Higgins DF, Kimura K, Iwano M, Haase VH. Hypoxia-inducible factor signaling in the development of tissue fibrosis. *Cell Cycle* 2008; 7(9):1128-32.

134. Kimura K, Iwano M, Higgins DF*, et al.* Stable expression of HIF-1alpha in tubular epithelial cells promotes interstitial fibrosis. *American journal of physiology Renal physiology* 2008; 295(4):F1023-9.

135. Higgins DF, Kimura K, Bernhardt WM*, et al.* Hypoxia promotes fibrogenesis in vivo via HIF-1 stimulation of epithelial-to-mesenchymal transition. *The Journal of clinical investigation* 2007; 117(12):3810-20.

136. Wang Z, Tang L, Zhu Q*, et al.* Hypoxia-inducible factor-1alpha contributes to the profibrotic action of angiotensin II in renal medullary interstitial cells. *Kidney international* 2011; 79(3):300-10.

137. Ortiz A, Ucero AC, Egido J. Unravelling fibrosis: two newcomers and an old foe. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2010; 25(11):3492-5.

138. Wang Q, Usinger W, Nichols B*, et al.* Cooperative interaction of CTGF and TGF-beta in animal models of fibrotic disease. *Fibrogenesis Tissue Repair* 2011; 4(1):4.

139. Wang S, Wilkes MC, Leof EB, Hirschberg R. Noncanonical TGF-beta pathways, mTORC1 and Abl, in renal interstitial fibrogenesis. *Am J Physiol Renal Physiol* 2010; 298(1):F142-9.

140. Yeh YC, Wei WC, Wang YK*, et al.* Transforming growth factor-{beta}1 induces Smad3-dependent {beta}1 integrin gene expression in epithelial-to-mesenchymal transition during chronic tubulointerstitial fibrosis. *Am J Pathol* 2010; 177(4):1743-54.

141. Zeng R, Han M, Luo Y*, et al.* Role of Sema4C in TGF-beta1-induced mitogen-activated protein kinase activation and epithelial-mesenchymal transition in renal tubular epithelial cells. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2011; 26(4):1149-56.

142. Zhu B, Wang YJ, Zhu CF*, et al.* Triptolide inhibits extracellular matrix protein synthesis by suppressing the Smad2 but not the MAPK pathway in TGF-beta1-stimulated NRK-49F cells. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2010; 25(10):3180-91.

143. Lan HY. Diverse roles of TGF-beta/Smads in renal fibrosis and inflammation. *Int J Biol Sci* 2011; 7(7):1056-67.

144. Meng XM, Huang XR, Chung AC*, et al.* Smad2 protects against TGF-beta/Smad3-mediated renal fibrosis. *Journal of the American Society of Nephrology : JASN* 2010; 21(9):1477-87.

145. Zhou Y, Mao H, Li S*, et al.* HSP72 inhibits Smad3 activation and nuclear translocation in renal epithelial-to-mesenchymal transition. *Journal of the American Society of Nephrology : JASN* 2010; 21(4):598-609.

146. Yang J, Zhang X, Li Y, Liu Y. Downregulation of Smad transcriptional corepressors SnoN and Ski in the fibrotic kidney: an amplification mechanism for TGF-beta1 signaling. *Journal of the American Society of Nephrology : JASN* 2003; 14(12):3167-77.

147. Leask A. Targeting the jagged/notch pathway: a new treatment for fibrosis? *J Cell Commun Signal* 2010; 4(4):197-8.

148. Sharma S, Sirin Y, Susztak K. The story of Notch and chronic kidney disease. *Curr Opin Nephrol Hypertens* 2011; 20(1):56-61.

149. Sirin Y, Susztak K. Notch in the kidney: development and disease. *J Pathol* 2011.

150. Bottinger EP, Bitzer M. TGF-beta signaling in renal disease. *Journal of the American Society of Nephrology : JASN* 2002; 13(10):2600-10.

151. Schnaper HW, Hayashida T, Hubchak SC, Poncelet AC. TGF-beta signal transduction and mesangial cell fibrogenesis. *American journal of physiology Renal physiology* 2003; 284(2):F243-52.

152. Ma LJ, Jha S, Ling H*, et al.* Divergent effects of low versus high dose anti-TGF-beta antibody in puromycin aminonucleoside nephropathy in rats. *Kidney international* 2004; 65(1):106-15.

153. Liu Y. Renal fibrosis: new insights into the pathogenesis and therapeutics. *Kidney Int* 2006; 69(2):213-7.

154. Zeisberg M. Bone morphogenic protein-7 and the kidney: current concepts and open questions. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2006; 21(3):568-73.

155. Zeisberg M, Bottiglio C, Kumar N*, et al.* Bone morphogenic protein-7 inhibits progression of chronic renal fibrosis associated with two genetic mouse models. *American journal of physiology Renal physiology* 2003; 285(6):F1060-7.

156. Zeisberg M, Hanai J, Sugimoto H*, et al.* BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nature medicine* 2003; 9(7):964-8.

157. Zeisberg M, Shah AA, Kalluri R. Bone morphogenic protein-7 induces mesenchymal to epithelial transition in adult renal fibroblasts and facilitates regeneration of injured kidney. *J Biol Chem* 2005; 280(9):8094-100.

158. Luo DD, Phillips A, Fraser D. Bone morphogenetic protein-7 inhibits proximal tubular epithelial cell Smad3 signaling via increased SnoN expression. *Am J Pathol* 2010; 176(3):1139-47.

159. Maciel TT, Kempf H, Campos AH. Targeting bone morphogenetic protein signaling on renal and vascular diseases. *Curr Opin Nephrol Hypertens* 2010; 19(1):26-31.

160. Yanagita M. Modulator of bone morphogenetic protein activity in the progression of kidney diseases. *Kidney international* 2006; 70(6):989-93.

161. Tanaka M, Asada M, Higashi AY*, et al.* Loss of the BMP antagonist USAG-1 ameliorates disease in a mouse model of the progressive hereditary kidney disease Alport syndrome. *The Journal of clinical investigation* 2010; 120(3):768-77.

162. Grgurevic L, Macek B, Healy DR*, et al.* Circulating bone morphogenetic protein 1-3 isoform increases renal fibrosis. *Journal of the American Society of Nephrology : JASN* 2011; 22(4):681-92.

163. Okada H. Tolloid-like proteinases orchestrate extracellular matrix formation. *Journal of the American Society of Nephrology : JASN* 2011; 22(4):588-9.

164. Xin BM, Wang XX, Jin W*, et al.* Activation of Toll-like receptor 9 attenuates unilateral ureteral obstruction-induced renal fibrosis. *Acta Pharmacol Sin* 2010; 31(12):1583-92.

165. Pulskens WP, Rampanelli E, Teske GJ*, et al.* TLR4 promotes fibrosis but attenuates tubular damage in progressive renal injury. *Journal of the American Society of Nephrology : JASN* 2010; 21(8):1299-308.

166. Hahm K, Lukashev ME, Luo Y*, et al.* Alphav beta6 integrin regulates renal fibrosis and inflammation in Alport mouse. *The American journal of pathology* 2007; 170(1):110-25.

167. Yan Q, Sui W, Xie S*, et al.* Expression and role of integrin-linked kinase and collagen IV in human renal allografts with interstitial fibrosis and tubular atrophy. *Transpl Immunol* 2010; 23(1-2):1-5.

168. Yang J, Liu Y. Blockage of tubular epithelial to myofibroblast transition by hepatocyte growth factor prevents renal interstitial fibrosis. *Journal of the American Society of Nephrology : JASN* 2002; 13(1):96-107.

169. Ostendorf T, Eitner F, Floege J. The PDGF family in renal fibrosis. *Pediatr Nephrol* 2011.

170. Floege J, Hudkins KL, Seifert RA*, et al.* Localization of PDGF alpha-receptor in the developing and mature human kidney. *Kidney international* 1997; 51(4):1140-50.

171. Floege J, Hudkins KL, Davis CL, Schwartz SM, Alpers CE. Expression of PDGF alpha-receptor in renal arteriosclerosis and rejecting renal transplants. *Journal of the American Society of Nephrology : JASN* 1998; 9(2):211-23.

172. Eitner F, Ostendorf T, Kretzler M*, et al.* PDGF-C expression in the developing and normal adult human kidney and in glomerular diseases. *Journal of the American Society of Nephrology : JASN* 2003; 14(5):1145-53.

173. Eitner F, Ostendorf T, Van Roeyen C*, et al.* Expression of a novel PDGF isoform, PDGF-C, in normal and diseased rat kidney. *Journal of the American Society of Nephrology : JASN* 2002; 13(4):910-7.

174. Eitner F, Bucher E, van Roeyen C*, et al.* PDGF-C is a proinflammatory cytokine that mediates renal interstitial fibrosis. *Journal of the American Society of Nephrology : JASN* 2008; 19(2):281-9.

