Key fibrogenic mediators: old players. Renin–angiotensin system

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Interstitial fibrosis represents the final common pathway of any form of progressive renal disease. The severity of tubular interstitial damage is highly correlated to the degree of decline of renal function, even better than the glomerular lesions do. Angiotensin II (Ang II), the main effector of the renin–angiotensin system, is a critical promoter of fibrogenesis. It represents a nexus among glomerular capillary hypertension, barrier dysfunction, and renal tubular injury caused by abnormally filtered proteins. Transforming growth factor (TGF)-β1 and reactive oxygen species (ROS) are the key mediators of the pro-fibrotic effect of Ang II causing apoptosis and epithelial-to-mesenchymal transition of the renal tubular epithelium. Recent studies link fibrosis to changes of microRNA (miRNA) modulated by Ang II through TGF-β1, unraveling that antifibrotic action of Ang II antagonism is attributable to epigenetic control of fibrosis-associated genes. Other mechanisms of Ang II-induced fibrosis include ROS-dependent activation of hypoxia-inducible factor-1. Finally, Ang II via angiotensin type 1 receptor regulates the activation and transdifferentiation of pericytes and fibrocytes into scar-forming myofibroblasts. Detachment and phenotypic changes of the former can lead to the loss of peritubular capillaries and also contribute to hypoxia-dependent fibrosis.

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Independently of the primary insult, progression of chronic kidney disease (CKD) to end-stage renal failure is the common outcome. The rate of decline of renal function varies among nephropathies and for the same disease in different individuals[1] '…the functional disturbances known to occur in human renal failure are precisely those that occur in animal experiment as a result of reduction in the amount of functioning renal substance—that is, loss of nephron.' (Robert Platt, Lumleian Lecture to the Royal College of Physicians, London 1952).[2] After 30 years of Platt's observation, Brenner and colleagues, while deeper investigating the structural and functional renal adaptation to nephron loss in the model of renal ablation in the rat,[3] identified glomerular capillary hypertension as a leading cause of progressive deterioration of remaining nephrons. Mathematical modeling of the size-selectivity function of the glomerular membrane suggested that elevated transcapillary hydraulic pressure increased the population of large unselective pores perforating the glomerular membrane by a mechanism at least partly mediated by angiotensin II (Ang II).[4] Mechanical strain, as results of glomerular hypertension, upregulates angiotensin type 1 receptor (AT1R) and increases the production of Ang II in cultured podocytes.[5] Loss of podocyte–podocyte contact and increased albumin permeability induced by Ang II in these cells[6] translate in the glomerular sieving dysfunction observed in proteinuric nephropathies. The proteinuric ultrafiltrate represents the way for spreading the disease from the glomerulus to the tubulointerstitial compartment.

ANG II-INDUCED PROTEINURIA AND INTERSTITIAL FIBROSIS

Ang II-induced proteinuria is a leading cause of CKD progression. Proteins abnormally filtered through the glomerular capillary have intrinsic toxicity on the proximal tubule contributing to the development of interstitial inflammation, fibrosis, and ultimately to kidney dysfunction.

Proximal tubular cells exposed *in vitro* to albumin undergo apoptosis through a mechanism involving reduced expression of its receptor megalin. Furthermore, overload of plasma proteins stimulates proximal tubular cells to synthesize and release pro-inflammatory substances including Monocyte Chemoattractant Protein-1 (MCP-1/CCL2), Regulated upon Activation, Normal T-cell Expressed and Secreted (RANTES/CCL5), fractalkine/CX3CL1 that are

potent chemoattractants for monocytes/macrophages, and T lymphocytes.

Changes observed *in vitro* parallel those obtained in proteinuric models *in vivo*. They showed apoptosis-induced glomerular–tubule disconnection and inflammation via release of cytokine including MCP-1 from activated proximal tubular cells.[7] A similar inflammatory pathway occurs in patients with severe proteinuria in whom renal upregulation of MCP-1, RANTES, and osteopontin, mainly in tubular epithelial cells, correlates with the severity of the disease.[8,9] Both injured tubules and inflammatory cells enhance fibrogenesis by activating interstitial fibroblasts via a paracrine mechanism that involves the release of transforming growth factor (TGF)-β1, the most potent inducer of epithelial-to-mesenchymal transition (EMT). Albumin induces the upregulation of TGF-β receptor type II expression in proximal tubular cells and makes them more susceptible to the matrix-stimulatory actions of TGF-β. Accumulation of extracellular matrix (ECM) proteins by proximal tubular cells occurs concomitantly with the induction of tissue inhibitors of metalloproteinases, (TIMP)-1 and TIMP-2, in response to albumin.[7] Filtered C3 is also recognized as a major promoter of injury in proteinuric nephropathies as highlighted by cross-transplantation experiments employing C3-deficient mice and wild-type littermates. Mechanism of injury induced by C3 is attributed to the formation of the C5b-9 membrane attack complex.[10]

