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Transforming growth factor-β1 and diabetic nephropathy

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Chang AS, Hathaway CK, Smithies O, Kakoki M. Transforming growth factor-β1 and diabetic nephropathy. *Am J Physiol Renal Physiol* 310: F689–F696, 2016. First published December 30, 2015; doi:10.1152/ajprenal.00502.2015.— Transforming growth factor-β1 (TGF-β1) is established to be involved in the pathogenesis of diabetic nephropathy. The diabetic milieu enhances oxidative stress and induces the expression of TGF-β1. TGF-β1 promotes cell hypertrophy and extracellular matrix accumulation in the mesangium, which decreases glomerular filtration rate and leads to chronic renal failure. Recently, TGF-β1 has been demonstrated to regulate urinary albumin excretion by both increasing glomerular permeability and decreasing reabsorption in the proximal tubules. TGF-β1 also increases urinary excretion of water, electrolytes and glucose by suppressing tubular reabsorption in both normal and diabetic conditions. Although TGF-β1 exerts hypertrophic and fibrogenic effects in diabetic nephropathy, whether suppression of the function of TGF-β1 can be an option to prevent or treat the complication is still controversial. This is partly because adrenal production of mineralocorticoids could be augmented by the suppression of TGF-β1. However, differentiating the molecular mechanisms for glomerulosclerosis from those for the suppression of the effects of mineralocorticoids by TGF-β1 may assist in developing novel therapeutic strategies for diabetic nephropathy. In this review, we discuss recent findings on the role of TGF-β1 in diabetic nephropathy.

nephrin; megalin; podocyte; proximal tubule; sodium-glucose cotransporter

TRANSFORMING GROWTH FACTOR-β1 (TGF-β1) is a multifunctional cytokine that controls numerous biological processes including immunity (92), differentiation (29), tumor suppression (2), tumor metastasis (107), senescence (68), migration (17), wound healing (105), apoptosis (108), cell division (2), adipogenesis (76), and osteogenesis (27). In addition, the renal expression of TGF-β1 mRNA and protein are increased in patients with diabetes mellitus (110), and it enhances the synthesis and cross-linking of extracellular matrix (ECM) (3, 81).

In the US population, the most frequent cause of chronic renal failure is diabetes mellitus (~44%). No more than 20~40% of diabetic subjects, however, develop nephropathy despite having similar blood glucose levels, suggesting the presence of genetic predisposition to the complication. The T869C polymorphism in the human TGF-β1 gene, leading to the L10P variant of the coding protein, is associated with an increased risk of diabetic nephropathy (71, 77, 109), but how the functional change in TGF-β1 protein increases the incidence of diabetic nephropathy remains elusive.

In incipient diabetic nephropathy, the glomerular filtration rate (GFR) increases by 25~50% (glomerular hyperfiltration) (1) by the reduction of tubuloglomerular feedback, which is caused by the increase in sodium/glucose reabsorption and hence the reduced sodium delivery in the macula densa (101). However, in the late stage of diabetic nephropathy, the GFR eventually declines as the number of functional nephrons decreases, which leads to the insufficiency of renal excretory function. The decline in GFR is associated with the expansion of mesangial area, which is caused by cell proliferation and accumulation of ECM.

Although the increase in urinary excretion of albumin is considered to be the early indicator of diabetic nephropathy, the mechanisms whereby hyperalbuminuria occurs in diabetic subjects are not fully understood. However, impairment of barrier function at the slit diaphragm between podocytes and the decrease in reabsorption of filtered albumin by proximal tubules have recently been identified as pivotal in the development of diabetic albuminuria.

Mice having the heterozygous Akita diabetogenic mutation expressing ~10, 50, 100, 200, and 300% normal *Tgfb1* mRNA levels have recently been generated. In these mice, the severity of glomerulosclerosis and albuminuria is enhanced as the expression of TGF-β1 is increased, despite blood pressure being negatively correlated with TGF-β1 expression (31). It is noteworthy that the diabetic mice with 10% normal TGF-β1 expression exhibit near-normal glomerular histology, GFR, and urinary albumin excretion, despite the presence of primary aldosteronism and hypertension. Additionally, the markedly increased urinary excretion of water, electrolytes, and glucose in Akita type 1 diabetes was diminished to levels comparable to those in nondiabetic wild-type mice by the genetic insufficiency of TGF-β1.

