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Antifibrotic Treatment and Other New Strategies for Improving Renal Outcomes

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Abstract

Diabetic nephropathy (DN) is clinically characterized by proteinuria and hypertension. Investigations suggest that matrix accumulation and inflammatory processes contribute to the pathological features of this progressive disease. This chapter reviews novel targeted approaches to the treatment of DN, with the goal of slowing the progression and improving renal function. Many studies support the use of agents that block the renin-angiotensin-aldosterone system in DN. Novel, oral agents that are promising in early clinical studies are agents such as pirfenidone and bardoxolone as they are associated with early improvement in renal function in patients with advanced diabetic kidney disease. Additionally, strategies that inhibit inflammatory cytokines, chemokines, adhesion molecules and mediators of the innate immune response may provide novel targets for the treatment of DN. Larger clinical studies are eagerly awaited to determine if new agents that specifically block kidney fibrosis and inflammation will delay, arrest and possibly reverse progressive renal failure.

Clinically, progressive diabetic nephropathy (DN) is characterized by heavy proteinuria, high blood pressure and decline in the glomerular filtration rate (GFR). Pathologically, the degree of mesangial matrix accumulation, tubulointerstitial fibrosis and inflammation portend the decline in renal function. Evaluating treatment approaches from the viewpoint of blocking matrix accumulation and inflammation within the kidney may offer more potent and targeted approaches to prevent decline and possibly improve renal function in advanced DN.

Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone system (RAAS) pathway has been the central component of therapeutic measures to prevent progression of diabetic kidney disease. In addition to angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ARBs), direct renin inhibition using agents like aliskiren are showing increasing potential

to prevent the progression of DN. Renin may promote kidney disease via the activation of the mitogen-activated protein kinases, ERK1 and ERK2, transforming growth factor- β (TGF- β) secretion and hypertrophic and proliferative effects independently of angiotensin II [1, 2]. In the diabetic transgenic (mRen-2)27 rat, which has greatly enhanced tissue RAS and plasma prorenin levels, aliskiren and perindopril were equally effective in reducing blood pressure and albuminuria, but aliskiren reduced tubulointerstitial fibrosis to a greater extent than perindopril [3]. In a separate study, using the same model system, aliskiren also reduced renal expression of TGF- β and collagen I, and attenuated the expression of the renin receptor [4].

In two placebo-controlled studies, aliskiren was as effective as the ARB irbesartan, and their combination was even more effective than monotherapy in reducing baseline albuminuria. The Aliskiren in the Evaluation of Proteinuria in Diabetes study is a multinational, randomized, double-blind, placebo-controlled study that included 599 patients with hypertension, type 2 diabetes and nephropathy. The double-blinded phase compared the combination of aliskiren and losartan with losartan plus placebo. The results showed that the combination of aliskiren and losartan was 20% more effective ($p < 0.001$) than losartan alone in reducing the mean urinary albumin-creatinine ratio [5]. Also, there is an ongoing international phase 3 trial looking at aliskiren 300 mg OD versus placebo in hypertensive diabetics looking at renal outcomes of time to dialysis, transplantation and creatinine >6 mg/dl or sustained doubling of creatinine above upper limit of the central laboratory (NCT00549757).

There is accumulating interest in a local aldosterone axis within the kidney and specifically within podocytes [6]. Several studies have suggested that aldosterone may be produced within the kidney independent of the adrenal gland [7]. Additionally, podocyte expression of the mineralocorticoid receptor (MR) appears to be activated even without exposure to aldosterone [8]. As aldosterone blockade may be more effective than an ARB to reduce proteinuria in patients on ACEI therapy [9], new approaches that block the MR or block local aldosterone production may be beneficial. However, systemic aldosterone or MR blockade can cause hyperkalemia necessitating close follow-up of electrolytes.

Profibrotic Growth Factors

Of the many growth factors that have been implicated as the direct effectors of the RAAS, TGF- β appears to be the central player mediating the fibrotic pathway in the diabetic kidney of type 1 and type 2 diabetes. In the streptozotocin-induced diabetes model, anti-TGF- β antibody was able to reduce hypertrophy and mRNA production of matrix proteins [10]. The protective effects seem to be independent of albuminuria reduction as anti-TGF- β therapy in the *db/db* mice prevented expansion of mesangial matrix, but not albuminuria. This suggests a renoprotective effect of anti-TGF- β antibodies independent of albuminuria [11]. The finding that an antifibrotic approach may confer renoprotection at a histologic level but may not reduce albuminuria, has been identified with several antifibrotic and anti-inflammatory approaches [reviewed in 12].