175. Taneda S, Hudkins KL, Topouzis S*, et al.* Obstructive uropathy in mice and humans: potential role for PDGF-D in the progression of tubulointerstitial injury. *Journal of the American Society of Nephrology : JASN* 2003; 14(10):2544-55.

176. Sheerin NS, Sacks SH. Leaked protein and interstitial damage in the kidney: is complement the missing link? *Clin Exp Immunol* 2002; 130(1):1-3.

177. Rangan GK, Pippin JW, Couser WG. C5b-9 regulates peritubular myofibroblast accumulation in experimental focal segmental glomerulosclerosis. *Kidney international* 2004; 66(5):1838-48.

178. Rangan GK, Pippin JW, Coombes JD, Couser WG. C5b-9 does not mediate chronic tubulointerstitial disease in the absence of proteinuria. *Kidney international* 2005; 67(2):492-503.

179. Boor P, Konieczny A, Villa L*, et al.* Complement C5 mediates experimental tubulointerstitial fibrosis. *Journal of the American Society of Nephrology : JASN* 2007; 18(5):1508-15.

180. Pan H, Shen Z, Mukhopadhyay P*, et al.* Anaphylatoxin C5a contributes to the pathogenesis of cisplatin-induced nephrotoxicity. *American journal of physiology Renal physiology* 2009; 296(3):F496-504.

181. Nakorchevsky A, Hewel JA, Kurian SM*, et al.* Molecular mechanisms of chronic kidney transplant rejection via large-scale proteogenomic analysis of tissue biopsies. *Journal of the American Society of Nephrology : JASN* 2010; 21(2):362-73.

182. Colvin RB, Chang A, Farris AB*, et al. Diagnostic Pathology: Kidney Diseases*, Vol., Amirsys: Salt Lake City, UT, 2011.

183. Liptak P, Ivanyi B. Primer: Histopathology of calcineurin-inhibitor toxicity in renal allografts. *Nat Clin Pract Nephrol* 2006; 2(7):398-404; quiz following

184. Klahr S, Morrissey J. Obstructive nephropathy and renal fibrosis: The role of bone morphogenic protein-7 and hepatocyte growth factor. *Kidney international Supplement* 2003; (87):S105-12.

185. Solez K, Colvin RB, Racusen LC*, et al.* Banff '05 Meeting Report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant* 2007; 7(3):518-26.

186. Farris AB, Adams CD, Brousaides N*, et al.* Morphometric and Visual Evaluation of Fibrosis in Renal Biopsies. *J Am Soc Nephrol* 2010.

187. Grimm PC, Nickerson P, Gough J*, et al.* Quantitation of allograft fibrosis and chronic allograft nephropathy. *Pediatr Transplant* 1999; 3(4):257-70.

188. 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* 1968; 2(7564):363-6.

189. Choi BS, Shin MJ, Shin SJ*, et al.* Clinical significance of an early protocol biopsy in living-donor renal transplantation: ten-year experience at a single center. *Am J Transplant* 2005; 5(6):1354-60.

190. Colvin RB, Nickeleit V. Renal transplant pathology. In: Jennette JC, Olson JL, Schwartz MM, Silva FG (eds). Heptinstall's Pathology of the Kidney, 6th edn, Vol. 2, Lippincott Williams and Wilkins: Philadelphia, PA, 2006, pp 1347-490.

191. Cosio FG, Grande JP, Wadei H*, et al.* Predicting subsequent decline in kidney allograft function from early surveillance biopsies. *Am J Transplant* 2005; 5(10):2464-72.

192. Liu Y, Yang J. Hepatocyte growth factor: new arsenal in the fights against renal fibrosis? *Kidney Int* 2006; 70(2):238-40.

193. Vilayur E, Harris DC. Emerging therapies for chronic kidney disease: what is their role? *Nat Rev Nephrol* 2009.

194. Moreso F, Lopez M, Vallejos A*, et al.* Serial protocol biopsies to quantify the progression of chronic transplant nephropathy in stable renal allografts. *Am J Transplant* 2001; 1(1):82-8.

195. Marcussen N, Olsen TS, Benediktsson H, Racusen L, Solez K. Reproducibility of the Banff classification of renal allograft pathology. Inter- and intraobserver variation. *Transplantation* 1995; 60(10):1083-9.

196. Furness PN, Taub N. International variation in the interpretation of renal transplant biopsies: report of the CERTPAP Project. *Kidney Int* 2001; 60(5):1998-2012.

197. Racusen LC, Solez K, Colvin RB*, et al.* The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; 55(2):713-23.

198. Servais A, Meas-Yedid V, Buchler M*, et al.* Quantification of interstitial fibrosis by image analysis on routine renal biopsy in patients receiving cyclosporine. *Transplantation* 2007; 84(12):1595-601.

199. Servais A, Meas-Yedid V, Toupance O*, et al.* Interstitial fibrosis quantification in renal transplant recipients randomized to continue cyclosporine or convert to sirolimus. *Am J Transplant* 2009; 9(11):2552-60.

200. Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J* 1979; 11(4):447-55.

201. Sund S, Grimm P, Reisaeter AV, Hovig T. Computerized image analysis vs semiquantitative scoring in evaluation of kidney allograft fibrosis and prognosis. *Nephrol Dial Transplant* 2004; 19(11):2838-45.

202. Feldman DL, Mogelesky TC, Chou M, Jeng AY. Enhanced expression of renal endothelin-converting enzyme-1 and endothelin-A-receptor mRNA in rats with interstitial fibrosis following ureter ligation. *J Cardiovasc Pharmacol* 2000; 36(5 Suppl 1):S255-9.

203. Satoh M, Kashihara N, Yamasaki Y*, et al.* Renal interstitial fibrosis is reduced in angiotensin II type 1a receptor-deficient mice. *J Am Soc Nephrol* 2001; 12(2):317-25.

204. Nicholson ML, Bailey E, Williams S, Harris KP, Furness PN. Computerized histomorphometric assessment of protocol renal transplant biopsy specimens for surrogate markers of chronic rejection. *Transplantation* 1999; 68(2):236-41.

205. Moreso F, Seron D, Vitria J*, et al.* Quantification of interstitial chronic renal damage by means of texture analysis. *Kidney Int* 1994; 46(6):1721-7.

206. De Heer E, Sijpkens YW, Verkade M*, et al.* Morphometry of interstitial fibrosis. *Nephrol Dial Transplant* 2000; 15 Suppl 6:72-3.

207. Servais A, Meas-Yedid V, Noel LH*, et al.* Interstitial Fibrosis Evolution on Early Sequential Screening Renal Allograft Biopsies Using Quantitative Image Analysis. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2011; 11(7):1456-63.

208. Servais A, Meas-Yedid V, Toupance O*, et al.* Interstitial fibrosis quantification in renal transplant recipients randomized to continue cyclosporine or convert to sirolimus. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2009; 9(11):2552-60.

209. Meas-Yedid V, Servais A, Noel LH*, et al.* New computerized color image analysis for the quantification of interstitial fibrosis in renal transplantation. *Transplantation* 2011; 92(8):890-9.

210. Miura Y, Satoh S, Saito M*, et al.* Factors increasing quantitative interstitial fibrosis from 0 hr to 1 year in living kidney transplant patients receiving tacrolimus. *Transplantation* 2011; 91(1):78-85.

211. Servais A, Meas-Yedid V, Noel LH*, et al.* Interstitial fibrosis evolution on early sequential screening renal allograft biopsies using quantitative image analysis. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2011; 11(7):1456-63.

212. Seron D, Moreso F. Protocol biopsies in renal transplantation: prognostic value of structural monitoring. *Kidney Int* 2007; 72(6):690-7.

213. Sis B, Mengel M, Haas M*, et al.* Banff '09 Meeting Report: Antibody Mediated Graft Deterioration and Implementation of Banff Working Groups. *Am J Transplant* 2010.

214. Halloran PF, de Freitas DG, Einecke G*, et al.* The molecular phenotype of kidney transplants. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2010; 10(10):2215-22.

215. Shishido S, Asanuma H, Nakai H*, et al.* The impact of repeated subclinical acute rejection on the progression of chronic allograft nephropathy. *J Am Soc Nephrol* 2003; 14(4):1046-52.

216. Mannon RB, Matas AJ, Grande J*, et al.* Inflammation in areas of tubular atrophy in kidney allograft biopsies: a potent predictor of allograft failure. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* 2010; 10(9):2066-73.

217. Park WD, Griffin MD, Cornell LD, Cosio FG, Stegall MD. Fibrosis with inflammation at one year predicts transplant functional decline. *Journal of the American Society of Nephrology : JASN* 2010; 21(11):1987-97.

218. Randhawa PS, Minervini MI, Lombardero M*, et al.* Biopsy of marginal donor kidneys: correlation of histologic findings with graft dysfunction. *Transplantation* 2000; 69(7):1352-7.

219. Seron D, Carrera M, Grino JM*, et al.* Relationship between donor renal interstitial surface and post-transplant function. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 1993; 8(6):539-43.