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These previous findings suggest that TGF-β1 plays a pathophysiological role not only in promoting glomerulosclerosis, interstitial fibrosis, and the decline in GFR but also in increasing urinary excretion of albumin, water, electrolytes, and glucose in diabetes. In the current review, we will discuss the mechanisms whereby TGF-β1 causes renal morphological and functional changes in diabetes mellitus. As such, therapeutic strategies targeting TGF-β signaling may be developed to effectively treat chronic excretory insufficiency in patients with diabetes.

TGF-β1 Facilitates Accumulation of ECM in Diabetic Nephropathy

Previous studies show that neutralizing anti-TGF-β antibodies prevented glomerulosclerosis and interstitial fibrosis, and reduced expression of ECM genes including fibronectin and type IV collagen in mice with type 1 and type 2 diabetes (90, 116), suggesting that TGF-β signaling plays a critical role in ECM accumulation in diabetic nephropathy. Both canonical and alternative signaling of TGF-β1 have been suggested to be involved in the development of diabetic nephropathy (15, 19).

Since glucose availability is impaired in diabetes, the metabolic shift occurs from glycolysis toward oxidative phosphorylation by using more fatty acids as an energy source (Fig. 1). As a result, the mitochondrial electron transport chain increases superoxide production and stimulates three major pathways of hyperglycemic damage (activation of the polyol pathway, advanced glycation end products generation, and protein kinase C activation) (72). Furthermore, high glucose induces reactive oxygen species (ROS) via NAD(P)H oxidase, mitochondrial electron transport chain, and protein kinase C (56).

Hydrogen peroxide upregulates TGF-β1 and its downstream ECM-related genes, including integrin-linked kinase, fibronectin, and collagen types I, III, and IV (25, 48). In the reverse direction, pharmacological inhibition of different ROS sources including NAD(P)H oxidase and mitochondrial respiratory chain decreases the transcription of TGF-β1 via reduced activity of activated protein-1 (26), indicating that ROS-induced enhancement of the transcription of TGF-β1 may at least in part account for the increase in TGF-β1 expression in diabetes.

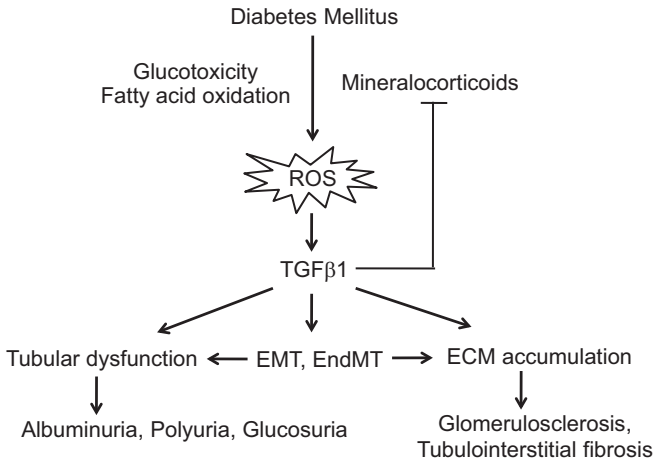

Fig. 1. Diagram depicting the proposed effects of transforming growth factor-β1 (TGF-β1) on the features of diabetic nephropathy. ROS, reactive oxygen species; EMT, epithelial-mesenchymal transition; EndMT, endothelial-mesenchymal transition.

Previous studies have demonstrated that TGF-β1 stimulates the transcription of the components of ECM, including collagen, fibronectin, and laminin (5, 41). It has been demonstrated that TGF-β1 also increases the expression of lysyl oxidase, which forms cross-links between collagen and elastin fibers that stabilizes their structure (3). TGF-β1 also stimulates the transcription of procollagen lysyl hydroxylase 2, which hydroxylates lysyl residues of collagen telopeptides and is essential for collagen cross-linking (24). In addition, TGF-β1 augments the expression of plasminogen activator inhibitor-1 (39) and tissue inhibitor of metalloproteinases-1 (100), both of which inhibit the activity of ECM-degradating matrix metalloproteinases.

