Renal tubulointerstitial fibrosis: common but never simple

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Hewitson TD. Renal tubulointerstitial fibrosis: common but never simple. Am J Physiol Renal Physiol 296: F1239–F1244, 2009. First published January 14, 2009; doi:10.1152/ajprenal.90521.2008.—Regardless of etiology, all patients with chronic renal disease show a progressive decline in renal function with time. Fibrosis, so-called scarring, is a key cause of this pathophysiology. Fibrosis involves an excess accumulation of extracellular matrix (primarily composed of collagen) and usually results in loss of function when normal tissue is replaced with scar tissue. While recent major advances have led to a much better understanding of this process, many problems remain. We for instance know little about why some wounds heal and others scar and little about how many putative antifibrotic agents work. This review discusses recent advances in our understanding of the mechanisms of tubulointerstitial fibrosis, focusing on the regulation and role of the myofibroblast in this process, the role of recently recognized endogenous antifibrotic factors, controversy surrounding the effects of metalloproteinases, and the opportunities presented by new treatment strategies that abrogate and may even reverse fibrosis.

extracellular matrix; fibroblast; fibrosis; kidney; myofibroblast

THE KIDNEY’S RESPONSE TO INJURY resembles the generalized wound healing response that occurs elsewhere. The intention of wound healing is the restoration of architecture and the recovery of function. However, in the adult, this is only ever partially successful. For reasons that are poorly understood, complete tissue regeneration, so-called scarless healing, only ever occurs in the fetus (48). Adult wound repair is variable, complicated by impaired healing in diseases such as diabetes and, more commonly, the accumulation of scar tissue (fibrosis or sclerosis). Why then do some wounds heal and others fibrose? The answer is complex, but a number of intrinsic and extrinsic factors are likely to influence outcomes.

Fibrotic disorders are commonplace, take many forms, and can be life-threatening. Fibrosis involves an excess accumulation of extracellular matrix (ECM) (primarily composed of collagen) and usually results in loss of function when normal tissue is replaced with scar tissue (65). No better example of this exists than the progressive fibrosis that accompanies all chronic renal disease. Regardless of etiology, all patients with chronic renal disease show a progressive decline in renal function with time. The process is largely irreversible, inevitably leading to end-stage renal failure, a condition that requires life-long dialysis or renal transplantation.

Pathogenesis of Fibrosis

Histologically end-stage renal disease manifests itself as glomerulosclerosis, vascular sclerosis, and tubulointerstitial fibrosis, with tubulointerstitial fibrosis having consistently been shown to be the best histological predictor of progression (5). We should not really be surprised by this; the tubulointerstitium comprises ~90% of the volume of the kidney. Nevertheless, a renewed interest in the biology of the tubulointerstitium has led to some significant findings that have advanced our understanding of both the pathophysiology of tubulointerstitial disease and mechanisms of renal disease in general.

This review addresses the following four issues: how fibrosis develops, the cellular basis of this process, how the function of these cells is regulated, and finally, what therapeutic strategies are being explored.

How Does Fibrosis Develop?

We perhaps most commonly associate scarring with an excess synthesis of ECM (Fig. 1), usually collagen, in response to sustained inflammation after injury. Although matrix synthesis is of course part of the normal repair process, excessive synthesis of ECM is itself deleterious, further exacerbating injury in a vicious cycle (13).

Keloids represent the best example of scarring that results from aberrant matrix synthesis (53). Notwithstanding genetic factors, keloids result from the uncontrolled matrix synthesis by dermal fibroblasts (20). There are certainly renal parallels of this process, such as the focal scarring that accompanies a localized tissue trauma.

What does now seem clear is that scarring is often a multifactorial process, with aberrant matrix synthesis only part of the process. Our temporal studies in experimental renal infection indicate that aberrant collagen synthesis is often transient, peaking in the first few days after infection (25). Histologically however, scarring, as defined by increasing matrix density, continues to increase (25).

How can we account for this discrepancy? Although it has long been known that end-stage kidneys are smaller than their...
unscarred counterparts (13), it is the focal lesions found in diseases such as reflux nephropathy that provide us with a clue. The irregular surface of these kidneys indicates underlying scar tissue, highlighting the fibrocontractive nature of renal scarring. In what we term the "balloon" hypothesis, fibrosis is due not only to an increase in matrix synthesis but also to the collapse of the renal parenchyma (Fig. 1). Analogous to deflating a balloon, we are effectively measuring the same amount of ECM in a smaller volume.