220. Wang HJ, Kjellstrand CM, Cockfield SM, Solez K. On the influence of sample size on the prognostic accuracy and reproducibility of renal transplant biopsy. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 1998; 13(1):165-72.

221. Roberts IS, Cook HT, Troyanov S*, et al.* The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility. *Kidney international* 2009; 76(5):546-56.

222. Yamamoto R, Imai E. A novel classification for IgA nephropathy. *Kidney international* 2009; 76(5):477-80.

223. Pape L, Henne T, Offner G*, et al.* Computer-assisted quantification of fibrosis in chronic allograft nephropaty by picosirius red-staining: a new tool for predicting long-term graft function. *Transplantation* 2003; 76(6):955-8.

224. Bains JC, Sandford RM, Brook NR*, et al.* Comparison of renal allograft fibrosis after transplantation from heart-beating and non-heart-beating donors. *Br J Surg* 2005; 92(1):113-8.

225. Hunter MG, Hurwitz S, Bellamy CO, Duffield JS. Quantitative morphometry of lupus nephritis: the significance of collagen, tubular space, and inflammatory infiltrate. *Kidney Int* 2005; 67(1):94-102.

226. Masseroli M, O'Valle F, Andujar M*, et al.* Design and validation of a new image analysis method for automatic quantification of interstitial fibrosis and glomerular morphometry. *Lab Invest* 1998; 78(5):511-22.

227. Pape L, Mengel M, Offner G*, et al.* Renal arterial resistance index and computerized quantification of fibrosis as a combined predictive tool in chronic allograft nephropathy. *Pediatr Transplant* 2004; 8(6):565-70.

228. Vleming LJ, Baelde JJ, Westendorp RG*, et al.* Progression of chronic renal disease in humans is associated with the deposition of basement membrane components and decorin in the interstitial extracellular matrix. *Clin Nephrol* 1995; 44(4):211-9.

229. Abrass CK, Berfield AK, Stehman-Breen C, Alpers CE, Davis CL. Unique changes in interstitial extracellular matrix composition are associated with rejection and cyclosporine toxicity in human renal allograft biopsies. *Am J Kidney Dis* 1999; 33(1):11-20.

230. Verkade MA, Bajema IM, De Heer E, Bruijn JA. Decorin and TGF-beta-1 protein expression in renal disease: a morphometric analysis. *J Am Soc Nephrol* 1999; 10:584 (abstract).

231. Kostadinova-Kunovska S, Petrusevska G, Jovanovic R*, et al.* Histomorphometric analysis of fibrosis in the renal interstitial compartment. *Prilozi* 2005; 26(1):51-9.

232. Ruiz P, Kolbeck PC, Scroggs MW, Sanfilippo F. Associations between cyclosporine therapy and interstitial fibrosis in renal allograft biopsies. *Transplantation* 1988; 45(1):91-5.

233. Ruiz P, Kolbeck PC, Scroggs MW, Bollinger RR, Sanfilippo F. Cyclosporine therapy and the development of interstitial fibrosis in renal allografts. *Transplant Proc* 1988; 20(3 Suppl 3):807-11.

234. Nicholson ML, McCulloch TA, Harper SJ*, et al.* Early measurement of interstitial fibrosis predicts long-term renal function and graft survival in renal transplantation. *Br J Surg* 1996; 83(8):1082-5.

235. Diaz Encarnacion MM, Griffin MD, Slezak JM*, et al.* Correlation of quantitative digital image analysis with the glomerular filtration rate in chronic allograft nephropathy. *Am J Transplant* 2004; 4(2):248-56.

Renal Fibroblasts: Origins, Activation and Their Role in Renal Fibrosis

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Please be noted that this syllabus is largely based on my recent review (*Nat Rev Nephrol* **7**: 684-696, 2011).

Introduction

Although many types of cells in renal tubulointerstitium, such as fibroblasts, tubular epithelial cells and subsets of macrophages, are capable of producing extracellular matrix (ECM), fibroblasts are commonly regarded as the principal matrix-producing cells that produce a large amount of interstitial matrix components including fibronectin, type I and type III collagens. Activated fibroblasts in diseased kidneys often express a molecular signature, α-smooth muscle actin (α-SMA), and are also referred as myofibroblasts. In this context, one of the fundamental issues in renal fibrosis field is to delineate the origin, activation and regulation of these matrix-producing myofibroblasts.[1-4]

There are at least 5 different sources have been proposed to contribute to myofibroblast pool in diseased kidneys. These include activation of interstitial fibroblasts and pericytes, phenotypic conversion of tubular epithelial and endothelial cells, and recruitment of circulating fibrocytes.[5] The relative contribution, and even the very existence, of each particular myofibroblast-generating pathway to renal fibrosis is a matter of intense debate and highly controversial. This is largely due to the inherent difficulty in identifying and tracking fibroblasts owing to the lack of specific markers for this cell type. Another major problem is that fibroblasts exhibit enormous phenotypic heterogeneity, probably reflecting their diverse origins, and change their phenotypes over the activation status, localization and times in renal fibrogenesis.

Activation of interstitial fibroblasts and pericytes

In normal adult kidneys, fibroblasts situate in the interstitial space between the capillaries and the epithelia.[6] Morphologically, these cells are stellate shaped and exhibit abundant rough endoplasmic reticulum, collagen-secreting granules and actin filaments. They possess multiple cell processes, which connect to the tubular and capillary basement membranes.[6] In the resting, quiescent state, interstitial fibroblasts express CD73, also known as ecto-5'-nucleotidase, in their plasma membrane, and produce erythropoietin.[6,7] They also express platelet-derived growth factor receptor β (PDGFR-β),[8-10] and fibroblast-specific protein-1 (Fsp1), a small cytoskeleton-associated and calcium-binding protein also

known as S100A4.[11-13] Upon activation, fibroblasts acquire a myofibroblast phenotype by expressing α-SMA and producing a large amount of ECM components.

Several markers have been used to characterize fibroblasts and myofibroblasts in the kidneys such as a-SMA, desmin, FSP1, CD73, PDGFRβ. Unfortunately, none of these markers is specific. In addition, they are rarely expressed by all fibroblasts/myofibroblasts or hardly ever present all the times. Even α-SMA, a classic hallmark for myofibroblast activation in organ fibrosis of all types,[14,15] is not without problem. The expression of α-SMA is not exclusive for myofibroblasts, as it is also present in vascular smooth muscle cells. In addition, not all activated fibroblasts express α-SMA all the time.

Activated fibroblasts are often characterized by two key features: proliferation and myofibroblastic activation. The latter is illustrated by α-SMA expression and matrix production. Both fibroblasts and myofibroblasts have the capacity to proliferate in response to cytokine cues, leading to the expansion of fibroblast population and interstitial space in diseased kidneys. Several fibrogenic growth factors including PDGF, transforming growth factor-β (TGF-β), basic fibroblast growth factor (FGF-2) and connective tissue growth factor (CTGF) are the well-known mitogens for fibroblasts.[8,16-20] In addition to these classic cytokines, tissue-type plasminogen activator (tPA) is another critical player promoting fibroblast survival, proliferation and myofibroblastic activation.[21-24]

Studies suggest that vascular pericytes are a major source of myofibroblasts in fibrotic kidneys.[4,25-27] Pericytes are a subset of stromal cells that partially cover capillary walls, thereby stabilizing endothelium. Following kidney injury, pericytes are detached from the endothelium, undergo migration and proliferation, and differentiate into myofibroblasts.[25,27] The story of pericytes is quite interesting, because pericyte detachment and differentiation into myofibroblasts under pathological conditions not only result in destabilization of microvasculature, but also contribute to myofibroblast activation, leading to interstitial fibrosis.

Phenotypic conversion of epithelial and endothelial cells

Another source of matrix-producing cells could come from tubular epithelium through EMT, a cell phenotypic conversion process occurs in embryonic development, tumor metastasis and organ fibrosis.[28-32] Similarly, fibroblasts/myofibroblasts may derive from capillary endothelium by endothelial to mesenchymal transition (EndoMT).[33,34] The contribution of EMT to renal fibrosis is controversial and is the focus of several recent reviews and debates.[26,35-41]

There is a broad agreement that tubular epithelial cells *in vitro* can undergo EMT, characterized by loss of epithelial feature and acquisition of mensenchymal markers, under the bombardment of various pro-fibrotic cytokines, particularly TGF-β1. However, whether this merely represents an *in vitro* artifact or it does occur *in vivo* is the center of argument. Using a genetic lineage-tracking, fate mapping technique, an early study demonstrates that more than one third of the $Fsp1^+$ interstitial fibroblasts are derived from tubular epithelia in obstructive nephropathy.[42] Likewise, two independent studies also show significant contribution of endothelial cells to the generation of fibroblasts/myofibroblasts via EndoMT in a variety of CKD.[33,34] However, these results are challenged by a number of similar cell fate mapping studies in which no epithelial or endothelial origin of fibroblasts is evident.[27,43] Thus far, the reason behind these discrepancies is unsettled.

