Mediators of Diabetic Renal Disease: The Case for TGF-β as the Major Mediator

FUAD N. ZIYADEH
Penn Center for Molecular Studies of Kidney Diseases, Renal-Electrolyte and Hypertension Division, Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Abstract. The critical role of hyperglycemia in the genesis of diabetic nephropathy has been established by cell culture studies, experimental animal models, and clinical trials. Certain cytokines and growth factors have been identified as likely mediators of the effects of high ambient glucose on the kidney, but prominent among these is TGF-β, a prototypical hypertrophic and fibrogenic cytokine. Overexpression of TGF-β has been demonstrated in the glomerular and tubulointerstitial compartments of experimental diabetic animals. The TGF-β receptor signaling system is also triggered, as evidenced by upregulation of the TGF-β type II receptor and activation of the downstream Smad signaling pathway. Treatment of diabetic mice with neutralizing anti–TGF-β antibodies prevents the development of renal hypertrophy, mesangial matrix expansion, and the decline in renal function. Antibody therapy also reverses the established lesions of diabetic glomerulopathy. These studies argue strongly in support of the hypothesis that overactivity of the TGF-β system in the kidney is a crucial mediator of diabetic renal hypertrophy and mesangial matrix expansion.

The structural renal changes in diabetes consist of glomerular and tubulointerstitial hypertrophy, followed by thickening of glomerular and tubular basement membranes and progressive accumulation of extracellular matrix proteins in the mesangium and the interstitium. Identifying mediators of increased synthesis or decreased degradation of matrix molecules in diabetic renal disease may help design novel, effective therapies to avert glomerulosclerosis and tubulointerstitial fibrosis and the development of proteinuria and progressive renal insufficiency.

TGF-β is one effector molecule that has been studied extensively as a major mediator of the hypertrophic and prosclerotic changes in diabetic kidney disease (1,2). TGF-β stimulates the synthesis of key extracellular matrix molecules including type I collagen, type IV collagen, fibronectin, and laminin (3). TGF-β also decreases matrix degradation by inhibiting proteases as well as activating protease inhibitors (e.g., plasminogen activator inhibitor-1) (4). In addition, TGF-β promotes cell–matrix interactions by upregulating integrins, the cell surface receptors for matrix (5).

Almost all of the molecular mediators and intracellular signaling pathways that have been identified in diabetic kidney injury have also been found to stimulate the renal TGF-β activity as an intermediary step. These mediators encompass the following: high glucose concentration (6); early and advanced products of nonenzymatic glycation of proteins (7,8); oxidative stress and overproduction of superoxide by the mitochondrial electron-transport chain (9,10); cyclical stretch and relaxation of mesangial cells in culture (mimicking intraglomerular hypertension) (11); de novo synthesis of diacylglycerol and protein kinase C activation (12,13) and stimulation of mitogen-activated protein kinase (14); glucosamine overproduction (15); and high levels of vasoactive substances such as intrarenal angiotensin II (16), endothelin (17), and thromboxane (18).

High glucose concentration in cell culture studies stimulates hypertrophy of proximal tubular and mesangial cells (19,20) and stimulates the production of matrix molecules such as fibronectin and collagens in these cells as well as in epithelial, endothelial, and interstitial-fibroblastic cells (6,12,19,21–24). In almost all renal cell types, high ambient glucose upregulates the expression and bioactivity of TGF-β (6,22,25,26) and in some cases upregulates the TGF-β type II receptor (27). In fact, antagonism of TGF-β by specific neutralizing monoclonal antibodies (6) or by antisense oligonucleotides (28) virtually abolishes the high glucose-induced rise in matrix expression, indicating that TGF-β is a major mediator of the profibrotic effect of high glucose on the kidney.

Extensive evidence in experimental animal models of both type 1 and type 2 diabetes further implicates TGF-β as an important mediator of diabetic kidney disease. TGF-β mRNA and protein levels are increased in both the glomerular and tubular compartments of various models of experimental diabetes in rats and mice (29–33). It is interesting that upregulation of the renal TGF-β type II signaling receptor is concomitantly upregulated in diabetes (27,33–36). Recently, we reported significant activation in the diabetic kidney of the intracellular Smad pathway, which transduces the TGF-β signal (23,36). All of these studies provide evidence of activation
of the TGF-β1 system and its intracellular signaling pathway in renal cell in diabetes.

Interventional studies in animals have shown that the development of diabetic renal disease is likely caused by the heightened activity of the renal TGF-β system. Short-term treatment of diabetic mice with neutralizing monoclonal antibodies against all three isoforms of TGF-β prevented glomerular hypertrophy and attenuated the increase in TGF-β1, α1(IV) collagen, and fibronectin mRNAs (34). Furthermore, long-term antibody therapy in the db/db mouse, a spontaneous model of type 2 diabetes, virtually prevented the mesangial matrix expansion and preserved the creatinine clearance (35). However, the anti–TGF-β antibody did not reduce albuminuria. Because the deleterious effects of proteinuria can be mediated by the TGF-β system itself (37), the apparent paradox of preserved renal function in the face of persistent albuminuria may be best explained by the effective inhibition of proteinuria–induced renal dysfunction with the neutralizing anti–TGF-β antibody regimen (2). Another likely possibility is that diabetic proteinuria, unlike other renal manifestations of diabetes, is mediated by increased vascular permeability factor/vascular endothelial growth factor (VEGF) rather than by TGF-β (24,35,38). Our preliminary data have shown upregulation of VEGF in the kidney of db/db mice and that treatment with anti–TGF-β antibody was ineffective in reducing VEGF levels (35).

Most recently, we addressed the question of whether anti–TGF-β therapy can reverse the histologic lesions of diabetic glomerulopathy once they are established (39). Diabetic db/db mice and their nondiabetic db/m littermates were allowed to grow until 16 wk of age, by which time the db/db mice had developed glomerular basement membrane (GBM) thickening and mesangial matrix expansion. The mice were then treated with an irrelevant control IgG or a panselective, neutralizing anti–TGF-β antibody for 8 more weeks. Compared with control db/db mice, the db/db mice that were treated with anti–TGF-β antibody showed attenuation of GBM thickening and regression of mesangial matrix accumulation (39). Thus, inhibiting renal TGF-β activity can partially reverse the GBM thickening and mesangial matrix expansion in this mouse model of type 2 diabetes.

Corroborative surveys of kidney specimens derived from patients with diabetes have also suggested an important role of the renal TGF-β system in the pathogenesis of diabetic renal disease (40,41). For instance, glomerular TGF-β1 mRNA is markedly increased in renal biopsy specimens from patients with proven diabetic kidney disease (42). In another study, we have shown that the gradient of TGF-β1 concentration across the renal vascular bed (venous minus arterial) was positive and the urinary level of bioassayable TGF-β was significantly increased in diabetic patients, indicating net production of TGF-β1 protein by the kidney in diabetes (43). Of clinical interest is the observation that angiotensin-converting enzyme inhibitor therapy may protect the kidney by lowering TGF-β1 production. In captopril-treated patients, especially in the subset of patients with an initial GFR of <75 ml/min, we demonstrated that a decrease in the circulating TGF-β1 level predicted a better preservation of the GFR (44).

In summary, the studies reviewed herein indicate that if novel therapies can be designed to intercept effectively the renal TGF-β axis, then it may be possible in the future to prevent, arrest, or even reverse the damaging effects of diabetes on the kidney.

Acknowledgments

Dr. Fuad N. Ziyadeh is supported by the Juvenile Diabetes Foundation and National Institutes of Health (NIH) grants DK-44513, DK-45191, and DK-54608.

References