Once again, there are good nonrenal examples of this process. Wound contraction has long been recognized as an integral part of skin wound healing, with the drawing together of wound edges an important part of wound closure (1). The observation that a decrease in renal size parallels loss of renal function (17) provides indirect evidence of this process in the kidney. More direct evidence comes from examining the histology in experimental renal infection and scarring. Being a primary tubulointerstitial fibrosis, increasing density (small arrows) of glomeruli (solid circles) is indicative of collapse fibrosis.

Remodeling. Posttranslational modifications of fibrillar collagen increase its rigidity and strength. Before these processes are complete, newly synthesized collagen is particularly prone to remodeling through contraction and proteolysis. Dogma suggests that the extent of fibrosis is a balance between net collagen synthesis and degradation and that a reduction in protease activity can result in fibrosis. Increasingly, we recognize that this is an oversimplification (54). Although the first is probably true, the second is much harder to accept.

Most collagen degradation in the kidney is under the control of matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases. Collectively, they are capable of degrading all ECM proteins. However, Ronco et al. (54) highlight a number of problems in the dogma. Only collagen IV (69) and aggrecan (19) have been shown to be cleaved in vivo. Other than membrane type 1 MMP-deficient mice (29), MMP knockout mice do not have a fibrotic phenotype (54), and overexpression of MMP-2 induces fibrosis (10). In addition, MMPs have many nonmatrix substrates (reviewed in Ref. 8).

A more realistic view recognizes that MMPs have antifibrotic and profibrotic roles. The ability to remodel collagen is an important counterbalance to upregulated synthesis. Conversely, the pathogenic role of MMPs is underscored by the importance of gelatinase (MMP-2 and -9)-mediated degradation of collagen IV in diseases such as crescentic glomerulonephritis (23) and Alport’s syndrome (52). Likewise, production of fibrillar MMPs (MMP-1 and -13) is requisite for cell migration through surrounding ECM (38).

Studies of MMP expression and activity in vivo reflect this controversy. Sustained MMP expression has consistently been seen in experimental models (44) and human renal disease (59). We are still unsure if this reflects compensatory mechanisms trying to limit matrix accumulation, or profibrotic activities.

What is the Cellular Basis of Fibrosis?

Sclerosis (or fibrosis) in all three settings is associated with the activities of a mesenchymal cell (4). The interstitial fibroblast, glomerular mesangial cell, and vascular smooth muscle cell are phenotypically similar, with the fibroblast (24) and mesangial cell (2) acquiring features of smooth muscle when activated.

In each form of renal scarring, not only is the mesenchymal cell the principal ECM-producing cell, but it also provides the force for contraction and reorganization of ECM, thereby increasing its density.

The renal interstitial fibroblast represents the quintessential example of this process and has been the focus of our studies of tubulointerstitial fibrosis. Recognized by its de novo synthesis of α-smooth muscle actin (αSMA), activated fibroblasts, so-called myofibroblasts, are a feature of all forms of progressive renal disease where their accumulation correlates strongly with disease progression (24).

Origin of the fibroblast. Fibroblasts, and by implication myofibroblasts, can be derived from a number of sources. These include migration from the perivascular region (64), local proliferation of a resident cell population (27), recruitment of blood-borne precursors (22), and transformation of both tubule epithelia and endothelial cells through epithelial-mesenchymal transition (EMT) (43) and endothelial-mesenchymal transition (EndMT) (68), respectively.
Much recent interest has focused on EMT. Long recognized in developmental biology, EMT facilitates the derivation of a multitude of functionally specialized cells, tissues, and organs in the developing embryo (37). Consistent with the recapitulation of developmental programs after tissue injury, epithelial cells acquire features of mesenchymal cells. During EMT, epithelial cells lose polarity and cell-cell contacts and undergo dramatic cytoskeletal remodeling. Concurrent with the loss of epithelial cell adhesion and cytoskeletal components, cells undergoing EMT acquire mesenchymal cell expression profiles and migratory phenotype.

