Reactive Oxygen Species and Matrix Remodeling in Diabetic Kidney

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Abstract. Excessive deposition of extracellular matrix (ECM) in the kidney is the hallmark of diabetic nephropathy. Although the amount of ECM deposited in the kidney depends on the balance between the synthesis and degradation of ECM, the role of ECM degradation in matrix remodeling has been less well appreciated. High glucose, advanced glycation end products, angiotensin II, and TGF-β1 all increase intracellular reactive oxygen species (ROS) in renal cells and contribute to the development and progression of diabetic renal injury. The role of ROS in increased ECM synthesis has been well documented. ROS may also play a critical role in decreased ECM degradation by mediating high glucose– and TGF-β1–induced inhibition of the proteolytic system, plasmin, and matrix metalloproteinases in the glomeruli. A recent observation suggests that ROS play an important role in tubulointerstitial fibrosis by mediating TGF-β1–induced epithelial-mesenchymal transition (EMT). Accelerated ECM degradation is required to disrupt tubular basement membrane and complete EMT. ROS thus seem to be involved in both decreased and increased ECM degradation. It is not clear how cells determine when and where to increase or decrease ECM degradation in response to ROS. Precise definition of ROS-activated signaling pathways leading to ECM remodeling in the kidney will provide new strategies to prevent or treat diabetic renal injury.

Excessive deposition of extracellular matrix (ECM) in the glomerular mesangium and tubulointerstitium is closely associated with progressive decline in renal function in diabetes (1,2). The amount of ECM deposited in the kidney depends on the balance between synthesis and degradation of ECM (Figure 1). Although the contribution of enhanced ECM synthesis in diabetic kidney is well recognized, the role of ECM degradation in matrix remodeling has been less well appreciated. The observation that mesangial fractional volume and the thickness of glomerular and tubular basement membranes are significantly reduced in diabetic kidneys 10 yr after pancreas transplantation (3) suggests the reversibility of renal fibrosis and the importance of ECM degradation in ECM remodeling in diabetic kidney. Epithelial-mesenchymal transition (EMT) of tubular epithelial cells is an alternative mechanism involved in tubulointerstitial fibrosis, in which increased ECM degradation is one of the key steps (4–6).

High glucose (7), advanced glycation end products (AGE) (8,9), angiotensin II (Ang II) (10), and TGF-β1 (11) all increase intracellular reactive oxygen species (ROS) in renal cells and contribute to the development and progression of diabetic renal injury. The role of ROS in increased ECM synthesis in diabetic kidney has previously been reviewed (12). In this article, we review the evidence that ROS also play a critical role in decreased ECM degradation and induction of EMT leading to glomerular mesangial expansion and tubulointerstitial fibrosis in diabetic kidney (Table 1).

Role of Plasminogen Activator Inhibitor-1 and Plasmin in ECM Degradation

Two major ECM protease systems, plasminogen activator (PA)/plasmin/PA inhibitors (PAI) system and matrix metalloproteinases (MMP)/tissue inhibitors of matrix metalloproteinases (TIMP) system, are interrelated and involved in matrix degradation (13–15). The physiology of each system is complex, and the activities are tightly regulated at many levels, including gene expression, activation, and inhibition by specific inhibitors (Figure 2). Plasmin is generated from plasminogen by the enzymatic activity of tissue-type PA (tPA) and urokinase-type PA (uPA). PA activity is inhibited by PAI. A strong positive correlation between plasmin activity and ECM degradation in cultured mesangial cells and the ability of plasmin inhibitors to inhibit ECM degradation suggest the importance of plasmin in ECM degradation by mesangial cells (16). Plasmin not only degrades several matrix proteins such as fibronectin, laminin, proteoglycan, and type IV collagen (17–19) but also activates pro-MMP (20,21). PAI-1 seems to play an important role in ECM degradation in mesangial cells, because a larger molar excess of PAI-1 over tPA and uPA is detected in mesangial cell culture supernatant and because anti–PAI-1 antibody increases ECM degradation (22). PAI-1 is not expressed in normal human kidney but is strongly induced in various forms of kidney diseases that lead to renal fibrosis and is now considered a potential target in renal fibrogenesis (23).

Both high glucose (24) and TGF-β1 (24,25) upregulate PAI-1 mRNA and protein expression in a time-dependent
manner and decrease plasmin activity in glomerular mesangial cells. TGF-β1 also increases PAI-1 protein synthesis and decreases PA activity in normal as well as diseased glomeruli (26) and in rat proximal tubular epithelial cells (27). TGF-β1 markedly reduces the conversion of latent MMP-2 to active form, leading to decreased ECM degradation in human mesangial cells (25). High glucose decreases MMP and increases TIMP expression possibly through TGF-β1 (28). These observations suggest that high glucose, directly or indirectly through TGF-β1, upregulates PAI-1 and decreases plasmin activity in renal cells. Our preliminary observation (24) suggests that ROS may play a critical role in both high glucose– and TGF-β1–induced PAI-1 expression and decreased plasmin activity.