The effect of TGF-β1 on the expression of matrix metalloproteinase 9 (MMP9) is controversial. TGF-β1 increases the expression of MMP9 in cultured cells and in isolated, perfused kidneys (13, 82), whereas transgenic overexpression of TGF-β1 decreases MMP9 expression in mice (100, 112). The exact reason for this discrepancy is unclear, but the suppressive effect of TGF-β1 on mineralocorticoid production may be related. Previous studies have demonstrated that aldosterone increases the transcription of MMP9 via phosphoinositide 3-kinase (PI3K), p38 MAPK, ERK (23), and oxidized Ca^{2+}/calmodulin-dependent protein kinase II (oxCaMKII) (32), suggesting that TGF-β1 decreases tissue MMP9 expression via suppressing circulating aldosterone produced in adrenocortical cells.

Recently, TGF-β type 1 receptor antagonists and aldosterone have also been found to increase the expression of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-1, which also degrades ECM (16, 54).

Thus TGF-β1 facilitates ECM accumulation by increasing production and stabilization of ECM and by suppressing its degradation, which plays a causative role in developing glomerulosclerosis and interstitial fibrosis in diabetic nephropathy.

TGF-β1 Facilitates Dedifferentiation of Renal Cells

Previous studies suggest that TGF-β1 induces epithelial-mesenchymal transition/transdifferentiation (EMT), a dedifferentiation process by which an epithelial cell is transformed into a myofibroblast, a type of mesenchymal stem cell (96). In the kidney, tubular cells are normally lining the tubular basement membrane and have a highly polarized structure for efficient reabsorption of solutes and proteins from urinary space into peritubular capillaries, but through the EMT process they lose cell polarity and gain migratory and fibrogenic properties of myofibroblasts (35). Indeed, TGF-β1 has been demonstrated to induce EMT in proximal tubules (115), collecting duct cells (43), glomerular podocytes (33, 88), and glomerular parietal epithelial cells (93).

It has been demonstrated that hypoxia-inducible factor 1α (HIF-1α) enhances EMT in murine proximal tubular epithelial cells (34). Since the effect of hypoxia on EMT is only partially inhibited by anti-TGF-β antibodies, TGF-β signaling is unlikely to be the only mechanism to induce EMT (34). Prolyl hydroxylase domain-containing proteins (PHDs) hydroxylate HIF-1α. Hydroxylated HIF-1α binds to von Hippel-Lindau tumor suppressor protein and is subject to polyubiquitination and degradation by proteasomes (44). TGF-β1 has been shown

to decrease both mRNA and protein levels of PHD2 and hence stabilize HIF-1α protein (66). Overexpression of the HIF-1α PHD2 transgene, which enhances degradation of HIF-1α, completely prevents TGF-β1-induced changes in the expression of HIF-1α and marker genes for EMT, suggesting that HIF-1α mediates TGF-β1-induced EMT (30).

It has also been shown that TGF-β1 activates the Jagged/Notch signaling pathway and that activated Notch triggers EMT (74). These results suggest that TGF-β1-induced EMT is also mediated at least in part by Jagged/Notch signaling.

Previous studies suggest that endothelial-mesenchymal transition/transdifferentiation (EndMT), a process by which endothelial cells are transformed into myofibroblasts, also contributes to renal fibrosis in streptozotocin (STZ)-induced type 1 diabetic mice (113). TGF-β1 has been demonstrated to induce EndMT (114). Overexpression of Snail induced EndMT, but suppression of Snail expression abrogated TGF-β2-induced EndMT in mouse endothelial cells, suggesting that Snail mediates TGF-β signaling-induced EndMT (50).

Thus TGF-β1 may be involved in the process of EMT and EndMT in diabetic nephropathy, which decreases the number of functional cells and increases the accumulation of ECM, leading to impairment of renal excretory and reabsorptive function. However, controversy remains over the contribution of each of these processes to renal fibrosis (18).

TGF-β1 Enhances Diabetic Albuminuria Via Both Glomerular and Postglomerular Mechanisms

A number of previous studies have demonstrated that TGF-β1 promotes the development of diabetic albuminuria. Several transgenic animal models overexpressing TGF-β1 exhibited increased urinary excretion of albumin (51, 100). Chronic treatment with the soluble human TGF-β type II receptor reduced albuminuria without changing blood glucose levels in STZ-induced diabetic mice (84). However, several studies did not show a significant effect of the suppression of TGF-β signaling on albuminuria in diabetic animals. For instance, chronic peritoneal infusion of neutralizing anti-TGF-β antibodies did not alter urinary albumin excretion in *db/db* type 2 diabetic mice (116). Furthermore, genetic deficiency of small mothers against decapentaplegic homolog (Smad) 3, an intracellular signaling molecule which is downstream of TGF-β receptors, did not affect albuminuria in STZ-induced diabetic mice (104).