Renal tubular EMT, by definition, is therefore a process in which renal tubular cells lose their epithelial phenotype and acquire features characteristic of fibroblasts (18, 43). Consistent with profibrotic activities of MMPs, degradation of tubular basement membranes is also key (10). The process of EMT has been found to be particularly significant in the pathogenesis of tubulointerstitial fibrosis that accompanies all progressive renal disease (43). Pioneering work from Strutz et al. (62) first indicated that, after injury, renal tubular epithelial cells acquire fibroblast-like features, consistent with epithelial-to-mesenchymal transition being an important source of cells in pathological kidneys. This was paralleled by the de novo expression of a novel protein they termed fibroblast specific protein-1 (FSP-1). MMP-2 (but not the closely related MMP-9) may exacerbate fibrosis by directly and indirectly promoting EMT. Degradation of tubular basement membranes both facilitates migration into interstitial spaces and more directly triggers EMT by creating bioactive collagen fragments (9). MMP-2 may also amplify EMT in a paracrine fashion by proteolytic activation of transforming growth factor (TGF)-β1, itself a potent stimulus for transformation (9). Overexpression of MMP-2 in mice results in pathological changes consistent with human chronic kidney disease (10).

Elegant renal experiments in a murine model of unilateral ureteric obstruction (UUO) have attempted to address the relative importance of the various potential sources of fibroblasts (32). Using bone marrow chimeras and transgenic reporter mice, the authors were able to estimate that EMT and circulating precursors contributed 38 and 9% of the FSP-1-positive cells, respectively (32), with many of the rest presumably derived from local proliferation (27) (Fig. 2). Expression of αSMA by tubular epithelium suggests that myofibroblast can be derived directly from tubules, as well as via prior transition into fibroblasts. The process is not confined to specific segments of the nephron; EMT has been described in both proximal tubules (32) and medullary collecting ducts (7).

Clearly, much of the work on EMT has used expression of FSP-1 as a marker of newly acquired fibroblast-like features. However, Le Hir et al. (39) suggest that FSP-1 may not be as specific for fibroblasts as first thought; antibodies for FSP-1, and its homologue s100A4, stain both fibroblasts and leukocytes in progressive renal injury (39). These findings therefore highlight the importance of using multiple criteria (FSP-1, αSMA, cytoskeletal changes, morphology, loss of cell adhesion) to define EMT both in vitro and in vivo.

Zeisberg et al. (69) have recently used lineage tracing techniques to demonstrate EndMT in a variety of murine models of renal disease. When tie-2-expressing endothelial cells were permanently labeled with yellow fluorescent protein (YFP), after injury, a substantial proportion of αSMA-positive and FSP-1-positive cells colabeled for YFP. Likewise, immunohistochemical techniques showed that 30–50% of cells co-express (myo)fibroblast markers and the endothelial antigen CD31.

Myofibroblast function. Myofibroblasts synthesize the ECM components that constitute interstitial fibrosis (27) and, like a deflating balloon, contract and reorganize matrix to increase its density (36) (Fig. 1).

Fibrogenesis. What was once described simply as reticulin is now known to be a complex mixture of proteoglycans and nonproteoglycan constituents, such as fibronectin, elastin, laminin, and collagens. Renal mesenchymal cells, in their various forms, are the principal matrix-producing cells in the kidney (15, 27). Accordingly, myofibroblasts produce a plethora of ECM constituents.

Contraction. In vitro studies of collagen gel contraction have provided valuable insights into matrix reorganization and the process of collapse (Fig. 1). In a process that is directly proportional to the number of cells present, fibroblasts embedded in solidified collagen progressively contract matrix to reduce gel diameter and increase matrix density (36). We know that this process is dependent upon β1-integrins found on the surface of renal fibroblasts (35). Blocking these receptors with specific antisera is sufficient to prevent fibroblasts binding to collagen I, and in doing so abrogate gel contraction (35). The process is complex and is due both to contraction in the surrounding matrix, and traction or migration of fibroblasts through surrounding matrix.

