Published in final edited form as:

Semin Nephrol. 2007 March ; 27(2): 153–160.

Regulation of Transforming Growth Factor–Beta in Diabetic Nephropathy: Implications for Treatment

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Abstract

The recognition of the drivers of matrix accumulation as a therapeutic target for diabetic nephropathy is accepted by the Nephrology and pharmaceutical community. Interventions focused around Transforming Growth Factor–beta (TGF–β) will likely be an important area of clinical investigation in the near future. Understanding the various pathways involved in stimulating TGF–β in the diabetic kidney is of paramount importance in devising strategies to combat the development and progression of diabetic nephropathy. In this review we highlight the major pathways involved in stimulating TGF–β production by elevated glucose and discuss the therapeutic implications.

Keywords

Protein Kinase C; reactive oxygen species; Upstream stimulatory factor; HETE; decorin; thrombospondin; macrophages; podocytes; glucose excursion

Transforming growth factor–β has been closely linked to the development and progression of diabetic nephropathy in cell culture, animal models, and the human condition^{1,2}. It is likely that there will be several strategies to target TGF–β production and action as a novel means of therapy for diabetic nephropathy within the next ten years. In this review, we will highlight several aspects regarding the regulation of TGF–β in the context of diabetic kidney disease. An aspect that will be highlighted is that there are multiple pathways by which TGF–β may be stimulated in diabetic kidney disease thus complicating approaches that attempt to block one pathway and not others.

Regulation of TGF–β by glucose

Elevations in glucose drives stimulation of TGF–β in cell culture, animal models and human studies. We recently demonstrated that a glucose infusion for 2h to raise plasma glucose to the 200–250 mg/dl range led to stimulation of urinary levels of TGF–β1 in normal human subjects³. This study demonstrates that transient elevation of blood glucose, even in healthy individuals, is sufficient to cause production of TGF–β. As urine levels of TGF–β1 increased and plasma levels were stable, the study suggested that the kidney may indeed be responding to hyperglycemia with increased TGF–β production. The mechanisms involved in this response

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remain to be proven in humans, however several pathways are likely involved based on experiments in cell culture and animal models.

Protein kinase C (PKC), reactive oxygen species (ROS), HETES, hexosamines, and the ERK-p38MAPK have all been implicated in mediating glucose induced stimulation of TGF- β . Protein kinase C has been found to be stimulated by glucose elevations in mesangial cells and vascular smooth muscle cells⁴⁻⁶. The isoform responsible for regulating TGF- β has not been conclusively demonstrated, although there is convincing data that the PKC- β isoform is intimately involved. The use of PKC- β inhibitors has been demonstrated to block TGF- β stimulation in animal models of diabetic kidney disease, as well as block matrix accumulation^{7,8}. These pre-clinical studies have contributed to recognizing the possible role of PKC- β inhibition in human diabetic kidney disease⁹. The PKC- α isoform is likely not involved in TGF- β stimulation as PKC- α knockout diabetic mice did not exhibit reduction of renal TGF- β levels as compared to wild type diabetic mice¹⁰.

Reactive oxygen species (ROS) production appears to be critical in the pathophysiology of diabetic vascular complications. High glucose induces ROS in mesangial cells¹¹ and ROS upregulates TGF- β and extracellular matrix (ECM) expression¹². In addition, Nath et al. reported that H₂O₂ was able to induce TGF- β mRNA expression in rat kidneys and isolated fibroblasts¹³. Scavenging of ROS by alpha-lipoic acid and manganese SOD dismutase leads to decreased TGF- β production in diabetic kidney disease and amelioration of diabetic renal pathology and albuminuria^{14,15}. Specific pathways that lead to ROS production by high glucose in renal cells will need to be determined and may pave the way for directed therapies to focus on ROS-induced TGF- β production. One such pathway is NADPH oxidase and the isoform Nox4. Specific inhibition of Nox 4 blocks glomerular matrix accumulation in diabetic rats¹⁶ and may be involved in stimulation of TGF- β . On the other hand, TGF- β itself could cause ROS production via NADH/NADPH oxidase¹⁷⁻¹⁹. We reported that TGF- β 1 induced ROS production in vascular smooth muscle and endothelial cells via NADPH oxidase and that Nox4 is involved in cytoskeletal alterations by TGF- β ^{18,19}.

Lipoxygenases (LO) are a family of enzymes that insert molecular oxygen into polysaturated fatty acids. They are classified as 5-, 8-, 12-, and 15-LO. 12-LO activation can produce 12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE]²⁰ and have been demonstrated to stimulate TGF- β in human macrophages²¹. High glucose has been demonstrated to stimulate 12/15-LO expression, and 12(S)-HETE (12-LO product) induces cellular hypertrophy and fibronectin expression in rat mesangial cells²². Recently, the Natarajan group²³ clarified the cross-talk between the TGF- β and 12/15-LO pathway in MCs. Direct addition of 12(S)-HETE to rat mesangial cells stimulated the murine TGF- β 1 promoter, TGF- β 1 mRNA and protein expression, along with p-Smad 2/3 activation. Reciprocally, TGF- β treatment of rat mesangial cells increased 12/15-LO mRNA expression and 12(S)-HETE production, significantly. In addition, mesangial cells from 12/15-LO knock out mice expressed less TGF- β , and mesangial cells overexpressing 12/15-LO produced more TGF- β . The authors suggested that 12/15-LO and TGF- β could cross-talk and activate each other during the initiation and progression of diabetic kidney disease²³.