Studies have demonstrated synergistic effects of the RAAS blockade and anti-TGF- β antibody on the progression of proteinuria and renal injury in experimental DN and other models. Phase I/II trials of human anti-TGF- β antibody (CAT-192) in 45 patients with scleroderma showed no evidence of antifibrotic effects; however, clinical studies with new neutralizing anti-TGF- β antibodies are in early phase [13]. Several large pharmaceutical companies are in the process of pursuing clinical trials with anti-TGF- β antibodies for progressive kidney disease, and these results are eagerly awaited (NCT00125385, NCT00464321). Strategies to manipulate TGF- β activity include upregulating Betaglycan, a soluble TGF- β coreceptor and decorin, a leucine-rich proteoglycan that can bind active TGF- β . Betaglycan has been shown to reduce structural and functional renal injury [14], and the decorin knockout mice exhibit enhanced diabetic kidney disease [15]. Another strategy would be to inhibit thrombospondin activity, as this molecule is stimulated in diabetic kidney disease and can enhance activation of latent TGF- β [16].

Pirfenidone [5-methyl-1-phenyl-2(1H)-pyridone] is a small synthetic molecule with potent antifibrotic and hydroxyl ion scavenging properties. In renal cells, pirfenidone blocks the TGF- β promoter, TGF- β protein secretion, and phosphorylation of the downstream TGF- β target SMAD 2 [17]. Pirfenidone reduces tubulointerstitial and glomerular lesions, collagen and TGF- β production in animal models of diabetes, subtotal nephrectomy and unilateral obstruction. Additionally, pirfenidone treatment significantly reduced mesangial matrix expansion and expression of renal matrix genes in *db/db* mice with DN without affecting albuminuria. Using a combined proteomics and protein-protein interaction networks, pirfenidone was found to inhibit the eukaryotic initiation factor 4E protein. Pirfenidone in clinical studies has shown promising effects in patients with idiopathic and advanced focal segmental glomerulosclerosis. In a recently completed placebo controlled randomized clinical trial with 77 patients with diabetic kidney disease and proteinuria, pirfenidone showed improvement in estimated GFR after one-year follow-up [18]. The major reported side effects were gastrointestinal symptoms and photosensitivity. These findings provide support for a larger clinical trial to determine the renoprotective benefit of this convenient oral drug.

Another novel pyridine agent, fluorofenodine, has recently been studied in a model of renal cells stimulated by angiotensin II. The data showed that fluorofenodine reduced the production of reactive oxygen species and inhibited TGF- β expression more potently than pirfenidone [19]. Finally, anthraquinone compounds isolated from rhubarb have shown a potential to improve diabetic kidney injury: in vitro, they reduced hypertrophy and extracellular matrix expansion in tubular epithelial cells; in vivo, they attenuated renal alterations in *db/db* mice [20, 21].

Bone morphogenic protein 7 (BMP-7) is a member of the TGF- β superfamily, and has been shown to play an important role in diabetic kidney disease [22]. Overexpression of BMP-7 and inhibition of gremlin (a BMP-7 antagonist) ameliorate features of diabetic kidney disease [23]. Another potential target is SMP-534, a molecule that inhibits p38 signaling downstream of TGF- β . In the *db/db* mice model, SMP-534 decreased extracellular matrix and ameliorated progression of glomerular fibrosis greater than losartan alone and had an additive effect when combined with losartan [24, 25].

Tranilast [N-(3,4-dimethoxycinnamoyl) anthranilic acid] suppresses collagen synthesis by interfering with the actions of TGF- β . In one study, tranilast reduced the slope of the reciprocal of serum creatinine and decreased urinary proteinuria and urinary type IV collagen [26]. Similar observations were made in a study on proteinuric (<1 g) diabetics with normal creatinine; there was no change in creatinine after one year of treatment with tranilast; however, the urinary protein and urinary type IV collagen levels decreased from baseline levels in the treatment group [27]. In diabetic rat models, tranilast decreased albuminuria, TGF- β excretion, and mesangial expansion [28].