Microalbuminuria is low-grade albuminuria, which ranges from 30 to 300 mg/day in humans, and is widely established as an early indicator of diabetic nephropathy. However, whether the elevated urinary excretion of albumin plays a causative role in diabetic tubular damage or is merely a consequence of diabetic nephropathy is still controversial (45).

The paradigm that the sieving of albumin at the slit diaphragm is the predominant mechanism in the prevention of albuminuria has been questionable because of the lack of a clearing mechanism of the sieved albumin (91). Indeed, the concentration of albumin at Bowman's capsule estimated with two-photon microscopy has been demonstrated to be within the nephrotic range (1–10 mg/ml) even in normal conditions (86). In contrast, others using a micropuncture technique have reported less concentrations of albumin (20–30 μg/ml) than the nephrotic range in Bowman's capsule (53, 97). Nevertheless, these results indicate that both incomplete glomerular barrier and subsequent tubular reabsorption/degradation are important in preventing albuminuria (10).

Nephrin is abundantly expressed in the slit diaphragm and considered to exert a barrier function against glomerular filtration of albumin (37, 106). The mutations in the nephrin gene lead to urinary overexcretion of high-molecular-weight proteins and albumin and congenital nephrotic syndromes (47, 79). However, tubular handling of protein is important as well. Megalin and cubilin in the brush border of proximal tubular cells contribute to the endocytosis of albumin and low-molecular-weight proteins (9, 22, 58, 67, 111).

The expression of nephrin is decreased in diabetes (12). TGF-β1 decreases renal mRNA levels of nephrin (31) and increases the permeability of albumin in the podocyte (55). Additionally, the presence of diabetes attenuates the expression of megalin (98). TGF-β1 decreases renal megalin mRNA levels in Akita type 1 diabetic mice (31), which might attenuate the albumin endocytosis mediated by megalin (22).

Therefore, it is likely that diabetic albuminuria is the result of a double insult in the kidney's ability to process albumin, increased permeability at the slit diaphragm, and reduced tubular reabsorption. However, in what proportion diabetic albuminuria is caused by tubular dysfunction and/or podocyte dysfunction is controversial (75, 85, 98, 99).

Albuminuria in Akita mice expressing 10% TGF-β1 mRNA was markedly reduced compared with that in Akita mice and was comparable to nondiabetic wild-type mice, despite similar glucose levels and increased systolic blood pressure in the 10% hypomorphic Akita mice (31). In the reverse direction, genetic overexpression of TGF-β1 markedly increased urinary albumin excretion despite no significant changes in blood glucose or blood pressure (31). The amount of urinary albumin excretion in the 10% hypomorphic Akita mice was found to be increased by 20-fold by overexpressing *Tgfb1* in proximal tubular cells, but only by 4-fold by overexpressing *Tgfb1* in podocytes.

Thus TGF-β1 plays a critical role in developing hyperalbuminuria. These findings demonstrate that attenuated tubular reabsorption of albumin appears to be the predominant factor leading to diabetic albuminuria, rather than enhanced glomerular filtration.

TGF-β1 Inhibits Production and Function of Mineralocorticoids

Plasma levels of aldosterone are increased in diabetic patients (36), and aldosterone has been shown to increase ROS by stimulating NAD(P)H oxidase (83). These findings suggest that the increased action of mineralocorticoids partly contributes to the development of diabetic complications. Indeed, chronic administration of spironolactone, a mineralocorticoid receptor antagonist, decreased albuminuria in patients with diabetes (89).

It has been shown that TGF-β1 potently inhibits the synthesis of aldosterone in vitro (28, 38, 61). TGF-β1 also suppresses the production of androstenedione, corticosterone, and cortisol (38). This broad suppressive effect of TGF-β1 on steroidogenesis may be partly because TGF-β1 decreases the expression of cytochrome *P*-450 side-chain cleavage (P450scc), adrenodoxin reductase, and adrenodoxin, which controls the initial step of

steroidogenesis (69). The mRNA levels for steroidogenic acute regulatory protein (StAR), which supplies cholesterol to P450scc by transporting it from the outer to the inner mitochondrial membrane, is also decreased by TGF-β1 (4). In bovine adrenocortical cells, TGF-β1 also reduced the transcript levels of hydroxyl-delta-5-steroid dehydrogenase, 3β- and steroid delta-isomerase 1 (Hsd3b1), and steroid 17-α-monooxygenase (Cyp17a1) (60).