Dendritic cells, the main professional antigen-presenting cell population of the kidney, accumulate in the renal parenchyma in proteinuric nephropathies in the vicinity of proximal tubular cells. Epithelial–dendritic cell interaction leads to albumin processing into antigenic peptides that activate immune cells. Overall proteinuric state and inflammatory environment enable dendritic cells to become immunogenic towards normally ignored self-antigens providing a link among proteinuria, autoimmunity, and renal disease progression.[11]

Targeting proteinuria by angiotensin-converting enzyme (ACE) inhibitors alone or as combined therapy prevents glomerular–tubule disconnection and atrophy and ameliorates interstitial inflammation and fibrosis.[12]

ANG II-INDUCED INTERSTITIAL FIBROSIS IS MEDIATED BY TGF-β

Besides protein load, Ang II directly stimulates the TGF-β1 gene, protein expression in proximal tubular cells,[13] and triggers the production of plasminogen activator inhibitor-1 (PAI-1), ECM synthesis and deposition in the interstitial space.[14,15] TGF-β1 signaling includes Smad and non-Smad pathways for the activation of its major effector and downstream target PAI-1. Kindlin-2, a β-integrin adaptor protein, has been recently discovered to work as a TGF-β type I receptor/Smad3 adapter protein that amplifies profibrogenic effects of TGF-β1 through Smad3 signaling in tubular cells *in vitro* and *in vivo*[16] (Figure 1).

The non-Smad pathway involves reactive oxygen species (ROS)-dependent-c-Src-mediated activation of epidermal growth factor receptor and downstream signaling cascade.[17] ROS also maintain receptor-activated Smads in a phoshorylated state and are crucial for p53 activation that interacts with Smads and transcriptional cofactors forming transcriptionally active multiprotein complexes important for maximal PAI-1 induction[17] (Figure 1). Smad and non-Smad signal integration contributes to renal fibrosis in unilateral ureteral obstruction (UUO) and in diabetic nephropathy.[18]

Control of ECM accumulation through the inhibition of TGF-β1/PAI-1 has been proposed as the underlying mechanism of the therapeutic effect of Ang II blockade in several models of progressive nephropathies.[19] In Munich Wistar Fromter (MWF) rats with advanced nephropathy, ACE inhibition induces regression of glomerular lesions and prevents worsening of interstitial changes through significant reduction of TGF-β1 expression.[20] Moreover, add-on anti-TGF-β antibody to ACE inhibitor attenuates interstitial inflammation and accumulation of type III collagen and abrogates tubular damage in rats with overt diabetic nephropathy.[21] Such studies paved the way for the use of fresolimumab, a human monoclonal antibody, which neutralizes the mammalian isoforms of TGF-β, in patients with primary focal segmental glomerulosclerosis.[22]

CROSS-TALK BETWEEN ANG II AND TGF-β IN RENAL FIBROSIS: MICRORNAS, POTENTIAL TARGETS OF THE ANTIFIBROTIC EFFECT OF ANG II ANTAGONISM

MicroRNAs (miRNA) are a class of short, single-stranded noncoding RNAs of ~20–22 nucleotides in length that act as post-transcriptional repressors. Tubulointerstitial fibrosis has been recently linked to the loss or activation of those miRNA. In this context, we have recently discovered miR-324-3p as a new mediator of renal fibrosis.[23] MiR-324-3p, identified as the most highly expressed miRNA in microdissected glomeruli from MWF rats with advanced nephropathy, localizes to the glomerulus and, most abundantly, to cortical tubules. The downstream target of miR-324-3p is prolyl endopeptidase (Prep), also known as prolyl oligopeptidase, a serine protease that is involved in the metabolism of Ang-I/Ang II into Ang-(1–7) and in the synthesis of the antifibrotic peptide N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP). Transfection of tubular epithelial cells with miR-324-3p results in downregulation of Prep expression and activity and increases susceptibility to developing pro-fibrotic phenotype in response to TGF-β1, suggesting a feedback loop sustaining fibrosis (Figure 1). In MWF rats, overexpression of miR-324-3p is associated with reduced expression of Prep in both glomeruli and tubular epithelium in fibrotic areas. Unbalance between miR-324-3p and Prep is normalized by ACE inhibitor that also increases urine and plasma Ac-SDKP levels, hence attenuating tubulointerstitial fibrosis. These data underline how dysregulation of the miR-324-3p and its target Prep greatly limits the activation of the Ac-SDKP antifibrotic pathway in the kidney, sensitizing MWF rats to Ang II and TGF-β, which in turn favor progressive renal fibrosis. Ac-SDKP that is formed from its precursor, thymosin β4, by