Nrf2 is a transcription factor controlling antioxidant genes that help maintain redox homeostasis. Nrf2 also inhibits TGF- β mRNA production. Nrf2-deficient mice have increased reactive oxygen species production and renal injury. Bardoxolone is an anti-inflammatory agent that acts via the Nrf2 pathway. A recent study presented at the American Society of Nephrology meeting in 2010, revealed encouraging benefits at week 24 of the ongoing 52-week trial with 200 diabetic proteinuric patients, mostly chronic kidney disease stage 3b. Bardoxolone treatment was associated with a fairly rapid increase in GFR that was seen as early as 4 weeks, with a continuous increase up to 12 weeks. The increase was sustained through week 24, with the average increase in GFR of 10.1 ml/min/1.73 m², compared with no change in the placebo group (NCT00811889).

Connective tissue growth factor (CTGF) is a downstream partner of TGF- β and may also play an independent role in the progression of renal fibrosis. Plasma CTGF level is an early marker of renal disease and may be a good predictor of end-stage renal disease in patients with DN [29]. Its inhibition by anti-sense oligonucleotides, siRNA or neutralizing antibodies can prevent the extracellular matrix expansion associated with several experimental models of kidney disease. CTGF is highly expressed in podocytes during the development of diabetic kidney disease and, in contrast to anti-TGF- β antibodies, there appears to be a reduction in albuminuria with anti-CTGF treatment in early phase studies. Diabetic mice models with CTGF attenuation have decreased fibrosis, proteinuria and progression of nephropathy [30–32]. In a recent phase I study, anti-CTGF antibodies were administered intravenously every 14 days for four doses and then followed up at day 62 and 365. Urinary albumin/creatinine ratio (ACR) decreased significantly from mean pretreatment ACR of 48 mg/g to mean posttreatment (day 56) ACR of 20 mg/g ($p = 0.027$) without evidence for a dose-response relationship and minimal side effects [33]. There seems to be minimal infusion adverse events and no significant drug-attributable adverse effects over 1 year of follow-up. Changes in albuminuria were promising but require validation in a prospective, randomized, blinded study.

Hepatocyte growth factor (HGF) is considered an endogenous inhibitor of TGF- β with multiple renoprotective properties that may find therapeutic application in the prevention of chronic kidney disease. Multiple animal studies have demonstrated that the administration of recombinant HGF protein or a corresponding gene therapy ameliorates several forms of chronic renal dysfunction [34]. A combination of exogenous HGF and an ARB (losartan) exhibited a synergistic efficacy in slowing the progression of renal fibrosis in a model of murine obstructive nephropathy [35]. Increased vascular endothelial growth factor (VEGF) production causes glomerular hypertrophy and is associated with proteinuria [36].

Semaxanib (SU5416) is a VEGF inhibitor that reduces albuminuria in mouse models of DN [37].

Alternate modes of drug delivery such as ultrasound microbubbles may be useful to target the kidney with specific neutralizing antibodies against key mediators involved in the progression of DN such as TGF- β . Although this technology was recently used in a model of liver fibrosis in rats, more studies are needed in DN [38].

Advanced Glycation End Product Pathway

The receptor for advanced glycation end products (RAGE) activates multiple intracellular pathways and amplifies the inflammatory effects of AGEs. Recently, the soluble form of the RAGE was shown to decrease AGE-induced vascular injury [39]. Anti-RAGE antibody administered in a type 1 diabetic animal model decreased the urine albumin excretion, kidney weight and creatinine clearance [40]. Pyridoxamine is a form of vitamin B₆ that blocks formation of preglycated amadori compounds, advanced lipoxidation protein end-products and scavenges reactive carbonyl species [41]. Pyridoxamine decreased proteinuria, TGF- β and laminin 1 mRNA expression in animal models of type 2 diabetes [42]. In phase 2 clinical studies, pyridoxamine showed benefit in decreasing serum creatinine and proteinuria, and a phase 2b trial is currently underway [43].

Other Novel Pathways

Studies suggest that vitamin D analogs downregulate the RAAS pathway. In murine models of diabetes, paricalcitol, an active analog of vitamin D, acts synergistically with losartan in decreasing albuminuria, glomerular inflammation and sclerosis both in type 1 and type 2 models [44]. Similar results were seen with doxercalciferol, another vitamin D analog, which impedes progression of diabetic kidney disease and downregulates the intrarenal RAAS [45]. A recent clinical study, VITAL, showed reduction in albuminuria in diabetic patients taking paricalcitol (2 μ g/day), when compared to placebo [46].