It has recently been reported that TGF-β1 decreases cortisol and 11-hydroxyandrostenedione production induced by forskolin by 85% and production of aldosterone induced by angiotensin II by 80%. The activity of steroid 11β-hydroxylase (Cyp11b1) and the transcript levels for *Cyp11b1* induced by forskolin, as well as the activity of aldosterone synthase (Cyp11b2) and the transcript levels for *Cyp11b2* induced by angiotensin II, were strongly inhibited by TGF-β1 in the NCI-H295R cell line (61). TGF-β1 suppressed *Cyp11b1* promoter activity, but the Smads-binding sequence was not responsible for the transcriptional inhibition. This result suggests that TGF-β1 indirectly represses *Cyp11b1* promoter activity (61). Intriguingly, the mRNA levels of steroidogenic factor 1, the genetic deficiency of which causes the absence of gonadal and adrenal steroidogenic cells (64), were inhibited by TGF-β1 (59). Steroidogenic factor 1 binds to a shared promoter element of steroidogenic enzymes, including Cyp11b1, Cyp11b2, steroid 21-hydroxylase (Cyp21a1), and Cyp11a1 (80), and enhances their expression (52). Thus numerous steroidogenic steps that are upstream of the synthesis of aldosterone are suppressed by TGF-β1.

Indeed, it has been shown that plasma levels of aldosterone and the adrenal expression of several steroidogenic enzymes involved in the production of aldosterone, including Cyp11b1, Cyp11b2, Star, Hsd3b1, and Cyp21a1, are decreased as TGF-β1 expression is increased in mice having graded expression of TGF-β1 (46). The suppressive effect of overexpression of TGF-β1 on plasma levels of aldosterone was also observed in Akita diabetic mice (31).

Although TGF-β1 exerts a suppressive effect on adrenocortical production of aldosterone, the stimulatory effect of aldosterone on the activity of epithelial sodium channel (ENaC) is also inhibited by TGF-β1. Aldosterone expands the volume of extracellular fluid by increasing ENaC activity. ENaC is expressed in the "aldosterone-sensitive distal nephron," which is composed of collecting ducts, connecting tubules, and late distal convoluted tubules (21, 62). The aldosterone-sensitive distal nephron also expresses two other components required for sodium reabsorption in response to aldosterone: Na^+-K^+-ATPase and mineralocorticoid receptors.

Although aldosterone is the major regulator of ENaC activity, TGF-β1 also regulates ENaC activity either in combination with aldosterone or independently (6, 62). The aldosterone-induced increase in sodium transport via the ENaC is inhibited by TGF-β1 (40). TGF-β1 enhances β-ENaC internalization, which facilitates destabilization of the cell surface ENaC complex (78). It has also been found that the total activity, functional expression, and open probability of ENaC in mice underexpressing TGF-β1 are all greater than those in wild-type (46).

Protease nexin-1 (PN-1) has recently been found to inhibit prostasin, which augments the activity of ENaC(8, 103). Intriguingly, aldosterone and TGF-β1 reciprocally regulate the expressions of PN-1 and prostasin. Thus, prostasin expression is increased by aldosterone and decreased by TGF-β1, whereas PN-1 expression is decreased by aldosterone and increased by TGF-β1 (103).

Aldosterone and TGF-β1 differentially control the reabsorption of sodium in proximal tubular cells. Na^+-K^+-ATPase α1-subunits and mineralocorticoid receptors are expressed in the proximal tubules of the mice, rats, and humans (87). The activity of sodium/proton exchange is stimulated, and the expression of sodium/proton exchanger 3 on the cell surface is increased by aldosterone in cultured proximal tubular cells (14). By contrast, Na^+-K^+-ATPase α- and β-subunit expression and Na^+-K^+-ATPase activity are dose dependently decreased by TGF-β1 (73, 94).

Thus TGF-β1 has been suggested to inhibit both aldosterone production in the adrenal cortex and aldosterone actions in the kidney. Although TGF-β1 has been shown to directly increase ROS in cultured cells (95), it counteracts production and action of aldosterone, which enhance ROS generation and tissue fibrosis (7, 65). Inhibition of aldosterone by TGF-β1 may be a negative feedback mechanism in diabetic nephropathy.