**ROS Upregulate PAI-1 Expression and Downregulate Plasmin Activity**

High glucose, TGF-β1, and H₂O₂ continuously generated by glucose oxidase (GO) upregulate PAI-1 mRNA expression and protein secretion and significantly suppress plasmin activity in rat mesangial cells (24). When cells were pretreated with dl-buthionine-(S,R)-sulfoximine (BSO) for 24 h to deplete the intracellular glutathione, basal PAI-1 expression was upregulated; plasmin activity was downregulated; and high glucose–, TGF-β1–, and GO-induced PAI-1 mRNA expression and protein secretion were exaggerated. In addition, antioxidants N-acetylcysteine (NAC), catalase, and trolox effectively reverse high glucose–, TGF-β1–, and GO-induced changes in PAI-1 mRNA and protein expression and plasmin activity without significant effect on basal expression. In this study, TGF-β1 at concentrations that upregulated PAI-1 expression increased intracellular ROS in mesangial cells. These observations suggest that ROS mediate high glucose– and TGF-β1–induced upregulation of PAI-1 mRNA expression and protein secretion, leading to decreased plasmin activity in mesangial cells (24). Previous studies demonstrated that ROS mediate hyperglycemia-induced (29) and cyclic strain–induced (30) PAI-1 expression in endothelial cells and radiation-induced PAI-1 expression in rat kidney tubular epithelial cells (31).

**ROS Induce EMT in Tubular Epithelial Cells**

Yang and Liu (4) demonstrated that EMT is an orchestrated, highly regulated process involving four key steps: (1) loss of epithelial cell adhesion, (2) de novo α-smooth muscle actin expression and actin reorganization, (3) disruption of tubular basement membrane, and (4) enhanced cell migration and invasion into the interstitium. Although EMT can be induced by TGF-β1 (32), AGE (33), and Ang II (34), the intracellular signaling pathways that lead to EMT remain largely unknown. Smad pathway (32), c-jun-NH₂-terminal kinase (JNK) (35), and p38 mitogen-activated protein kinase (MAPK) (36) seem to be involved in TGF-β1–induced EMT. In normal rat tubular epithelial cell line NRK-52E, Li et al. (32) showed that TGF-β1 induced Smad2 phosphorylation and resulted in the transformation of epithelial cell into myofibroblast phenotype with the loss of E-cadherin and de novo expression of α-smooth muscle actin and collagens I, III, and IV and that

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**Table 1.** Evidence for critical role of ROS in ECM remodeling in diabetic kidney

| ROS are generated by high glucose, AGE, Ang II, and TGF-β1 in renal cells. High glucose, TGF-β1, and H₂O₂ upregulate ECM synthesis and secretion by renal cells. High glucose, TGF-β1, and H₂O₂ upregulate PAI-1 expression and downregulate plasmin and MMP activity in renal cells. AGE, TGF-β1, and H₂O₂ induce EMT in tubular epithelial cells. Antioxidants reverse high glucose– and TGF-β1–induced changes in ECM synthesis, PAI-1 and plasmin activity, and TGF-β1–induced EMT. |

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**AGE,** advanced glycation end products; Ang II, angiotensin II; EMT, epithelial-mesenchymal transition; PAI-1, plasminogen activator inhibitor-1.
overexpression of Smad7 resulted in marked inhibition of TGF-β-induced Smad2 activation with the prevention of EMT and collagen synthesis. Hashimoto et al. (35) showed that a specific inhibitor of JNK-mediated signaling pathway (CEP-1347) but not an inhibitor of ERK (PD 98059) or p38 MAPK (SB 203580) attenuated TGF-β1–induced phenotypic modulation of human lung fibroblasts. In mouse mammary epithelial cells, however, p38 MAPK is required for TGF-β–mediated fibroblastic transdifferentiation and cell migration (36). Bakin et al. (36) showed that a direct inhibitor of p38 MAPK inhibited TGF-β–mediated changes in cell shape and reorganization of the actin cytoskeleton and that dominant negative MAPK kinase 3 (MKK3) inhibited TGF-β–mediated activation of p38 MAPK and EMT. We (11) recently observed that exogenous H2O2 as well as TGF-β1 induces EMT in tubular epithelial cells and that antioxidants NAC and catalase effectively inhibit TGF-β1–induced EMT. These data suggest that ROS may also play an important role in induction of EMT leading to tubulointerstitial fibrosis in diabetic kidney. It is not known whether ROS are involved in Smad activation or in JNK or p38 MAPK activation leading to EMT.

Whereas decreased ECM degradation plays an important role in ECM deposition in the kidney, accelerated ECM degradation is required during the process of EMT to effectively disrupt tubular basement membrane. In this regard, Yang et al. (5) demonstrated that deficiency of tPA in mice selectively block EMT and significantly decrease MMP-9 induction, leading to a dramatic preservation of the structural and functional integrity of tubular basement membrane and that disruption of tPA gene reduced deposition of interstitial collagen III and fibronectin as well as total tissue collagen in the kidneys after sustained ureteral obstruction. This study underscores the importance of ECM degradation to complete EMT. The role of ROS in increased ECM degradation is suggested by the observation that v-Ha-Ras oncogene induces ROS generation, NF-κB activation, and upregulation of MMP-9 mRNA along with downregulation of TIMP-1, an inhibitor of MMP-9, and PAI-1 mRNA in tubular epithelial cells (37).

**Conclusion**

Diabetes favors ECM accumulation in the kidney during remodeling process. High glucose and TGF-β1 increase ECM synthesis and secretion and at the same time decrease ECM degradation by inhibiting proteolytic systems plasmin and MMP in the glomeruli through ROS. However, ROS mediate TGF-β1–induced EMT through increasing MMP activity and ECM degradation in tubular epithelial cells, leading to tubulointerstitial fibrosis. It is not clear how cells determine when and where to increase or decrease ECM degradation in response to ROS. More precise definition of ROS-activated signaling pathways leading to ECM remodeling in the kidney will provide us with new strategies to treat diabetic renal injury.

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**References**