EMT is a dynamic program in which epithelial cells and fibroblasts represent two extremes of a continual spectrum of a variety of intermediate cell phenotypes. The frequency that epithelial cells that complete the entire EMT course and ultimately become fibroblasts probably is limited, and heavily depends on the disease models, stages and persistence of elevated cytokine pressure in the inflamed milieu.[44] In most circumstances, tubular cells undergo a partial EMT, in which epithelial cells only change one or two phenotypic markers, while the transcriptional program of EMT is activated. Such a partial EMT, however, is closely associated with poor outcomes and predicts the progression toward interstitial fibrosis in humans.[45] Not surprisingly, blockade of EMT by a variety of agents ameliorates renal fibrosis and preserves kidney function.

Recruitment of circulating fibrocytes

Fibrocytes are a subset of bone marrow-derived, circulating monocytes with fibroblast-like feature in the peripheral blood.[46] They are spindle-shaped, express hematopoietic cell marker CD45, and are capable of producing type I collagen.[47,48] Fibrocytes also express certain chemokine receptors such as CCR7. In response to kidney injury, fibrocytes mobilize and infiltrate into renal parenchyma and participate in fibrogenesis.

The relative importance of fibrocytes in renal fibrogenesis is another area full of controversy. Because of the lack of specific markers for these cells, clear discrimination of them from monocytes, macrophages, fibroblasts and myofibroblasts is a great challenge. In addition, fibrocytes appear to exhibit different subpopulations.[48] Thus far, experimental results on the involvement of fibrocytes, and bone marrow-derived cells in general, in renal fibrosis are inconsistent.[25,42,49,50] Clarification of this issue needs more investigations.

The different origins of fibroblasts likely contribute to their phenotypic heterogeneity. The relative contribution of each lineage to the myofibroblast pool may depend on the disease model and specific stages. It is conceivable that myofibroblast activation from fibroblasts, pericytes or fibrocytes is an early event,[6] while EMT often takes place at a late stage after a sustained injury (Figure 2).[44] The pathological impact of the early activation of fibroblasts versus EMT to renal fibrosis may be different. While fibroblast activation is important for the onset of renal fibrosis, EMT could be a major determinant for fibrosis progression and irreversibility (Figure 2).

Molecular machinery that integrates fibrogenic signals and orchestrates matrix production

The expression and synthesis of ECM proteins by activated fibrobleasts is primarily controlled at the gene transcriptional levels in response to various extracellular fibrogenic cues. Key fibrogenic factors include TGF-β1, PDGF, FGF-2, CTGF and angiotensin II, while HGF and BMP-7 inhibit matrix production primarily by antagonizing TGF-β1 action.[8,51-55] Through their respective receptors and specific downstream intracellular signal cascades, these fibrogenic cytokines activates a host of transcription factors that act on the cognate elements in the promoter regions of the collagen and

fibronectin genes to activate their transcription. Such signal transduction cascades and expression of matrix genes are also regulated by a variety of microRNA.[56-60]

Activated fibroblasts contain stress fibers and display abundant transmembrane connections, also known as fibronexus,[61] between extracellular fibronectin-containing matrix and actin microfilaments, suggesting that an increased interaction of ECM and integrins.[9] Integrins transmit their signals by activating the downstream effector kinase, FAK and ILK, as they possess no enzymatic or actin-binding activity. Extensive studies indicate that ILK, a scaffolding/adaptor protein and a serine/threonine protein kinase, is especially suited to serve as a molecular platform that integrates various fibrogenic signals.[62]

It becomes increasingly clear that integrins/ILK and their associated proteins constitute a fibrogenic molecular machinery that orchestrates matrix production and its extracellular assembly. Analogous to the concept of inflammasome,[63] we propose this multi-component, integrin-associated protein complex as 'matrisome', which is molecular platform activated upon injury that integrates various fibrogenic signal inputs and triggers the production and assembly of matrix components. It appears that almost all key fibrogenic cues promote matrix production somehow by regulating, directly or indirectly, this molecular machinery.

Summary

Renal fibrogenesis is an enormously complex, dynamic process in which activation of fibroblasts is arguably the most important event leading to excessive ECM production and scar formation. We now have a better appreciation for the diverse origins and phenotypic heterogeneity of the matrix-producing fibroblasts, as well as their roles in renal fibrogenesis. As a variety of strategies targeting fibroblast activation are effective in animal models, we are optimistic that some of these remedies will become clinically relevant for CKD patients in the future.

References

1. Liu, Y. Cellular and molecular mechanisms of renal fibrosis. *Nat Rev Nephrol* **7**, 684-696 (2011).
2. Meran, S. & Steadman, R. Fibroblasts and myofibroblasts in renal fibrosis. *Int J Exp Pathol* **92**, 158-167 (2011).
3. Grande, M.T. & Lopez-Novoa, J.M. Fibroblast activation and myofibroblast generation in obstructive nephropathy. *Nat Rev Nephrol* **5**, 319-328 (2009).
4. Schrimpf, C. & Duffield, J.S. Mechanisms of fibrosis: the role of the pericyte. *Curr Opin Nephrol Hypertens* **20**, 297-305 (2011).
5. Barnes, J.L. & Gorin, Y. Myofibroblast differentiation during fibrosis: role of NAD(P)H oxidases. *Kidney Int* **79**, 944-956 (2011).
6. Kaissling, B. & Le Hir, M. The renal cortical interstitium: morphological and functional aspects. *Histochem Cell Biol* **130**, 247-262 (2008).
7. Paliege, A., *et al.* Hypoxia-inducible factor-2alpha-expressing interstitial fibroblasts are the only renal cells that express erythropoietin under hypoxia-inducible factor stabilization. *Kidney Int* **77**, 312-318 (2010).
8. Boor, P. & Floege, J. Special Series: Chronic kidney disease growth factors in renal fibrosis. *Clin Exp Pharmacol Physiol* **38**, 391-400 (2011).
9. Boor, P., Ostendorf, T. & Floege, J. Renal fibrosis: novel insights into mechanisms and therapeutic targets. *Nat Rev Nephrol* **6**, 643-656 (2010).
10. Floege, J., Eitner, F. & Alpers, C.E. A new look at platelet-derived growth factor in renal disease. *J Am Soc Nephrol* **19**, 12-23 (2008).
11. Zeisberg, M. & Neilson, E.G. Mechanisms of tubulointerstitial fibrosis. *J Am Soc Nephrol* **21**, 1819-1834 (2010).
12. Boye, K. & Maelandsmo, G.M. S100A4 and metastasis: a small actor playing many roles. *Am J Pathol* **176**, 528-535 (2010).
13. Grigorian, M., Ambartsumian, N. & Lukanidin, E. Metastasis-inducing S100A4 protein: implication in non-malignant human pathologies. *Curr Mol Med* **8**, 492-496 (2008).
14. Wynn, T.A. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest* **117**, 524-529 (2007).
15. Hinz, B., *et al.* The myofibroblast: one function, multiple origins. *Am J Pathol* **170**, 1807-1816 (2007).
16. Phanish, M.K., Winn, S.K. & Dockrell, M.E. Connective tissue growth factor-(CTGF, CCN2)--a marker, mediator and therapeutic target for renal fibrosis. *Nephron Exp Nephrol* **114**, e83-92 (2010).
17. Hewitson, T.D. Renal tubulointerstitial fibrosis: common but never simple. *Am J Physiol Renal Physiol* **296**, F1239-F1244 (2009).
18. Bottinger, E.P. TGF-beta in renal injury and disease. *Semin Nephrol* **27**, 309-320 (2007).
19. Strutz, F., *et al.* Basic fibroblast growth factor expression is increased in human renal fibrogenesis and may mediate autocrine fibroblast proliferation. *Kidney Int* **57**, 1521-1538 (2000).
20. Ostendorf, T., Eitner, F. & Floege, J. The PDGF family in renal fibrosis. *Pediatr Nephrol*, in press (2011).
21. Hu, K., *et al.* tPA protects renal interstitial fibroblasts and myofibroblasts from apoptosis. *J Am Soc Nephrol* **19**, 503-514 (2008).
22. Hao, S., Shen, H., Hou, Y., Mars, W.M. & Liu, Y. tPA is a potent mitogen for renal interstitial fibroblasts: role of β1 integrin/focal adhesion kinase. *Am. J. Pathol.* **177**, 1164-1175 (2010).
23. Lin, L., *et al.* tPA activates LDL receptor-related protein 1-mediated mitogenic signaling involving the p90RSK and GSK3beta pathway. *Am J Pathol* **177**, 1687-1696 (2010).
24. Hu, K., Wu, C., Mars, W.M. & Liu, Y. Tissue-type plasminogen activator promotes murine myofibroblast activation through LDL receptor-related protein 1-mediated integrin signaling. *J Clin Invest* **117**, 3821-3832 (2007).