Significance of αSMA expression. Despite the now-widespread acceptance of myofibroblasts per se, controversy surrounds the significance of αSMA expression. Although de novo expression of αSMA is used as a marker of fibroblast activation, why it should be so is poorly understood. The αSMA response to injury is very rapid; 45 min of renal ischemia are sufficient to upregulate αSMA expression in mesangial cells (21). We accept that αSMA is a contractile protein, the formation of αSMA rich stress fibers being key contractile machinery. Interestingly, myofibroblast-mediated contraction of wounds occurs in internal organs in a similar manner to skin wound healing (25). However, the association between αSMA expression and enhanced fibroblast synthesis, proliferation, and activity is more controversial. Indeed, those
nonrenal studies that have attempted to address this question suggest that SMA may actually inhibit fibroblast migration (16, 55). Unexpectedly, studies of glomerulosclerosis (snake venom glomerulonephritis) and tubulointerstitial fibrosis (UUO) in the αSMA-deficient mouse showed that αSMA null mice had more, not less, sclerosis and fibrosis (63).

Regulation of Fibroblast Behavior

Control of interstitial fibroblasts after injury is inherently complex. A variety of factors have been shown to directly influence or interfere with the in vitro behavior of renal profibrotic mesenchymal cells.

Myofibroblast differentiation, proliferation, and collagen synthesis are stimulated by a variety of circulating agents and factors derived from stimulated tubular cells, leukocytes, and fibroblasts themselves. A hierarchy exists among the profibrotic growth factors, with very compelling evidence for the importance of TGF-β1, ANG II, and platelet-derived growth factor (14). It is also recognized that fibroblasts are stimulated by disease-specific factors (e.g., the high glucose concentrations in diabetes). Extrapolation from other organs suggests that mechanical strain and the composition of surrounding ECM are important.

Are there endogenous renoprotective factors? Increasingly, we realize that there are also a number of naturally occurring endogenous renoprotective factors that limit fibrosis by interacting with profibrotic growth factors. Endogenous hepatocyte growth factor (HGF) is linked to decreased TGF-β1 expression and progression (49). In a similar manner, bone morphogenic protein-7, a member of the TGF-β superfamily, preserves renal function and prevents interstitial fibrosis after UUO, acting as a counterbalance to TGF-β1 activity (50). Mice deficient in the hormone relaxin develop age-related fibrosis in a number of organs (57, 58), whereas progression after UUO is accelerated in the absence of relaxin (26).

Potential Therapeutic Strategies

A number of different strategies are being employed to ameliorate, and hopefully even abrogate, progression. Several excellent articles have reviewed candidate molecules in detail (6, 66). What follows below is a summary of key strategies.

Indirect therapies. Therapies that reduce hypertension, proteinuria, hyperlipidemia, and hyperglycemia may potentially slow progression by improving the operating environment of the kidney (4). Administration of angiotensin-converting enzyme inhibitors, or angiotensin type I receptor blockers, have consistently been shown to slow progression of renal failure in experimental animals and patients with chronic renal disease (28, 33).

Interestingly, many of the agents used have been shown to have quite specific effects on fibrogenesis. Such observations highlight an inherent difficulty in evaluating antifibrotic strategies; we are often uncertain if the benefits are because of effects on injury, inflammation, or downstream direct cellular effects.

Anti-inflammatory. Conventional wisdom suggests that the inflammatory response is the major driving factor for recruitment and activation of fibroblasts. Certainly a growing list of inflammatory mediators has been shown to upregulate fibroblast function.

Interference with inflammatory chemokines, cytokines, and growth factors should therefore limit downstream fibroblast activation and fibrogenesis (3). However, although inflammation typically precedes the development of fibrosis, a variety of nonrenal models suggests that fibrosis is not always characterized by persistent inflammation (66). Several nonrenal studies have documented profibrotic activities for Th-2 cell responses, and antifibrotic activities for the Th-1-associated cytokines (66), while the presence of different macrophage subpopulations likewise differentially promote fibrosis or repair (12).

Antifibrogenesis. Theoretically at least, we should be able to use antagonists to directly interfere with the profibrotic activities of (myo)fibroblasts. Alteration of fibroblast kinetics (proliferation and cell death), differentiation (αSMA expression), migration, matrix production, and matrix reorganization all have the potential to limit progressive scarring.