Hexosamines, such as glucosamine-6-phosphate, can be formed under the control of the rate limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT). Immunostaining of GFAT demonstrated increased staining in diabetic kidneys, primarily in glomerular mesangial and epithelial cells²⁴. Inhibition of GFAT by chemical inhibitors can block TGF- β expression in mesangial cells treated with high glucose²⁵. The PKC and p38 pathway appears to be involved in mediating hexosamine induced TGF- β production in human mesangial cells²⁶.

ERK as well as p38 MAPK are activated in mesangial cells exposed to high glucose and in rat glomeruli of early type 1 diabetes^{27,28}. In a type 2 diabetic model, ERK activity was reported to be significantly activated in renal cortex of *db/db* mice as compared with non-diabetic mice²⁹. ERK activation may well be due to upstream PKC activation as PKC inhibition also inhibits ERK activation³⁰. There is a growing body of evidence supporting a role for p38 in diabetic kidney disease³¹ and its regulation of TGF- β ³². The mechanism of high glucose-induced p38 MAPK in monocytes and mesangial cells may be mediated via reactive oxygen species^{33,34} and possibly independent of PKC. High glucose has been shown to stimulate p38 in mesangial cells, podocytes, endothelial cells³⁵ as well as glomeruli in diabetic kidney disease³⁶. Inhibition of p38 may mediate renal TGF- β production in several models of kidney disease³⁷ and in proximal tubular cells³².

Transcription factors involved in glucose induced TGF- β stimulation

The major transcription factors involved in mediating glucose induced TGF- β 1 promoter activity has been postulated to include the AP-1 complex and the family of Upstream Stimulatory Factors (USF). Activation of PKC is well known to stimulate the *c-fos* and *c-jun* proto-oncogenes that form complexes for the AP1 binding site of the human and murine TGF- β 1 promoter^{38,39}. Mutagenesis of either one of the two or both AP-1 binding sites abolished the high glucose and PMA effect in the human promoter³⁸. Furthermore, addition of the AP-1 inhibitor curcumin blocked the glucose response³⁸. Interestingly, curcumin treatment of diabetic rats ameliorates kidney disease although no measurements of TGF- β 1 was performed^{40,41}. However, to date there are no *in vivo* studies to clearly implicate AP1 in glucose mediated renal TGF- β production.

Our group had initially identified the CACGTG element or E-box as an important site for glucose regulation in the murine TGF- β 1 promoter⁴². High glucose increased binding of mesangial cell nuclear proteins to this E-box element. Additional studies by our lab and others have now clearly determined that USF1 and USF2 are involved in binding the murine TGF- β 1 promoter⁴³ and the human TGF- β 1 promoter⁴⁴. In human mesangial cells, there is evidence of binding of both USF1 and USF2 to the human promoter⁴⁴. High glucose induced stimulation of the human TGF- β promoter via USFs and the E-box appears has been demonstrated to involve the hexosamine pathway⁴⁴. In murine mesangial cells, chromatin immunoprecipitation assay revealed *in vivo* binding of USF1, but not USF2, to a glucose-responsive region of the TGF- β 1 promoter. Furthermore, glucose fluctuation with high carbohydrate feeding led to stimulation of renal TGF- β 1 mRNA level in wild type and USF2 knockout mice but not in the USF1 knockout mice⁴³. Therefore, there is convincing data that both USF1 and USF2 are involved in regulating TGF- β 1 production by high glucose in mesangial cells and, at least in the murine system, the available evidence favors a dominant role for USF1. Future studies in various diabetic models are required to identify modulators of USF1 and USF2 to better understand the role of this family of transcription factors in the development of diabetic nephropathy.

TGF- β Activation: LAP, thrombospondin, and decorin

TGF- β is usually secreted in large latent complexes without biological activity. It consists of three components: a disulphide bonded homodimer of mature TGF- β , noncovalently bound to the latency-associated protein (LAP; homodimers of the N terminal fragment of precursor TGF- β) and a covalently attached molecule of latent TGF- β binding protein (LTBP)⁴⁵⁻⁴⁷. LAP and TGF- β compose the small latent complex. In this latent complex, TGF- β cannot bind to its surface receptors. Thus, the dissociation of TGF- β from LAP is a critical regulatory mechanism⁴⁵. Administration of LAP reduces glucose induced fibronectin production in

mesangial cells⁴⁸, however no studies to date have comprehensively examined regulation of latency associated peptide with active TGF- β in the setting of diabetic kidney disease.

Thrombospondin 1 (TSP1) is a homotrimeric multifunctional glycoprotein expressed by a variety of cell types such as platelets, vascular smooth muscle cells and mesangial cells. It is frequently expressed at sites of inflammation and wound healing⁴⁹, overexpressed in diabetic vessels⁵⁰, diabetic kidneys⁵¹ and secreted by mesangial cells⁵². Murphy-Ullrich et al.⁵³ first reported that TGF- β could bind to TSP1 under physiological conditions. More detailed studies identified two important sites in the TSP1 molecule that were responsible for this complex interaction. One is the WxxW (WSHW, WSPW or WGPW) motif from the type I repeats of the TSP1 that binds active TGF- β and the other site is the (K)RFK-sequence that binds the N-terminal LSKL-sequence of the LAP^{54,55}. Additional studies indicate that high glucose-induced activation of TGF- β is largely dependent on TSP-1. There is convincing data that high glucose stimulates TSP1 gene transcription via the USF2 transcription factor⁵⁶ and TSP1 then binds to the LAP thus dissociating active TGF- β . Interruption of this step may have therapeutic implications.