25. Lin, S.L., Kisseleva, T., Brenner, D.A. & Duffield, J.S. Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. *Am J Pathol* **173**, 1617-1627 (2008).
26. Duffield, J.S. & Humphreys, B.D. Origin of new cells in the adult kidney: results from genetic labeling techniques. *Kidney Int* **79**, 494-501 (2011).
27. Humphreys, B.D., *et al.* Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol* **176**, 85-97 (2010).
28. Thiery, J.P., Acloque, H., Huang, R.Y. & Nieto, M.A. Epithelial-mesenchymal transitions in development and disease. *Cell* **139**, 871-890 (2009).
29. Lopez-Novoa, J.M. & Nieto, M.A. Inflammation and EMT: an alliance towards organ fibrosis and cancer progression. *EMBO Mol Med* **1**, 303-314 (2009).
30. Kalluri, R. & Weinberg, R.A. The basics of epithelial-mesenchymal transition. *J Clin Invest* **119**, 1420-1428 (2009).
31. Acloque, H., Adams, M.S., Fishwick, K., Bronner-Fraser, M. & Nieto, M.A. Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. *J Clin Invest* **119**, 1438-1449 (2009).
32. Sleeman, J.P. & Thiery, J.P. SnapShot: The epithelial-mesenchymal transition. *Cell* **145**, 162 (2011).
33. Li, J., Qu, X. & Bertram, J.F. Endothelial-myofibroblast transition contributes to the early development of diabetic renal interstitial fibrosis in streptozotocin-induced diabetic mice. *Am J Pathol* **175**, 1380-1388 (2009).
34. Zeisberg, E.M., Potenta, S.E., Sugimoto, H., Zeisberg, M. & Kalluri, R. Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. *J Am Soc Nephrol* **19**, 2282-2287 (2008).
35. Kriz, W., Kaissling, B. & Le Hir, M. Epithelial-mesenchymal transition (EMT) in kidney fibrosis: fact or fantasy? *J Clin Invest* **121**, 468-474 (2011).
36. Zeisberg, M. & Duffield, J.S. Resolved: EMT produces fibroblasts in the kidney. *J Am Soc Nephrol* **21**, 1247-1253 (2010).
37. Liu, Y. New insights into epithelial-mesenchymal transition in kidney fibrosis. *J Am Soc Nephrol* **21**, 212-222 (2010).
38. Li, J. & Bertram, J.F. Endothelial-myofibroblast transition, a new player in diabetic renal fibrosis. *Nephrology* **15**, 507-512 (2010).
39. Burns, W.C. & Thomas, M.C. The molecular mediators of type 2 epithelial to mesenchymal transition (EMT) and their role in renal pathophysiology. *Expert Rev Mol Med* **12**, e17 (2010).
40. Grgic, I., Duffield, J.S. & Humphreys, B.D. The origin of interstitial myofibroblasts in chronic kidney disease. *Pediatr Nephrol*, In press (2011).
41. Quaggin, S.E. & Kapus, A. Scar wars: mapping the fate of epithelial-mesenchymal-myofibroblast transition. *Kidney In*, **80**, 41-50 (2011).
42. Iwano, M., *et al.* Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J Clin Invest* **110**, 341-350 (2002).
43. Li, L., Zepeda-Orozco, D., Black, R. & Lin, F. Autophagy is a component of epithelial cell fate in obstructive uropathy. *Am J Pathol* **176**, 1767-1778 (2010).
44. Liu, Y. Epithelial to mesenchymal transition in renal fibrogenesis: pathologic significance, molecular mechanism, and therapeutic intervention. *J Am Soc Nephrol* **15**, 1-12 (2004).
45. Hertig, A., *et al.* Early epithelial phenotypic changes predict graft fibrosis. *J Am Soc Nephrol* **19**, 1584-1591 (2008).
46. Herzog, E.L. & Bucala, R. Fibrocytes in health and disease. *Exp Hematol* **38**, 548-556 (2010).
47. Wada, T., *et al.* Involvement of bone-marrow-derived cells in kidney fibrosis. *Clin Exp Nephrol* **15**, 8-13 (2011).
48. Pilling, D., Fan, T., Huang, D., Kaul, B. & Gomer, R.H. Identification of markers that distinguish monocyte-derived fibrocytes from monocytes, macrophages, and fibroblasts. *PLoS One* **4**, e7475 (2009).

49. Niedermeier, M., *et al.* CD4+ T cells control the differentiation of Gr1+ monocytes into fibrocytes. *Proc Natl Acad Sci U S A* **106**, 17892-17897 (2009).
50. Roufosse, C., *et al.* Bone marrow-derived cells do not contribute significantly to collagen I synthesis in a murine model of renal fibrosis. *J Am Soc Nephrol* **17**, 775-782 (2006).
51. Yang, J., Dai, C. & Liu, Y. Hepatocyte growth factor suppresses renal interstitial myofibroblast activation and intercepts Smad signal transduction. *Am. J. Pathol.* **163**, 621-632 (2003).
52. Schnaper, H.W., *et al.* TGF-beta signal transduction in chronic kidney disease. *Front Biosci* **14**, 2448-2465 (2009).
53. Dai, C. & Liu, Y. Hepatocyte growth factor antagonizes the profibrotic action of TGF-beta1 in mesangial cells by stabilizing Smad transcriptional corepressor TGIF. *J Am Soc Nephrol* **15**, 1402-1412 (2004).
54. Liu, Y. Hepatocyte growth factor in kidney fibrosis: therapeutic potential and mechanisms of action. *Am J Physiol Renal Physiol* **287**, F7-F16 (2004).
55. Luo, D.D., Phillips, A. & Fraser, D. Bone morphogenetic protein-7 inhibits proximal tubular epithelial cell Smad3 signaling via increased SnoN expression. *Am J Pathol* **176**, 1139-1147 (2010).
56. Wang, B., *et al.* miR-200a Prevents renal fibrogenesis through repression of TGF-beta2 expression. *Diabetes* **60**, 280-287 (2011).
57. Kato, M., *et al.* A microRNA circuit mediates transforming growth factor-beta1 autoregulation in renal glomerular mesangial cells. *Kidney Int* **80**, 358-368 (2011).
58. Inui, M., Martello, G. & Piccolo, S. MicroRNA control of signal transduction. *Nat Rev Mol Cell Biol* **11**, 252-263 (2010).
59. Chung, A.C., Huang, X.R., Meng, X. & Lan, H.Y. miR-192 mediates TGF-beta/Smad3-driven renal fibrosis. *J Am Soc Nephrol* **21**, 1317-1325 (2010).
60. Zhou, Q., *et al.* TGF-{beta}-induced MiR-491-5p expression promotes Par-3 degradation in rat proximal tubular epithelial cells. *J Biol Chem* **285**, 40019-40027 (2010).
61. Eyden, B. Fibronexus junctions associated with in vivo human endothelium. *Ultrastruct Pathol* **33**, 28-32 (2009).
62. Legate, K.R., Montanez, E., Kudlacek, O. & Fassler, R. ILK, PINCH and parvin: the tIPP of integrin signalling. *Nat Rev Mol Cell Biol* **7**, 20-31 (2006).
63. Schroder, K. & Tschopp, J. The inflammasomes. *Cell* **140**, 821-832 (2010).

SAM Questions

1) There is no specific marker that is exclusively expressed in activated fibroblasts. But investigators often use all of the following to identify interstitial fibroblasts, except:

a. α-smooth muscle actin

b. Desmin

c. CD31

d. PDGFRβ

Correct answer: CD31. Fibroblasts do not express CD31 in any circumstances. CD31 is a marker for endothelial cells, although it is also found on platelets, macrophages, granulocytes, T / NK cells, lymphocytes, megakaryocytes, osteoclasts, neutrophils.

2) Several different sources have been proposed to contribute to (myo)fibroblast pool in diseased kidneys. During renal fibrogenesis, an activated fibroblast can, in theory, come from:

a. Interstitial fibroblasts and vascular pericytes

b. Tubular epithelial cells and endothelial cells

c. Circulating fibrocytes

d. All of them

Correct answer: all of them. Based on published literature, activated fibroblasts (myofibroblasts) can come from a wide variety of cells, including quiescent interstitial fibroblasts, vascular pericytes, tubular epithelial cells, endothelial cells and circulating fibrocytes.

3) All of the following statements regarding matrix production in renal fibrosis are true, except:

a. The expression of ECM proteins by activated fibrobleasts is primarily controlled at the gene transcriptional levels in response to various extracellular fibrogenic cues.

b. All growth factors such as TGF-β1, PDGF, CTGF, HGF and BMP-7 are fibrogenic and promote matrix production by fibroblasts.

c. Matrix genes are also regulated by a variety of microRNA

d. Integrins and its downstream ILK signaling are important for matrix production and deposition.

Correct answer: b. While TGF-β1, PDGF and CTGF are well known fibrogenic cytokines, HGF and BMP-7 have been demonstrated to be anti-fibrotic and inhibit matrix production.