Fibroblasts, mesangial cells, and vascular smooth muscle cells all express receptors for the vasoactive factors (46, 56). For some time, we have realized that the effect of angiotensin inhibition is greater than would be expected from blood pressure alone (28), implicating both glomerular hemodynamics and direct cellular effects.

Collagen degradation. Is regression of established fibrosis possible? The recognition that renal scarring involves both increased collagen synthesis and decreased breakdown has inevitably led to a search for agents that promote collagen degradation. Such strategies have been given further impetus by the clinical reality that extensive scarring has often already occurred at presentation. Agents or therapies that remove established fibrosis offer great potential benefits.

Pirfenidone, a pyridone compound, reduces ongoing fibrosis in both chronic anti-thy-1 glomerulonephritis (60) and UUO (61), seemingly through a variety of mechanisms indirectly increasing collagen degradation. A recent open-label study in patients with focal segmental glomerulosclerosis has confirmed its clinical utility (11). Likewise, relaxin has shown potential in animal studies, albeit not confirmed clinically (57).

Regeneration. Importantly, it is uncertain if removal of fibrosis will in itself be sufficient to improve renal function. For restoration of function to occur, several steps are necessary: resolution of damaging inflammation and fibrogenesis, followed by regeneration and reconstruction.

In acute injury, inflammation and therefore fibrogenesis are transient, and an organ as largely epithelial as the kidney has good capacity for regeneration and reconstruction (47). Although recent genetic mapping techniques suggest that proliferation of surviving epithelia is the main way in which epithelium is regenerated after acute renal failure (30), several other mechanisms contribute (reviewed in Ref. 47). These include recruitment of intrarenal stem cells (51), dedifferentiation of resident epithelia (42), and immigration of external stem cells (41). Likewise, using bone marrow chimeras, Ito et al. (31) were able to show that transplanted stem cells migrate to the glomerulus in thy-1 nephritis, repopulating the mesangium after mesangiolysis. These cells retained features of mesangial cells, even after isolation and culture (31). Such findings highlight the as yet unrealized potential of cell-based therapies in renal disease.

Although we now recognize that the kidney has a good capacity for regeneration after acute injury, regeneration after prolonged (chronic) or extensive injury is much more prob-
lematic. The principal difference is that fibrogenesis persists, and the loss of basement membranes means that a scaffold for regeneration no longer exists (47). Furthermore, once a variety of cell types are injured, the complexity of reconstruction increases exponentially. In such circumstances, scarring predominates. The persistence of fibrogenesis in chronic injury also suggests a failure of clearance mechanisms such as apoptosis. Apoptosis of myofibroblasts in skin wound healing is well described, but evidence in the kidney is largely lacking (14).

Progression is paralleled by a loss of peritubular capillaries, although it remains unclear if this is a cause or effect of fibrosis. Vascular endothelial growth factor upregulates angiogenesis, with a commensurate improvement in structure and function (34), suggesting that maintenance of blood supply is a limiting factor. Again bone marrow-derived endothelial cell precursors are a source of endothelial cells in renal angiogenesis (40) but also, in chronic injury, a source of myofibroblasts (40).

The transition between acute and chronic outcomes is poorly understood, although the failure of a number of endogenous mechanisms seems to contribute. The renoprotective properties of HGF relate in part to the ability of HGF to maintain an epithelial phenotype, and therefore prevent EMT after UUO (67), while in vitro studies suggest that the myofibroblast phenotype can be reversed by the hormone relaxin (45).

Conclusion
Recent major advances have led to a much better understanding of the scarring process, and the first tentative steps in developing rational treatment strategies have been made. However, important challenges remain.

As can clearly be seen above, so much of what we know about renal fibrosis has come from the study of UUO. The reason for this is simple: it has a rapid time course and is species independent and can therefore be easily used with genetically manipulated mice. The model does have inherent species independent and can therefore be easily used with genetically manipulated mice. The model does have inherent deficiencies, not least of which is the inability to monitor mechanisms seems to contribute. The renoprotective properties of HGF relate in part to the ability of HGF to maintain an epithelial phenotype, and therefore prevent EMT after UUO (67), while in vitro studies suggest that the myofibroblast phenotype can be reversed by the hormone relaxin (45).

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