Decorin is a multi-functional extracellular proteoglycan⁵⁷, and its core protein neutralizes TGF- β and antagonizes its prosclerotic effect^{58,59}. Exogenous decorin suppressed TGF- β mRNA expression of the kidney⁵⁸. Mogyrosi et al. reported a rapid and sustained increase of decorin mRNA expression in an animal model of type 1 diabetes⁶⁰. Furthermore, decorin expression was enhanced by high glucose in mesangial cells and proximal tubular cells⁶⁰. Stimulation of decorin by high glucose has been shown by several studies^{61,62} and appears to be via a CREB dependent pathway⁶³. It was notable that decorin expression of mesangium and proximal tubular cells were suppressed by exogenous TGF- β treatment, both in high and normal glucose condition⁶⁰. As both decorin and TGF- β were upregulated in diabetic state, Mogyrosi et al. speculated that TGF- β and decorin act in negative feedback loop with each other⁶⁰.

In a human study, Schaefer et al. revealed that small proteoglycans including decorin mRNA were upregulated in all stages of diabetic nephropathy, both in the tubulointerstitium and in glomeruli⁶⁴. The authors pointed out the glomerular expression and protein accumulation of decorin were not prominent, compared with mRNA expression. The authors suggested that this phenomenon could be explained by assuming that decorin were secreted into the mesangial matrix and then cleared via the vasculature or the urinary tract, in part as complexes with TGF- β . In advanced diabetic nephropathy, decorin deposition was found in fibrotic area and was co-localized with deposits of type I collagen⁶⁴. Decorin may modulate progression of TGF- β mediated renal fibrosis through the formation of complexes of decorin-type I collagen-TGF- β . Decorin deficiency could result in an imbalance of decorin and TGF- β counter-regulation and lead to excess TGF- β activity and progressive matrix accumulation. Further studies are needed to clarify the relative role of decorin in mediating TGF- β activity and progression in diabetic nephropathy.

Role of Macrophage in TGF- β production

Most studies have focused on mesangial cells and proximal tubular cells as the cells responsible for TGF- β production in diabetic kidney disease^{32,65-67}. However, there are a variety of other cell types that may contribute as much or more to renal TGF- β production in diabetic kidney disease. Macrophage are a rich source of TGF- β and it is now clear that macrophage infiltration is a characteristic feature of diabetic nephropathy^{68,69}.

The role of macrophages in diabetic kidney disease remains to be clarified. Depletion of leukocytes by irradiation in the diabetic rat leads to reduced $\alpha 3$ (IV) collagen mRNA expression

in the glomeruli⁷⁰. Moreover, diabetic ICAM-1 knock out mice demonstrated reduced macrophage infiltration and decreased TGF- β and type IV collagen expression coincident with reduced mesangial matrix expansion and reduced albuminuria⁶⁸. It is likely there is an important cell-cell interaction between macrophages and mesangial cells, as it has been reported that the culture supernatant of macrophages stimulates mesangial cells to produce fibronectin⁷¹. Together with these findings, one could speculate that infiltrated glomerular macrophages and mesangial cells conspire to both secrete TGF- β and promote a positive feedback loop. A potential protective role may also apply to macrophages in early diabetic kidney disease. Glomerular mesangial cell production of hyaluronan leads to attraction of macrophages in early diabetes in the rat⁷². Macrophages appear to be responsible for removal of glomerular hyaluronan and macrophages may then depart. If macrophages remain it is likely that they may contribute to pathology. As it is likely true for all infiltrating cell types the context in which the cell is present is critical in understanding its role. A complex dual role for macrophages has also been suggested in the development of atherosclerosis⁷³ and in models of glomerulonephritis^{74,75}.

TGF- β and podocytes

There is a growing body of evidence that podocytes occupy a significant role in the pathogenesis of diabetic nephropathy, diabetic glomerular proteinuria and matrix accumulation⁷⁶⁻⁷⁸. A further discussion of the effect of TGF- β on podocytes is discussed in another chapter. The role of the podocytes as a cell type producing TGF- β in diabetic nephropathy has not been clarified. Human diabetic nephropathy kidney sections have been demonstrated to have increased glomerular mRNA and protein for TGF- β 1 in podocytes as well as mesangial cells⁵¹. It remains to be established whether the high-glucose condition stimulates TGF- β expression in podocytes. In human podocytes, high glucose induced increased TGF- β expression in both protein and mRNA levels⁷⁹. However, Iglesias-de la Cruz et al.⁸⁰ reported that high glucose did not stimulate the production of TGF- β 1 in cultured mouse podocytes, although high glucose did stimulate the expression of TGF- β type II receptor. In addition, Chen et al. demonstrated that AngII could induce podocyte dysfunction via TGF- β and VEGF activation, and these factors could lead to GBM thickness and albuminuria in diabetic nephropathy^{81,82}. Of note, it is intriguing that anti-TGF- β approaches are associated with reduction of albuminuria in some experimental studies of diabetic nephropathy⁸³ but not in others^{84,85}.