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.[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. [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. [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.[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.[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.[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.[25] On the other hand, there is convincing growing evidence that tubular hypoxia precedes development of tubulointerstitial fibrosis. [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).[26-32] 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.[26] Studies of explants of native or transplant kidneys well documented reduced microvascular density.[27;29;30] 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.[31] 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. [33] 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. [34]

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 mm^2); 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 O_2 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 catergories 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

1. Herrera, G. A. Experimental models for renal diseases: pathogenesis and diagnosis. (169), 51-72. 2011. Basel, Karger. Contrib Nephrol. Ronco, C.
   Ref Type: Sound Recording

2. Zeisberg M, Neilson EG: Mechanisms of tubulointerstitial fibrosis. *J Am Soc Nephrol* 21:1819-1834, 2010

3. Mannon RB: Therapeutic targets in the treatment of allograft fibrosis. *Am J Transplant* 6:867-875, 2006

4. Nath KA: Tubulointerstitial changes as a major determinant in the progression of renal damage. *Am J Kidney Dis* 20:1-17, 1992

5. Einecke G, Sis B, Reeve J, Mengel M, Campbell PM, Hidalgo LG, Kaplan B, Halloran PF: Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant* 9:2520-2531, 2009

6. Cosio FG, Grande JP, Larson TS, Gloor JM, Velosa JA, Textor SC, Griffin MD, Stegall MD: Kidney allograft fibrosis and atrophy early after living donor transplantation. *Am J Transplant* 5:1130-1136, 2005

7. Brenner BD, 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* 309:652-659, 1982

8. Nangaku M: Mechanisms of tubulointerstitial injury in the kidney: final common pathways to end-stage renal failure. *Intern Med* 43:9-17, 2004

9. Liu Y: Cellular and molecular mechanisms of renal fibrosis. *Nat Rev Nephrol* 7:684-696, 2011

10. Lin SL, Kisseleva T, Brenner DA, Duffield JS: Pericytes and Perivascular Fibroblasts Are the Primary Source of Collagen-Producing Cells in Obstructive Fibrosis of the Kidney. *Am J Pathol* 173:1617-1627, 2008

11. Zeisberg EM, Potenta SE, Sugimoto H, Zeisberg M, Kalluri R: Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. *J Am Soc Nephrol* 19:2282-2287, 2008

12. Melk A, Halloran PF: Cell senescence and its implications for nephrology. *J Am Soc Nephrol* 12:385-393, 2001

13. Sis B, Tasanarong A, Khoshjou F, Dadras F, Solez K, Halloran PF: Accelerated expression of senescence associated cell cycle inhibitor p16(INK4A) in kidneys with glomerular disease. *Kidney Int* 71:218-226, 2007

14. 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

15. Kang DH, Joly AH, Oh SW, Hugo C, Kerjaschki D, Gordon KL, Mazzali M, Jefferson JA, Hughes J, Madsen KM, Schreiner GF, Johnson RJ: Impaired angiogenesis in the remnant kidney model: I. Potential role of vascular endothelial growth factor and thrombospondin-1. *J Am Soc Nephrol* 12:1434-1447, 2001

16. 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

17. Wong MG, Suzuki Y, Tanifuji C, Akiba H, Okumura K, Sugaya T, Yamamoto T, Horikoshi S, Tan SY, Pollock C, Tomino Y: Peritubular ischemia contributes more to tubular damage than proteinuria in immune-mediated glomerulonephritis. *J Am Soc Nephrol* 19:290-297, 2008

18. Ohashi R, Shimizu A, Masuda Y, Kitamura H, Ishizaki M, Sugisaki Y, Yamanaka N: Peritubular capillary regression during the progression of experimental obstructive nephropathy. *J Am Soc Nephrol* 13:1795-1805, 2002

19. 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

20. Basile DP, Donohoe D, Roethe K, Osborn JL: Renal ischemic injury results in permanent damage to peritubular capillaries and influences long-term function. *Am J Physiol Renal Physiol* 281:F887-F899, 2001

21. Horbelt M, Lee SY, Mang HE, Knipe NL, Sado Y, Kribben A, Sutton TA: Acute and chronic microvascular alterations in a mouse model of ischemic acute kidney injury. *Am J Physiol Renal Physiol* 293:F688-F695, 2007

22. Thomas SE, Anderson S, Gordon KL, Oyama TT, Shankland SJ, Johnson RJ: Tubulointerstitial disease in aging: evidence for underlying peritubular capillary damage, a potential role in renal ischemia. *J Am Soc Nephrol* 9:231-242, 1998

23. Kang DH, Anderson S, Kim YG, Mazzalli M, Suga S, Jefferson JA, Gordon KL, Oyama TT, Hughes J, Hugo C, Kerjaschki D, Schreiner GF, Johnson RJ: Impaired angiogenesis in the aging kidney: vascular endothelial growth factor and thrombospondin-1 in renal disease. *Am J Kidney Dis* 37:601-611, 2001

24. Shimizu A, Yamada K, Sachs DH, Colvin RB: Persistent rejection of peritubular capillaries and tubules is associated with progressive interstitial fibrosis. *Kidney Int* 61:1867-1879, 2002

25. Pillebout E, Burtin M, Yuan HT, Briand P, Woolf AS, Friedlander G, Terzi F: Proliferation and remodeling of the peritubular microcirculation after nephron reduction: association with the progression of renal lesions. *Am J Pathol* 159:547-560, 2001

26. Bohle A, kensen-Haen S, Wehrmann M: Significance of postglomerular capillaries in the pathogenesis of chronic renal failure. *Kidney Blood Press Res* 19:191-195, 1996

27. Bishop GA, Waugh JA, Landers DV, Krensky AM, Hall BM: Microvascular destruction in renal transplant rejection. *Transplant* 48:408-414, 1989

28. 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

29. Kaukinen A, Lautenschlager I, Helin H, Karikoski R, Jalanko H: Peritubular capillaries are rarefied in congenital nephrotic syndrome of the Finnish type. *Kidney Int* 75:1099-1108, 2009

30. Adair A, Mitchell DR, Kipari T, Qi F, Bellamy CO, Robertson F, Hughes J, Marson LP: Peritubular capillary rarefaction and lymphangiogenesis in chronic allograft failure. *Transplant* 83:1542-1550, 2007

31. Ishii Y, Sawada T, Kubota K, Fuchinoue S, Teraoka S, Shimizu A: Injury and progressive loss of peritubular capillaries in the development of chronic allograft nephropathy. *Kidney Int* 67:321-332, 2005

32. Lindenmeyer MT, Kretzler M, Boucherot A, Berra S, Yasuda Y, Henger A, Eichinger F, Gaiser S, Schmid H, Rastaldi MP, Schrier RW, Schlondorff D, Cohen CD: Interstitial vascular rarefaction and reduced VEGF-A expression in human diabetic nephropathy. *J Am Soc Nephrol* 18:1765-1776, 2007

33. Steegh FM, Gelens MA, Nieman FH, van Hooff JP, Cleutjens JP, Van Suylen RJ, Daemen MJ, van Heurn EL, Christiaans MH, Peutz-Kootstra CJ: Early loss of peritubular capillaries after kidney transplantation. *J Am Soc Nephrol* 22:1024-1029, 2011

34. Ozdemir BH, Ozdemir FN, Gungen Y, Haberal M: Role of macrophages and lymphocytes in the induction of neovascularization in renal allograft rejection. *Am J Kidney Dis* 39:347-353, 2002

35. Osasan S, de Freitas DG, Sellares J, Chang J, Ozluk Y, Hidalgo LG, Mengel M, Halloran PF, Sis B: Kidney Transplant Fibrosis Is Not Dependent on Peritubular Capillary Loss [Abstract]. *Am J Transplant* 11:54, 2011

36. Nishi T, Bond C, Jr., Brown G, Solez K, Heptinstall RH: A morphometric study of arterial intimal thickening in kidneys of dialyzed patients. *Am J Pathol* 95:597-610, 1979

37. Mengel M, Gwinner W, Schwarz A, Bajeski R, Franz I, Brocker V, Becker T, Neipp M, Klempnauer J, Haller H, Kreipe H: Infiltrates in protocol biopsies from renal allografts. *Am J Transplant* 7:356-365, 2007

38. Park WD, Griffin MD, Cornell LD, Cosio FG, Stegall MD: Fibrosis with inflammation at one year predicts transplant functional decline. *J Am Soc Nephrol* 21:1987-1997, 2010

Inflammation and Fibrosis – Interactions and Impact on the Kidney

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Introduction

Chronic kidney disease (CKD) is characterized by progressive interstitial fibrosis, tubular atrophy and glomerulosclerosis. The mechanisms of fibrosis are complex, and may involve excess synthesis of collagen with decreased degradation, in association with parenchymal injury and loss of functional tubules and glomeruli. The extracellular matrix (ECM) that makes up the fibrotic kidney has numerous sources, but in particular is contributed to by activated myofibroblasts. Regardless of the type of CKD, inflammatory cells are associated with the interstitial fibrosis. These inflammatory cells are diverse, including macrophages, various T-cells, dendritic cells, plasma cells and even granulocytes in more acute phases of injury. The impact and interaction of these cells with the parenchyma and effect on fibrosis are complex and stage specific.