TGF-β1 Suppresses Reabsorption of Water and Glucose in Proximal Tubules

Notably, the urine volume in TGF-β1 hypomorphic Akita diabetic mice was comparable to that in nondiabetic wild-type mice (31). In addition, the urinary output of glucose in diabetes was substantially decreased by genetic insufficiency of TGF-β1, despite unchanged plasma concentrations of insulin and glucose. The marked reduction in urinary water and glucose excretion by genetic insufficiency of TGF-β1 was also observed in their nondiabetic counterparts (31, 46). In addition, genetic disruption of Smad3, which is the intracellular signaling molecule downstream of TGF-β receptors, also reduced urine volume despite little change in plasma levels of glucose in STZ-induced diabetic mice (19). These findings indicate that TGF-β1 mediates hyperglycemia-induced polyuria and glucosuria, which has been attributed to osmotic diuresis.

The marked decrease in urinary water excretion in TGF-β1 hypomorphic mice cannot be explained merely by enhanced aldosterone function, because chronically high aldosterone levels have been demonstrated to increase urine volume due to impaired urine concentration ability (11). The diminished diuresis of the 10% hypomorphs was found to be fully reversed by overexpressing *Tgfb1* in the proximal tubule (31), and partially restored by overexpressing *Tgfb1* in the collecting duct cells (our unpublished observations), indicating that TGF-β1 suppresses the reabsorption of water in both proximal tubular cells and collecting ducts.

The finding that glucosuria and polyuria are both absent in the TGF-β1 hypomorphic diabetic mice may be related to the previous observation that the sodium/glucose cotransporters (SGLTs), which the proximal tubule highly expresses, cotransport each sugar molecule with >200 water molecules (63). In concordance with this inference, the urinary output of glucose was partially restored in Akita mice with the expression of *Tgfb1* augmented in proximal tubules (31). Furthermore, high glucose, hydrogen peroxide, and TGF-β1 have been shown to decrease the SGLT activity measured with α-methyl-D-gluco-

pyranoside, a metabolically inert analog of D-glucose, and the expression of SGLT 1 and 2 and sodium-hydrogen exchanger (NHE) 1 and 3 in primary cultured rabbit proximal tubule cells, which is associated with genetic expression profile of EMT (57).

Although the chronic efficacy of the inhibitors of SGLT 1 and 2, which are being used for the purpose of controlling blood glucose, on diabetic nephropathy is still controversial (20, 102), several studies suggest that SGLT inhibitors prevent cardiovascular and renal complications via reducing blood glucose levels (49, 70). Thus the facts that both diabetes by itself and TGF-β1 decrease the reabsorption of glucose in proximal tubular cells may be the compensatory mechanism preventing further tissue damage.

Conclusion

A number of features of diabetic nephropathy were absent in the TGF-β1 hypomorphic Akita mice, indicating that decreasing the expression of TGF-β1 may have therapeutic benefits in diabetics. However, the globally low expression of TGF-β1 resulted in primary aldosteronism (31, 46). Consequently, lowering the expression of TGF-β1 throughout the body could be deleterious in patients with diabetes. Nevertheless, these problems of TGF-β1 insufficiency might be circumvented if kidney-specific reduction of the expression of TGF-β1 were achieved.

Experimental results with tissue-specific TGF-β1 overexpression suggest that suppressing TGF-β1 expression in the proximal tubule is more likely to decrease albuminuria and interstitial fibrosis than to prevent the decrease in renal excretory function. In contrast, reducing expression of TGF-β1 in the podocyte may be more effective in avoiding the GFR decrease than in decreasing albuminuria and interstitial fibrosis. Thus manipulations which lead to decreased TGF-β1 expression in the podocyte may be useful for preventing/treating the decline in renal function in diabetic nephropathy.

Further studies are needed to unravel the mechanisms for the TGF-β1-induced accumulation of ECM and for the suppression of the effects of mineralocorticoids by TGF-β1, and this understanding might be useful in developing new therapeutic options for preventing and/or treating diabetic nephropathy.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: A.S.C. drafted manuscript; C.K.H. prepared figures; O.S. and M.K. edited and revised manuscript; O.S. and M.K. approved final version of manuscript.

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