Implications for Anti-TGF- β Approaches

Based on the convincing body of work implicating stimulation of TGF- β in the diabetic milieu and its critical role in progressive nephropathy⁸⁶, a new theoretical basis for therapy emerges^{2,87}. Emerging from the hemodynamic and metabolic control paradigms, the next decade or so may be characterized by an anti-fibrotic and cell based therapeutic approach. The multiple pathways by which TGF- β may be stimulated in the diabetic condition leads to consideration of a multi-pronged strategy to improve metabolic control, decrease hemodynamic stress, and reduce local angII effects. However, with the understanding of reactive oxygen species and glucose-induced intracellular signaling pathways to stimulate TGF- β 1, it is clear that even sub-optimal glycemic control would lead to ongoing production of TGF- β in diabetes. Accumulating evidence suggests that blood glucose fluctuations are correlated with complications of diabetes⁸⁸. Future studies that determine the metabolic pathways activated by repeated glycemic fluctuations are likely to be important in understanding the basis for renal TGF- β production and serve as targets for therapeutic interventions. Furthermore, there will also likely be heterogeneity in the pathways involved among various ethnic groups and genders. Translational studies in humans will facilitate future personalized approaches for interventions to block TGF- β production in the kidney.

Acknowledgements

We would like to acknowledge funding for our work from the NIH (R01DK053867-07, R01DK063017-04, U01DK076133-02 to KS), the American Diabetes Association, and the Juvenile Diabetes Research Foundation.

References

1. Sharma K, Ziyadeh F. Biochemical events and cytokine interactions linking glucose metabolism to the development of diabetic nephropathy. *Semin Nephrol* 1997;17:80–92.
2. McGowan T, Zhu Y, Sharma K. Transforming growth factor-beta: a clinical target for the treatment of diabetic nephropathy. [Review]. *Current Diabetes Reports* 2004;4:447–454. [PubMed: 15539010]
3. McGowan TA, Dunn SR, Falkner B, Sharma K. Stimulation of Urinary TGF- β and Isoprostanes in Response to Hyperglycemia in Humans. *Clin J Am Soc Nephrol* 2006;1(2):263–268.
4. Whiteside C, Dlugosz JA. Mesangial cell protein kinase C isozyme activation in the diabetic milieu. *Am J Physiol-Renal Physiol* 2002;282:F975–F980. [PubMed: 11997313]
5. Studer RK, Craven PA, DeRubertis FR. Role for protein kinase C in the mediation of increased fibronectin accumulation by mesangial cells grown in high glucose medium. *Diabetes* 1993;42:118–126. [PubMed: 8420808]
6. Lee TS, Saltsman KA, Ohashi H, King GL. Activation of protein kinase C by elevation of glucose concentration: Proposal for a mechanism in the development of diabetic vascular complications. *Proc. Natl. Acad. Sci. USA* 1989;86:5141–5145. [PubMed: 2740348]
7. Koya D, Jirousek M, Lin Y, Ishii H, Kuboki K, King G. Characterization of protein kinase C beta isoform activation on the gene expression of transforming growth factor-beta, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats. *J Clin Invest* 1997;115:126.
8. Koya D, Haneda M, Nakagawa H, Isshiki K, Sato H, Maeda S, Sugimoto T, Yasuda H, Kashiwagi A, Watanabe DK. Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes. *FASEB J* 2000;14(3):439–47. [PubMed: 10698958]
9. Tuttle KR, Bakris GL, Toto RD, McGill JB, Hu K, Anderson PW. The Effect of Ruboxistaurin on Nephropathy in Type 2 Diabetes. *Diabetes Care* 2005;28(11):2686–2690. [PubMed: 16249540]
10. Menne J, Park J-K, Boehne M, Elger M, Lindschau C, Kirsch T, Meier M, Gueler F, Fiebeler A, Bahlmann FH. Diminished Loss of Proteoglycans and Lack of Albuminuria in Protein Kinase C- α -Deficient Diabetic Mice. *Diabetes* 2004;53(8):2101–2109. [PubMed: 15277392]
11. Hua H, Munk S, Goldberg H, Fantus IG, Whiteside CI. High Glucose-suppressed Endothelin-1 Ca²⁺ + Signaling via NADPH Oxidase and Diacylglycerol-sensitive Protein Kinase C Isozymes in Mesangial Cells. *J. Biol. Chem* 2003;278(36):33951–33962. [PubMed: 12821678]
12. Iglesias-De La Cruz MC, Ruiz-Torres P, Alcamí J, Díez-Marques L, Ortega-Velázquez R, Chen S, Rodríguez-Puyol M, Ziyadeh FN, Rodríguez-Puyol D. Hydrogen peroxide increases extracellular matrix mRNA through TGF- β in human mesangial cells. *Kidney International* 2001;59(1):87–95. [PubMed: 11135061]
13. Nath KA, Grande J, Croatt A, Haugen J, Kim Y, Rosenberg ME. Redox regulation of renal DNA synthesis, transforming growth factor- β and collagen gene expression. *Diabetes* 1998;47(2):367.
14. Melhem MC, Liachenko J, DeRubertis FR. Alpha-lipoic acid attenuates hyperglycemia and prevents glomerular mesangial matrix expansion in diabetes. *J Am Soc Nephrol* 2002;13:108–116. [PubMed: 11752027]
15. DeRubertis F, Craven P, Melhem M, Salah E. Attenuation of renal injury in db/db mice overexpressing superoxide dismutase: evidence for reduced superoxide-nitric oxide interaction. *Diabetes* 2004;53:762–768. [PubMed: 14988262]
16. Gorin Y, Block K, Hernandez J, Bhandari B, Wagner B, Barnes JL, Abboud HE. Nox4 NAD(P)H oxidase mediates hypertrophy and fibronectin expression in the diabetic kidney. *J. Biol. Chem* 2005;280:12200.
17. Thannickal VJ, Fanburg BL. Activation of an H₂O₂-generating NADH Oxidase in Human Lung Fibroblasts by Transforming Growth Factor β 1*. *J Biol Chem* 1995;270:30334–30338. [PubMed: 8530457]