Monocyte/macrophage subsets

Similar to evolution of our understanding of the complex various subsets of lymphocytes over the last many decades, monocyte/macrophages are now recognized to consist of unique subsets with varying cell surface markers, chemokine receptors and roles in injury and repair vs. fibrosis. Cells with varying expression of the cell surface marker Ly6C show functionally important differences in receptor expression of key chemokines. Monocytes that express Ly6C show high levels of the chemokine receptor CCR2 but low levels of CXCR1 ($CCR2^{high}$ $CXCR1^{low}$). In contrast, monocytes without Ly6C on their surface have low levels of CCR2 and high levels of CXCR1. These $CCR2^{high}$ $CXCR1^{low}$ monocytes are the typical cells that infiltrate tissue and are implicated in disease processes and have been termed inflammatory or M1 macrophages. The Ly6C negative cells (Ly6C-$CCR2^{low}CXCR1^{high}$ cells) are also termed resident monocytes and may replenish tissue macrophages and dendritic cells. Tissue macrophages also have discrete phenotypes. Classically activated macrophages, M1, and alternatively activated macrophages, M2, were initially distinguished by their responses in vitro to interferon γ (IFN-γ) and lipopolysaccharide for an M1 phenotype, with an M2 phenotype observed after treatment with interleukin (IL)-4 or IL-13. M1 macrophages are proinflammatory and secrete TNF-α, IL-1β, reactive oxygen species and nitric oxide. In contrast, the M2 macrophages are anti-inflammatory and secrete IL-6 and insulin-like growth factor 1. M2 macrophages may promote tissue healing and angiogenesis. Further subcategories of these inflammatory cells are evolving based on in vivo responses.

Various theories for the origin(s) of these subtypes have been put forth, including the Hume hypothesis of one macrophage population that may assume infinite phenotypes, vs. the Geissman hypothesis, postulating that each subpopulation has its own unique precursor, and a third model where sequential subpopulations evolve based on tissue environment and activating stimuli. More recent evidence suggests that in various settings, there is evidence to support each of these possibilities. Examination of

the impact of macrophages on parenchymal cells and ultimate response to injury are beginning to emerge from experimental models. Examination of specific activation state and subset of macrophages in human tissue in vivo is still limited. However, overall glomerular or interstitial macrophage number correlates with worse outcome in various diseases, including fibrosis and tubular atrophy. More recent studies suggest that this link may be modulated and macrophage phenotype altered with a beneficial impact on outcome.

Activation of macrophages

When activated by various stimuli, including pathogen associated molecular patterns (PAMPs), which activate macrophages through Toll-like receptors (TLRs) and other receptors. Signaling is activated through NF kappa-B and MAP kinase, and proinflammatory cytokines are produced by macrophages include TNFα, interleukin IL-1 β, IL-12, IL-12, IL-13, and IL-16. Further inflammation and influx of other inflammatory cells is enhanced by macrophage production of chemokines including MIP-1, MIP-2, and monocyte chemotactic protein. Increased reactive oxygen species generated from activated macrophages also may contribute to injury. These activated macrophages expressed Mac2 (galactin-3) in tissue, along with NOS2 or IL-1 β. In contrast to these injurious activation patterns, macrophages also can elaborate vascular endothelial growth factor (VEGEF), transforming growth factor-β (TGF-β), IL-10, angiopoietin 1, hepatocyte growth factor, fibroblast growth factor 2, which together could promote angiogenesis, wound healing and anti-inflammatory responses. Macrophages may also scavenge fragments of injured cells and abnormal matrix and thus promote tissue remodeling and regeneration following injury. In injury settings where there are no immune complexes or external pathogens, macrophages may be activated by release of factors from injured cells, which could serve as ligands for activating receptors on the monocytes. These danger-associated molecular patterns (DAMPs) are factors that may bind to Toll-like receptors and also promote macrophage activation. DAMPs include advanced glycation end products, HMGB1, adenosine and others.

Studies now indicate that macrophage polarization also exists in vivo, and not just in the artifactual in vitro situation. In addition to M1 and M2 macrophages, a regulatory macrophage appears in response to the anti-inflammatory cytokine IL-10. Serum amyloid P, also known as pentraxin-2, or apoptotic cells and immune complexes may in specific contexts result in a macrophage subpopulation that generates increased IL10 and thus suppresses further immune responses. These regulatory macrophages therefore may dampen ongoing response of other infiltrating cells.

Macrophages, angiotensin and effects on tissue injury

The consequence of macrophages on tissue injury has been studied in various ways, with depletion of macrophages by transgenic models where these cells expressed diphtheria toxin on their surface, or with bone marrow transplant experiments, or with chemical ablation. The renin angiotensin system is a major activator of progressive kidney disease, and antagonism and/or inhibition of its actions are the mainstay of treatment of CKD. We therefore investigated the potential role of the angiotensin type-1 receptor (AT1R) on macrophages in promoting fibrosis. To this end, we generated chimeric mice that lacked the AT1a subtype receptor only on

macrophages, by performing bone marrow transplant with AT1a deficient marrow after lethal radiation. We then exposed the reconstituted chimeric mice to injury that induced progressive tubulointerstitial fibrosis, namely unilateral ureteral obstruction (UUO). In mice with intact AT1 receptor on parenchymal cells, but with bone marrow-derived cells with absence of AT1, there was surprisingly increased interstitial fibrosis, although macrophage infiltration was decreased. These AT1 deficient macrophages showed defective phagocytosis, and impaired migratory capacity ex vivo. We postulated that a defective phagocytic scavenging by the AT1 receptor deficient macrophages could contribute to a profibrotic phenotype.

Macrophages, TGF-β and fibrosis

When macrophages infiltrate tissue, tissue signaling interaction pathways must be intact for fibrosis to be effected. In addition, numerous therapies that decrease fibrosis, including ARBs, PPAR-γ agonists, decrease fibrosis and macrophage infiltration in parallel. Of note, inhibition of TGF-β may have complex actions. In addition to promoting macrophage infiltration and matrix synthesis, TGF-β has important immune modulatory effects. We found that inhibition of TGF-β with a pan-antibody decreased fibrosis at low does, but higher doses were ineffective, linked to higher levels of infiltrating macrophages.

TGF-β circulates in latent form. Activation of TGF-β by cleaving it from latency-associated peptide can be mediated by e.g. thrombospondin-1 and the integrin α_V β6. α_V β6 integrin is expressed in epithelium in the lung, the kidney and the skin. β6 deficient mice were protected from lung fibrosis and interstitial fibrosis induced by UUO. Of interest, infiltrating macrophages were even more abundant in β6 knockout mice, but no increase in collagen content was observed after UUO, and TGF-β was not activated by UUO in these mice. However, activation of other pathways by adding exogenous angiotensin II, could restore fibrosis but through a non-TGF-β-dependent pathway, which we linked to increased plasminogen activator inhibitor-1 (PAI-1) and thymosin β4. Thymosin β4 is a G-actin sequestering protein with numerous effects on cell migration, angiogenesis, and activates PAI-1 and TGF-β. We showed that thymosin β4 is necessary for angiotensin to induce PAI-1 in endothelial cells. PAI-1 could influence fibrosis in part by effects on cell migration through vitronectin, and in part by inhibiting plasmin-dependent proteolysis of accumulated ECM proteins. Thymosin β4 promotes wound healing in skin and corneal wounds, by promoting cell migration and ECM and angiogenesis. In early stages after UUO, thymosin β4 increased injury, but at later stages thymosin β4 was protective. Our most recent data with conditional knockout of thymosin β4 in macrophages supports that expression of thymosin β4 may influence polarization of macrophages.

Modulating macrophages to decrease fibrosis

Additional recent exciting studies from the laboratory of Duffield shows that treatment with exogenous pentraxin-2 (serum amyloid P) also is anti-fibrotic in the UUO model. The postulated mechanism may be opsonization of cell debris with serum amyloid P, and interaction with activated Fcγ receptors on macrophages, resulting in an anti-inflammatory IL10 expression. These and other studies have led to the theoretical consideration of yet another macrophage population, a potentially fibrolytic macrophage.

In additional studies, mice were genetically manipulated to express the diphtheria toxin only on macrophages, under the CD11b promoter. Injection of the toxin then resulted in specific monocyte/macrophage depletion. Ablation of monocyte/macrophages at day 4 through 6 after UUO resulted in decreased fibrosis. Similar results were seen when macrophage ablation was induced at a later stage after UUO from day 7 to 10. Fibrosis could be restored by adoptive transfer of monocytes from normal mice. Interestingly, when resident renal macrophages were depleted there was no effect on fibrosis- only depletion of circulating monocytes and recruited macrophages blunted fibrosis. In the nephrotoxic serum nephritis model, macrophage ablation also decreased injury, with decreased interstitial myofibroblasts and fibrosis. Of note, depletion of macrophages from mice with skin wounds resulted in decreased ECM deposition and cell proliferation and delayed healing. These mechanisms were tied to interference with macrophage production of PDGF, which in turn led to decreased activation of fibroblasts and fibroblast osteopontin expression. A balance of macrophage effects therefore appears to be important in early wound healing.

