Wound Healing in the Kidney: Complex Interactions in Renal Interstitial Fibrogenesis

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The degree of renal interstitial fibrosis, i.e., the poorly organized accumulation of extracellular matrix (ECM) proteins such as collagens (types I, III, and IV), fibronectin, laminins, and others, has emerged as the pathobiologic entity that correlates better with the degree of renal filtration failure than any other histologic descriptor and better predicts the rate of progression to end-stage renal failure in chronic renal diseases (1,2). Although injured tubular cells moderately contribute to ECM production, the primary matrix-generating cells in the renal interstitium seem to be myofibroblasts. Normal kidneys are devoid of myofibroblasts, and only a few of their precursor cells, fibroblasts, can be found in interstitial spaces. Effective fibrogenesis requires multiplication of fibroblasts and their transformation into the matrix-producing myofibroblast phenotype. Since the pivotal experiments in Border’s laboratory, TGF-β has emerged as a master regulator of fibrogenesis in the renal interstitium and in other parenchymal organs (3,4). However, other cells and intercellular mediators play important roles. As in many processes in nature, the baseline state of tissue maintenance is regulated by a balance of promoting factors and antagonists, and departure from this baseline requires a relative increase in permissive activity and loss of inhibitory factors. Increasing fibrosis-propagating cytokines include TGF-β and PDGF. Bone morphogenetic protein 7 (BMP7) and hepatocyte growth factor have emerged recently as inhibitors of fibrosis.

Renal fibrogenesis may resemble processes that occur during wound healing of the skin. In this latter condition, a cut through the skin is followed by degranulation of platelets, which provides to the wound a cocktail of growth factor-cytokines (EGF, IGF-I, TGF-β, and PDGF) that orchestrate repair. Immigrating neutrophils secrete IL-1 and TNF-α, and macrophages provide TGF-β, heparin-binding–EGF, fibroblast growth factor, TNF-α, and other factors to the wound. Fibroblast proliferation is regulated by TGF-β and PDGF, and myofibroblast transition is especially regulated by TGF-β in collaboration with IGF, ED-A splice variant of fibronectin, and other factors (5).

In this simplified model of wound healing, macrophages play key roles. Heavy and prolonged inflammatory infiltrates lead to more severe scarring (5,6). Of note, even large fetal skin wounds heal without scars, and fetal wound healing occurs with far fewer or even without inflammatory infiltrates, highlighting the role of macrophages and other inflammatory cells in fibrogenesis.

The importance of the macrophage infiltrate has also been appreciated in renal fibrogenesis. These cells are thought to be attracted into the renal interstitium by peritubular chemokine gradients that are formed by basolateral secretion of compounds such as the c-c-chemokines monocyte chemoattractant protein-1 (MCP-1) and RANTES as NF-κB–mediated responses to tubular cell injury. MCP-1 may also activate macrophages, which in response increases their expression of TGF-β and perhaps other cytokines (7). Through these peptides, macrophages are thought to act on tubular cells, fibroblasts, and themselves by autocrine and paracrine modes of actions. TNF-α and IL-1α target tubular cells, raising MCP-1 secretion, which may attract and activate more macrophages in a vicious cycle that maintains and worsens the infiltrate (8). TGF-β and PDGF cause fibroblast proliferation, and the former contributes to myofibroblast transition of injured tubular cells, a mechanism for the recruitment of myofibroblasts by epithelial-mesenchymal transformation (9), and to the expression of ECM proteins. Thus, interactions between macrophages and residential cells are thought to involve primarily humoral mechanisms.

In recent experimental studies, Zhang et al. examined the contribution of direct interactions between immigrating macrophages and tubular cells (10). These investigators generated in vitro experimental evidence in support of a model of competition between two avenues of direct cell–cell interactions between tubular cells and macrophages, one resulting in profibrogenic activation of tubular cells and another geared toward preventing this profibrogenic cell–cell contract (11,12). In this model, engagement of leukocyte function antigen-1 (LFA-1) on the surface of macrophages with tubular cell intercellular adhesion molecule-1 (ICAM-1) leads to TGF-β-mediated activation of a fibrogenic program in tubular cells, whereas binding of macrophage CD44 to tubular cell membrane hyaluronic acid extensions (“cables”) prevents this physical association via ICAM-1 and, hence, profibrogenic tubular cell activation (10–12). Moreover, these authors described that the hyaluronic acid cables on tubular cell surfaces are regulated by BMP7 (13). Increased hyaluronic acid cables reduce engagement of ICAM-1 by macrophages perhaps by geographically preventing close contacts between macrophages and the basolateral...
membrane of tubular cells. Although these findings will require in vivo confirmation, they elucidate a novel mechanism by which macrophage infiltrates may directly engage tubular cells and, in addition, describe a novel mechanism of antifibrogenic action of BMP7.