18. Hu T, RamachandraRao SP, Siva S, Valancius C, Zhu Y, Mahadev K, Toh I, Goldstein BJ, Woolkalis M, Sharma K. Reactive oxygen species production via NADPH oxidase mediates TGF- β -induced cytoskeletal alterations in endothelial cells. *Am J Physiol Renal Physiol* 2005;289(4):F816–825. [PubMed: 16159901]
19. Sharma K, Cook A, Smith M, Valancius C, Inscho EW. TGF- β impairs renal autoregulation via generation of ROS. *Am J Physiol Renal Physiol* 2005;288(5):F1069–1077. [PubMed: 15644487]
20. Yamamoto S. Mammalian lipoxygenases: molecular structures and functions. *Biochim Biophys Acta* 1992;1128:117–131. [PubMed: 1420284]
21. Leonarduzzi G, Scavazza A, Biasi F, Chiarpotto E, Camandola S, Vogel S, Dargel R, Poli G. The lipid peroxidation end product 4-hydroxy-2,3-nonenal up-regulates transforming growth factor β 1 expression in the macrophage lineage: a link between oxidative injury and fibrosclerosis. *Faseb J* 1997;11:851–857. [PubMed: 9285483]
22. Reddy M, Adler SG, Kim YS, Lanting L, Rossi J, Kang SW, Nadler JL, Shahed A, Natarajan R. Interaction of MAPK and 12-lipoxygenase pathways in growth and matrix protein expression in mesangial cells. *Am J Physiol Renal Physiol* 2002;283:F985–994. [PubMed: 12372774]
23. Kim Y-S, Xu Z-G, Reddy MA, Li S-L, Lanting L, Sharma K, Adler SG, Natarajan R. Novel Interactions between TGF- β 1 Actions and the 12/15-Lipoxygenase Pathway in Mesangial Cells. *J Am Soc Nephrol* 2005;16(2):352–362. [PubMed: 15615821]
24. Nerlich A, Sauer U, Kolm-Litty V, Wagner E, Koch M, Schleicher ED. D. Expression of glutamine:fructose-6-phosphate amidotransferase in human tissues: evidence for high variability and distinct regulation in diabetes. *Diabetes* Feb;1998 47:170–178. [PubMed: 9519709]
25. Kolm-Litty V, Sauer U, Nerlich A, Lehmann R, Schleicher E. High glucose-induced transforming growth factor- β 1 production is mediated by the hexosamine pathway in porcine glomerular mesangial cells. *J. Clin. Invest* 1998;101:160–169. [PubMed: 9421478]
26. Burt D, Gruden G, Thomas SM, Tutt P, Dell'Anna C, Viberti GC, Gnudi L. p38 mitogen-activated protein kinase mediates hexosamine-induced TGF β 1 mRNA expression in human mesangial cells. *Diabetologia* 2003;46:531–537. [PubMed: 12679868]
27. Haneda M, Koya D, Kikkawa R. Cellular Mechanisms in the development and progression of diabetic nephropathy: activation of the DAG-PKC-ERK Pathway. *American Journal of Kidney Diseases* 2001;38(N 4 Supplement 1 October):S178–S181. [PubMed: 11576950]
28. Kang HS, Adler S, LaPage J, Natarajan R. p38 MAPK and MAPK kinase 3/6 mRNA mRNA and activities are increased in early diabetic glomeruli. *Kidney Int* 2001;60:543–552. [PubMed: 11473637]
29. Feliars D, Duraisamy S, Faulkner JL, Duch J, Lee AV, Abboud H, Choudhury G, Kasinath BS. Activation of renal signaling pathways in *db/db* mice with type 2 diabetes. *Kidney Int* 2001;60:495–504. [PubMed: 11473632]
30. Haneda M, Araki S, Togawa M, Sugimoto T, Isono M, Kikkawa R. Mitogen-activated protein kinase cascade is activated in glomeruli of diabetic rats and glomerular mesangial cells cultured under high glucose conditions. *Diabetes* 1997;46:847–853. [PubMed: 9133554]
31. Adhikary L, Chow F 2, Nikolic-Paterson DJ, Stambe C, Dowling J, Atkins RC, Tesch GH. Abnormal p38 mitogen-activated protein kinase signalling in human and experimental diabetic nephropathy. *Diabetologia* 2004;47:1210–1222.
32. Zhang M, Fraser D, Phillips A. ERK, p38, and Smad Signaling Pathways Differentially Regulate Transforming Growth Factor- β 1 Autoinduction in Proximal Tubular Epithelial Cells. *Am J Pathol* 2006;169(4):1282–1293. [PubMed: 17003485]
33. Guha M, Bai W, Nadler JL, Natarajan R. Molecular Mechanisms of Tumor Necrosis Factor α Gene Expression in Monocytic Cells via Hyperglycemia-induced Oxidant Stress-dependent and -independent Pathways. *J. Biol. Chem* 2000;275(23):17728–17739. [PubMed: 10837498]
34. Wilmer W, Dixon C, Herbert C. Chronic exposure to high glucose environment activates the p38 MAPK pathway. *Kidney Int* 2001;60:858–871. [PubMed: 11532081]
35. Kuki S, Imanishi T, Kobayashi K, Matsuo Y, Obana M, Akasaka T. Hyperglycemia accelerated endothelial progenitor cell senescence via the activation of p38 mitogen activated protein kinase. *Circulation J* 2006;70:1076–1081.