A key step in macrophage modulation of fibrosis is modulation of monocyte recruitment to become tissue macrophages. The monocyte chemoattractant protein (MCP-1, or CCL2) and its receptor CCR2, are key for activation and chemotaxis. In mice genetically deficient in CCL2, macrophage infiltration was decreased and fibrosis was diminished in models of UUO and early diabetic injury. However, the specific phenotypes of resulting macrophages were not explored. Similarly, CCR2 receptor inhibitors ameliorated renal fibrosis. Interestingly, blockade of CCR1, which is present on M2 macrophages, decreased macrophages and fibrosis in the Alport mouse model, but CCR2 blockade was ineffective, while CCR1 blockade was effective in db/db mice, adriamycin nephropathy and UUO.

In addition to experimental maneuvers to deplete macrophages, infusion of modified macrophages has been examined for potential therapeutic impact on fibrosis. The impact of modulating macrophages will likely vary depending upon whether it is initiated in the injury or healing phase. When macrophages were transferred to mice at a late stage after UUO, there was attenuation of fibrosis. Manipulation of specific cell populations will influence the responsiveness. For instance, depletion of dendritic cells selectively did not affect renal fibrosis, while depletion of all monocyte lineage cells could have this beneficial effect in the UUO model. Specific injection of M2 macrophages primed with IL-4 and IL-13 ameliorated adriamycin nephropathy and reduced endogenous macrophage infiltration, whereas injection of M1 macrophages worsened injury and increased inflammatory gene expression, and unmodified macrophages did not alter the disease course.

In addition to promoting or regulating angiogenesis and myofibroblast production of matrix, macrophages have effects on parenchymal cells. Macrophages can promote activation to cell cycle entry and death in injured parenchymal cells. Entry of injured cells into cell cycle may lead to their death by pausing at DNA checkpoints and thus promoting apoptosis whereas cells with intact functionality will be able to continue through cell cycle without cell death.

Galactin-3 macrophage expression was necessary for them to transduce fibrosis. Interestingly, the kidney parenchyma itself may be important in mediating the renal

accumulation of specific macrophage phenotypes. The transcription factor Krüppel-like factor-5 (KLF-5) is expressed mostly in collecting duct epithelial cells, and haplo-insufficient mice (KLF-5 +/-) had less injury after UUO than wild type, with decreased macrophage infiltration of the M1 phenotype, and increased accumulation of M2. KLF-5, in concert with C/EBPα, mediated expression of chemotactic proteins S100a8 and S100a9, resulting in recruitment of inflammatory monocytes to the kidney and their activation into M1 type macrophages. This collecting duct-derived transcription factor thus played a major role in influencing the phenotype and consequences of inflammatory cell infiltration.

Other parenchymal receptors also contribute to tissue inflammation. Discoidin domain receptor-1 (DDR-1) is a tyrosine kinase transmembrane receptor for collagen constitutively expressed in several organs including the kidney, where it is found predominantly on vascular smooth muscle cells, mesangial cells and epithelial cells. DDR-1 is activated by binding to collagens I to VI and VIII and regulates cell differentiation, adhesion, proliferation and ECM remodeling. It is upregulated after UUO. DDR-1 deficient mice had decreased collagen and TGF-β and remarkable decrease in macrophages, but no shift in M1/2 polarization.

Summary

In summary, macrophages show diverse phenotypes that are context dependent. The ultimate effect on response to injury depends upon balance of macrophage phenotypes and their persistence or resolution, and whether injury and inflammatory stimuli persist or resolve. Inflammatory macrophages may play beneficial roles in response to transient inflammation with induction of macrophages that can suppress further inflammatory response and promote repair. Later in wound healing, profibrotic macrophages promote fibroblast proliferation and fibrogenesis with elaboration of TGF-β. Macrophages may also promote clearance of ECM, through the postulated fibrolytic macrophage phenotype. Anti-inflammatory M2 macrophages may promote tubular repair.

References:

Steinman RM and Moberg CL: Zanvil Alexander Cohn 1926-1993. J Exp Med 179:1-30, 1994

Ma LJ, Marcantoni C, Linton MF, Fazio S, Fogo AB: Peroxisome proliferator-activated receptor-gamma agonist troglitazone protects against nondiabetic glomerulosclerosis in rats. Kidney Int 59:1899-1910, 2001

Anders HJ, Vielhauer V, Frink M, Linde Y, Cohen CD, Blattner SM, Kretzler M, Strutz F, Mack M, Gröne HJ, Onuffer J, Horuk R, Nelson PJ, Schlöndorff D: A chemokine receptor CCR-1 antagonist reduces renal fibrosis after unilateral ureter ligation. J Clin Invest 109:251-259, 2002

Nishida M, Fujinaka H, Matsusaka T, Price J, Kon V, Fogo AB, Davidson JM, Linton MF, Fazio S, Homma T, Yoshida H, Ichikawa I: Absence of angiotensin II type 1 receptor in bone marrow-derived cells is detrimental in the evolution of renal fibrosis." J Clin Invest 110:1859-1868, 2002

Ma LJ, Yang H, Gaspert A, Carlesso G, Barty MM, Davidson JM, Sheppard D, Fogo AB: Transforming growth factor-beta-dependent and -independent pathways of induction of tubulointerstitial fibrosis in beta6(-/-) mice. Am J Pathol 163:1261-1273, 2003

Ma LJ, Jha S, Ling H, Pozzi A, Ledbetter S, Fogo AB: Divergent effects of low versus high dose anti-TGF-beta antibody in puromycin aminonucleoside nephropathy in rats. Kidney Int 65:106-115, 2004

Xu BJ, Shyr Y, Liang X, Ma LJ, Donnert EM, Roberts JD, Zhang X, Kon V, Brown NJ, Caprioli RM, Fogo AB: Proteomic patterns and prediction of glomerulosclerosis and its mechanisms. J Am Soc Nephrol 16: 2967-75, 2005

Nishida M, Okumura Y, Fujimoto S, Shiraishi I, Itoi T, Hamaoka K: Adoptive transfer of macrophages ameliorates renal fibrosis in mice. Biochem Biophys Res Commun 332:11-16, 2005

Wang Y, Wang YP, Zheng G, Lee VWS, Ouyang L, Chang DHH, et al: Ex vivo programmed macrophages ameliorate experimental chronic inflammatory renal disease. Kidney Int 72:290-299, 2007

Henderson NC, Mackinnon AC, Farnworth SL, Kipari T, Haslett, C, Iredale JP, Fu-Tong L, Hughes J and Sethi T: Galectin-3 expression and secretion links macrophages to the promotion of renal fibrosis. Am J Pathol 172:288-298, 2008

Ferenbach D and Kluth D: Macrophage cell therapy in renal disease. Semin Nephrol 30: 345-353, 2010

Rees AJ: Monocyte and macrophage biology: an overview. Semin Nephrol 30:216-233, 2010

Vielhauer V, Kulkarni O, Reichel CA, Anders HJ: Targeting the recruitment of monocytes and macrophages in renal disease. Semin Nephrol 30:318-333, 2010

Machida Y, Kitamoto K, Izumi Y, Shiota M, Uchida J, Kira Y, Nakatani T, Miura K: Renal fibrosis in murine obstructive nephropathy is attenuated by depletion of monocyte lineage, not dendritic cells. J Pharmacol Sci 114:464-473, 2010

Vernon MA, Mylonas KJ, Hughes J: Macrophages and renal fibrosis." Semin Nephrol 30:302-317, 2010

Duffield, JS: Macrophages and immunologic inflammation of the kidney. Semin Nephrol 30:234-254, 2010

Anders HJ and Ryu M: Renal microenvironments and macrophage phenotypes determine progression or resolution of renal inflammation and fibrosis. Kidney Int 80:915-925, 2011

Conway B and Hughes J: Cellular orchestrators of renal fibrosis. QJM 2011 (epub head of print)

Guerrot D, Kerroch M, Placier A, Vandermeersch S, Trivin C, Mael-Ainin M, Chatziantoniou C and Dussaule, JC: Discoidin domain receptor 1 is a major mediator of inflammation and fibrosis in obstructive nephropathy. Am J Pathol 179: 83-91, 2011

Fujiu K, Manabe I, Nagai R: Renal collecting duct epithelial cells regulate inflammation in tubulointerstitial damage in mice. J Clin Invest 121: 3425-3441, 2011
Anders HJ and Ryu M: Renal microenvironments and macrophage phenotypes determine progression or resolution of renal inflammation and fibrosis. Kidney Int 80:915-925, 2011

Snelgrove SL, Kausman JY, Lo C, Lo C, Ooi JD, Coates PT, Hickey MJ, Holdsworth SR, Kurts C, Engel DR, Kitching AR: Renal dendritic cells adopt a pro-inflammatory phenotype in obstructive uropathy to activate T cells but do not directly contribute to fibrosis. Am J Pathol 180:91-103, 2012