BMP7 has recently emerged as an antifibrogenic, endogenously expressed growth factor in kidney, which is down-regulated before or early at the onset of fibrogenesis in experimental renal diseases (14,15). Exogenously administered recombinant human BMP7 (rhBMP7) reduces renal interstitial fibrosis in experimental obstructive and diabetic nephropathy (14,16,17). The studies by Zhang et al. provide insights into one mechanism of BMP7’s antifibrogenic function, namely increasing hyaluronic acid cables on tubular cell surface (10). However, there is also evidence that a broad range of effects of BMP7 in other cell types reduce TGF-β-mediated fibrosis programs (18). Recent findings suggest that BMP7-dependent, smad5-mediated, transcriptionally activated smad6 blocks the TGF-β/smad2/3 signal transduction pathway by reducing the nuclear translocation of phosphorylated smad2/3 (19). Such opposing interaction between BMP7-dependent smad (smad1 and smad5) and the TGF-β–induced smad signals (smad2 and smad3) has also been elucidated as a mechanism by which BMP7 blocks TGF-β–induced epithelial-mesenchymal transformation (20). In vitro, BMP7 reduces the increased expression of several ECM proteins and matrix accumulation–promoting regulatory molecules that are induced by TGF-β via smad2/3 pathways (18).

Exogenous administration of rhBMP7 in rodents with obstructive nephropathy also reduces the interstitial macrophage infiltrate (14). It remains unclear whether and how the effects of BMP7 on tubular cell surface hyaluronic acid cables may contribute to reduced interstitial infiltration with macrophages. Perhaps interactions between macrophages and tubular cells via ICAM-1 increase tubular cell chemokine secretion, which would attract and activate more macrophages, but this is speculative.

The interstitial macrophage infiltrate seems to be of prominent importance for interstitial fibrogenesis as inferred by lessons from studies in wound healing. Some experts believe that the absence of fibrogenesis in fetal wound healing results from the virtual absence of an early inflammatory (macrophage) infiltrate (5,6,21). Perhaps prevention of the renal interstitial macrophage infiltrate or reduction of the paracrine or direct cell–cell interactions of inflammatory cells with renal cells reduces or prevents renal interstitial fibrogenesis. Indeed, in proof-of-concept in vivo gene therapy experiments, the reduction of MCP-1 activity reduces both interstitial macrophage infiltration and fibrosis (22,23). As a more practical approach, the small molecule proteasome inhibitors PS-341 (Velcade) and PS-519, which specifically prolong the half-life of the endogenous NF-κB inhibitor IκB, may emerge as a clinically useful therapy to reduce interstitial macrophage infiltration and, hence, fibrogenesis (24).

Insights into the mechanisms of renal interstitial fibrogenesis have generated novel therapeutic targets, and interventions have been tested experimentally in in vitro and in vivo studies. In addition to rhBMP7, hepatocyte growth factor reduces fibrogenesis in the kidney by inducing TGF-β–induced factor (TGIF) and SnoN, two smad3-transcriptional co-repressors that oppose TGF-β signals (25). Small molecule inhibitors of activin receptor–like kinases (Alk4, -5, and -7), which inhibit the TGF-β receptor I (Alk5) kinase but not the BMP-activated receptor kinases, block global TGF-β functions in vitro, but their in vivo efficacy on renal fibrogenesis has not yet been demonstrated (26,27). Inhibition of the TGF-β–induced fibrosis mediator connective tissue growth factor, such as with monoclonal antibodies, could be a suitable therapy. There is experimental evidence, albeit very limited, that simvastatin reduces TGF-β–dependent transcriptional activation of connective tissue growth factor in fibroblasts (28). If confirmed, then statins might gain yet another indication in patients with renal disease. TGF-β–induced renal fibroblast proliferation requires activation of abelson, and PDGF also contributes to this process. Indeed, imatinib mesylate, an inhibitor of abelson and the PDGF receptor kinase, reduces fibrogenesis in experimental obstructive nephropathy (29).

The study of mechanisms of interactions between cells in the renal interstitium in several laboratories has recently provided several new insights that may translate into novel antifibrogenic therapies for the management of patients with chronic renal disease.

References

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