36. Dai T, Natarajan R, Nast C, LaPage J, Chuang P, Sim J, Tong L, Chamberlin M, Wang S, Adler S. Glucose and diabetes: Effects on podocyte and glomerular p38MAPK, heat shock protein 25, and actin cytoskeleton. *Kidney Int* 2006;69:806–814. [PubMed: 16421517]
37. Wada TAH, Furuichi K, Sakai N, Kitagawa K, Iwata Y, Matsushima K, Takahara SYH, Kaneko S. Reduction in chronic allograft nephropathy by inhibition of p38 mitogen-activated protein kinase. *Am J Nephrol* 2006;26:319–325. [PubMed: 16816533]
38. Weigert C, Sauer U, Brodbeck K, Pfeiffer A, Haring H, Schleicher ED. AP-1 proteins mediate hyperglycemia-induced activation of the human TGF- β 1 promoter in mesangial cells. *J Am Soc Nephrol* 2000;11:2007–2016. [PubMed: 11053476]
39. Geiser AG, Kim S-J, Roberts AB, Sporn MB. Characterization of the mouse transforming growth factor- β 1 promoter and activation by the Ha-ras oncogene. *Mol Cellular Biol* 1991;11:84–92. [PubMed: 1986255]
40. Suresh BP, Srinivasan K. Amelioration of renal lesions associated with diabetes by dietary curcumin in streptozotocin diabetic rats. *Mol Cell Biochem* 1998;181:87–96. [PubMed: 9562245]
41. Sharma S, Kulkarni SK, Chopra K. CURCUMIN, THE ACTIVE PRINCIPLE OF TURMERIC (CURCUMA LONGA), AMELIORATES DIABETIC NEPHROPATHY IN RATS. *Clinical and Experimental Pharmacology and Physiology* 2006;33(10):940–945. [PubMed: 17002671]
42. Hoffman B, Sharma K, Zhu Y, Ziyadeh FN. Transcriptional activation of transforming growth factor- β 1 in mesangial cell culture by high glucose concentration. *Kidney Int* 1998;54:1107–1116. [PubMed: 9767526]
43. Zhu Y, Casado M, Vaulont S, Sharma K. Role of Upstream Stimulatory Factors in Regulation of Renal Transforming Growth Factor- β 1. *Diabetes* 2005;54(7):1976–1984. [PubMed: 15983197]
44. Weigert C, Brodbeck K, Sawadogo M, Haring HU, Schleicher ED. Upstream Stimulatory Factor (USF) Proteins Induce Human TGF- β 1 Gene Activation via the Glucose-response Element-1013/-1002 in Mesangial Cells: Up-regulation of USF activity by the hexosamine biosynthetic pathway. *J. Biol. Chem* 2004;279(16):15908–15915. [PubMed: 14757763]
45. Miyazano, K.; Heldin, C-H., editors. Wiley; Chichester, UK: 1991. Latent forms of TGF- β : Molecular structure and mechanisms of activation; p. 81-92.
46. Roberts AB. TGF- β signaling from receptors to the nucleus. *Microbes Infect* 1999;1(15):1265–73. [PubMed: 10611754]
47. Bottlinger E, Factor V, Tsang M, Weatherbee J, Kopp J, Qian S, Wakefield L, Roberts A, Thorgeirsson S, Sporn M. The recombinant proregion of transforming growth factor β 1 (latency-associated peptide) inhibits active transforming growth factor β 1 in transgenic mice. *Proc Natl Acad Sci U S A* 1996;93:5877–5882. [PubMed: 8650186]
48. Nomura KTH, Kuboki K, Inokuchi T. Transforming growth factor- β 1 latency-associated peptide and soluble betaglycan prevent a glucose-induced increase in fibronectin production in cultured human mesangial cells. *Nephron* 2002;91:606–611. [PubMed: 12138262]
49. Bornstein P. Diversity of function is inherent in matricellular proteins: an appraisal of thrombospondin 1. *J Cell Biol* 1995;130:503–506. [PubMed: 7542656]
50. Stenina OI, Krukovets I, Wang K, Zhou Z, Forudi F, Penn MS, Topol EJ, Plow EF. Increased Expression of Thrombospondin-1 in Vessel Wall of Diabetic Zucker Rat. *Circulation* 2003;107(25):3209–3215. [PubMed: 12810612]
51. Wahab N, Schaefer L, Weston BS, Yiannikouris O, Wright A, Babelova A, Schaefer R, Mason RM. Glomerular expression of thrombospondin-1, transforming growth factor β and connective tissue growth factor at different stages of diabetic nephropathy and their interdependent roles in mesangial response to diabetic stimuli. *Diabetologia* 2005;48:2650–2660. [PubMed: 16270194]
52. Raugi G, Lovett D. Thrombospondin secretion by cultured human glomerular mesangial cells. *Am J Pathol* 1987;129:364–372. [PubMed: 3314532]
53. Murphy-Ullrich JES-CS, Hook M. Transforming growth factor- β complexes with thrombospondin. *Mol Biol Cell* 1992;3:181–188. [PubMed: 1550960]
54. Schultz-Cherry S, Chen H, Mosher DF, Misenheimer TM, Krutzsch HC, Roberts DD, Murphy-Ullrich JE. Regulation of transforming growth factor- β activation by discrete sequences of thrombospondin 1. *J Biol Chem* 1995;270:7304–7310. [PubMed: 7706271]

55. Ribeiro S, Poczaek M, Schultz-Cherry S, Villain M, Murphy-Ullrich JE. The activation sequence of thrombospondin-1 interacts with the latency-associated peptide to regulate activation of latent transforming growth factor-beta. *J Biol Chem* 1999;274:13586–13593. [PubMed: 10224129]
56. Wang S, Skorzewski J, Feng X, Mei L, Murphy-Ullrich JE. Glucose Up-regulates Thrombospondin 1 Gene Transcription and Transforming Growth Factor- β Activity through Antagonism of cGMP-dependent Protein Kinase Repression via Upstream Stimulatory Factor 2. *J. Biol. Chem* 2004;279(33):34311–34322. [PubMed: 15184388]
57. Weber I, Harrison R, Iozzo R. Model structure of decorin and implications for collagen fibrillogenesis. *The Journal of Biological Chemistry* 1996;271(50):31767–31770. [PubMed: 8943211]
58. Yamaguchi Y, Mann DM, Ruoslahti E. Negative regulation of transforming growth factor- β by the proteoglycan decorin. *Nature* 1990;346:281–284. [PubMed: 2374594]
59. Hildebrand AR, Rasmussen LM, Heinegard D, Twardzik DR, Border WA, Ruoslahti E. Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor beta. *Biochemical Journal* 1994;302:527–534. [PubMed: 8093006]
60. Mogyrosi A, Ziyadeh FN. Increased decorin mRNA in diabetic mouse kidney and in mesangial and tubular cells cultured in high glucose. *American Journal of Physiology-Renal Physiology* 1998;275:F827–F832.
61. Holmes D, Wahab N, Mason R. Identification of glucose-regulated genes in human mesangial cells by mRNA differential display. *Biochemical and Biophysical Research Communications* 1997;238:179–184. [PubMed: 9299475]
62. Murphy M, Godson C, Cannon S, Kato S, Mackenzie HS, Martin F, Brady HR. Suppression subtractive hybridization identifies high glucose levels as a stimulus for expression of connective tissue growth factor and other genes in human mesangial cells. *J Biol Chem* 1999;274(9):5830–4. [PubMed: 10026205]
63. Wahab N, Parker S, Sraer J-D, Mason R. The decorin high glucose response element and mechanism of its activation in human mesangial cells. *J Am Soc Nephrol* 2000;11:1607–1619. [PubMed: 10966485]
64. Schaefer L, Raslik I, Grone H-J, Schonherr E, Macakova K, Ugorcakova J, Budny S, Schaefer R, Kresse H. Small proteoglycans in human diabetic nephropathy: discrepancy between glomerular expression and protein accumulation of decorin, biglycan, lumican, and fibromodulin. *The FASEB Journal*. 2001
65. Wolf G, Sharma K, Chen Y, Ericksen M, Ziyadeh FN. High glucose-induced proliferation in mesangial cells is reversed by autocrine TGF- β . *Kidney Int* 1992;42:647–656. [PubMed: 1357223]
66. Sharma K, Ziyadeh FN. The emerging role of transforming growth factor- β in kidney diseases. *Am J Physiol* 1994;266:F829–F842. [PubMed: 8023963]
67. Ziyadeh FN, Sharma K, Ericksen M, Wolf G. Stimulation of collagen gene expression and protein synthesis in murine mesangial cells by high glucose is mediated by activation of transforming growth factor- β . *J Clin Invest* 1994;93:536–542. [PubMed: 8113392]
68. Okada S, Shikata K, Matsuda M, Ogawa D, Usui H, Kido Y, Nagase R, Wada J, Shikata Y, Makino H. Intercellular Adhesion Molecule-1-Deficient Mice Are Resistant Against Renal Injury After Induction of Diabetes. *Diabetes* 2003;52(10):2586–2593. [PubMed: 14514644]
69. Usui H, Shikata K, Matsuda M, Okada S, Ogawa D, Yamashita T, Hida K, Satoh M, Wada J, Makino H. HMG-CoA reductase inhibitor ameliorates diabetic nephropathy by its pleiotropic effects in rats. *Nephrol Dial Transplant* 2003;18:265–272. [PubMed: 12543879]
70. Sassy-Prigent C, Heudes D, Mandet C, Belair MF, Michel O, Perdereau B, Bariety J, Bruneval P. Early glomerular macrophage recruitment in streptozotocin-induced diabetic rats. *Diabetes* 2000;49:466–475. [PubMed: 10868970]
71. Pawluczyk I, Harris KP. Macrophages promote prosclerotic responses in cultured rat mesangial cells: a mechanism for the initiation of glomerulosclerosis. *J Am Soc Nephrol* 1997;8:1525–1536. [PubMed: 9335380]
72. Wang A, Hascall VC. Hyaluronan Structures Synthesized by Rat Mesangial Cells in Response to Hyperglycemia Induce Monocyte Adhesion. *J. Biol. Chem* 2004;279(11):10279–10285. [PubMed: 14679194]

73. van Berkel TJOR, Hoekstra M, Kuiper J, Biessen E, van Eck M. Oct. Scavenger receptors: friend or foe in atherosclerosis? *Curr Opin Lipidol* 2005;16:525–535. [PubMed: 16148537]
74. Nishida M, Fujinaka H, Matsusaka T, Price J, Kon V, Fogo AB, Davidson JM, Linton MF, Fazio S, Homma T. Absence of angiotensin II type 1 receptor in bone marrow-derived cells is detrimental in the evolution of renal fibrosis. *J. Clin. Invest* 2002;110(12):1859–1868. [PubMed: 12488436]others
75. Oliver JA. Unexpected news in renal fibrosis. *J. Clin. Invest* 2002;110(12):1763–1764. [PubMed: 12488424]
76. Pagtalunan ME, Miller PL, Jumping-Eagle S, Nelson RG, Myers BD, Rennke HG, Coplon NS, Sun L, Meyer TW. Podocyte loss and progressive glomerular injury in type II diabetes. *Journal of Clinical Investigation* 1997;99(2):342–8. [PubMed: 9006003]
77. Wolf G, Chen S, Ziyadeh FN. From the periphery of the glomerular capillary wall toward the center of disease: podocyte injury comes of age in diabetic nephropathy. *Diabetes* 2005;54:1626–1634. [PubMed: 15919782]
78. Steffes MW, Schmidt D, McCrery R, Basgen JM. Glomerular cell number in normal subjects and in type 1 diabetic patients. *Kidney Int* 2001;59(6):2104–13. [PubMed: 11380812]
79. van Det N, Verhagen NA, Tamsma JT, Berden JH, Bruijn JA, Daha MR, van der Woude FJ. Diabetes. Regulation of glomerular epithelial cell production of fibronectin and transforming growth factor-beta by high glucose, not by angiotensin II. *Diabetes* 1997;46:834–840. [PubMed: 9133552]
80. De La Cruz MCI, Ziyadeh F, Isono M, Kouahou M, Han D, Kalluri R, Mundel P, Chen S. Effects of high glucose and TGF-B1 on the expression of collagen IV and vascular endothelial growth factor in mouse podocytes. *Kidney Int* 2002;62:1–13. [PubMed: 12081558]
81. Chen S, Kasama Y, Lee JS, Jim B, Marin M, Ziyadeh FN. Podocyte-derived vascular endothelial growth factor mediates the stimulation of alpha3(IV) collagen production by transforming growth factor-beta1 in mouse podocytes. *Diabetes* 2004;53:2939–2949. [PubMed: 15504975]
82. Chen S, Lee JS, Iglesias-de la Cruz MC, Wang A, Izquierdo-Lahuerta A, Gandhi NK, Danesh FR, Wolf G, Ziyadeh FN. Angiotensin II stimulates alpha3(IV) collagen production in mouse podocytes via TGF-beta and VEGF signalling: implications for diabetic glomerulopathy. *Nephrol Dial Transplant* 2005;20:1320–1328. [PubMed: 15840669]
83. Benigni A, Zoja C, Corna D, Zatelli C, Conti S, Campana M, Gagliardini E, Rottoli D, Zanchi C, Abbate M. Add-on anti-TGF-beta antibody to ACE inhibitor arrests progressive diabetic nephropathy in the rat. *J Am Soc Neph* 2003;14:1816–1824.others
84. Ziyadeh F, Hoffman B, Han D, Iglesias-de la Cruz C, Hong S, Isono M, Chen S, McGowan T, Sharma K. Long-term prevention of renal insufficiency excess matrix gene expression and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-b antibody in db/db diabetic mice. *Proc Natl Acad Sci U S A* 2000;97:8015–8020. [PubMed: 10859350]
85. Chen S, Iglesias-de la Cruz M, Hong S, Isono M, Ziyadeh F. Reversibility of established diabetic glomerulopathy by anti-TGF-b antibodies in db/db mice. *Biochem Biophys Res Commun* 2002;300in press
86. Sharma K, Ziyadeh FN. Hyperglycemia and diabetic kidney disease. The case for transforming growth factor-beta as a key mediator. *Diabetes* 1995;44(10):1139–1146. [PubMed: 7556948]
87. Ziyadeh FN, Sharma K. Overview: Combating Diabetic Nephropathy. *J Am Soc Nephrol* 2003;14(5):1355–1357. [PubMed: 12707405]
88. Del Prato S, Tiengo A. The importance of first-phase insulin secretion: implications for the therapy of type 2 diabetes mellitus. *Diabetes Metab Res Rev* 2001;17:164–174. [PubMed: 11424229]