The sodium glucose cotransporter (SGLT2) is a high-capacity, low-affinity proximal tubular cell transporter that reabsorbs filtered glucose. Mutations in this transporter lead to renal glycosuria. In diabetic animal models, there appears to be upregulation of the SGLT2 receptors and GLUT2 mRNA. SGLT2 antagonism provides an interesting therapeutic option to decrease glucose loads in diabetes. Nonspecific SGLT2 inhibitors like phlorizin have disturbing gastrointestinal side effects. More specific inhibitors like dapagliflozin and sergliflozin are being tested in small clinical trials. The effect on the progression of diabetic kidney disease remains to be studied [47].

Glucagon-like peptide is a gut hormone that improves insulin secretion in pancreatic cells, and its agonist exendin-4 (Eventide) ameliorates albuminuria, glomerular hyperfiltration, hypertrophy and essential matrix expansion in diabetic rats without changing blood pressure or body weight. Exendin-4 also prevented macrophage infiltration, and decreased protein levels of intercellular adhesion molecule-1 (ICAM-1) and type IV collagen, as well as decreasing oxidative stress and nuclear factor- κ B activation in kidney tissue [48]. Long-term treatment of *db/db* mice with exendin-4 decreased albuminuria. Glomerular hypertrophy,

mesangial matrix expansion, TGF- β_1 expression, and type IV collagen accumulation and associated glomerular lipid accumulation were significantly decreased [49]. Taken together, exendin-4 treatment seems to ameliorate DN as well as the metabolic abnormalities.

Inflammatory Pathway

The progression and manifestations of DN are likely associated with activation of the innate and adaptive immune systems [50]. Therefore, investigators have employed multiple strategies to interfere with inflammatory signaling networks. The innate immune response is activated by Toll-like receptors (TLRs) that bind to pattern recognition peptides, promoting a rapid response to microbial pathogens. TLRs are present on many intrinsic renal cells and infiltrating leukocytes, and TLR expression is augmented in type 1 and type 2 diabetic patients [51] and in the kidneys of diabetic rodents. Given the link between diabetes and inflammation, modulation of TLR expression may be an attractive target for treatment of DN.

Overexpression of cytokines including tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , IL-6 and IL-18 has been implicated in the progression of DN [51]. In rodent models of diabetes, reduction in these cytokines has improved renal outcomes. Multiple cytokines signal through STAT-3; reduction in STAT-3 activity dampens inflammation (IL-6, MCP-1, activated NF- κ B, TGF- β , ICAM-1) and abnormal matrix synthesis in early DN. Suppressors of cytokine signaling negatively regulate activation of cytokine-stimulated JAK/STAT pathways in DN and may prove to be another efficient therapeutic target [52]. The immunosuppressant mycophenolate mofetil and pentoxifylline (decreases TNF- α) reduce cytokine elaboration and ameliorate manifestations of diabetic kidney disease.

Chemokines are molecules that drive the tissue migration of inflammatory cells. One of the most actively studied chemokines is monocyte chemoattractant protein-1 (MCP-1)/CCL2. MCP-1 attracts and activates monocytes and macrophages, and plays a central role in promoting renal injury. Blockade of MCP-1 or its receptor CCR2 improves DN, and pharmacologic blockers of MCP-1 activity are currently in preclinical human studies. Inhibition of the RAAS (ACEI, ARBs, spironolactone) and nuclear receptor ligands (thiazolidinediones, vitamin D) can also block MCP-1 activity and improve renal abnormalities in diabetic kidney disease. Other chemokines that may play a role in the progression of chronic inflammatory diseases include fractalkine/CX3CL1, CXCL12, IL-8, macrophage inflammatory protein-1 α , interferon- γ inducible protein-10 and RANTES [53].

Migration and infiltration of inflammatory cells into tissues requires leukocyte adhesion molecules, including ICAM-1 and vascular adhesion molecule. Inhibitors of ICAM-1 are currently being developed for treatment in chronic inflammatory disease. Furthermore, uric acid upregulates ICAM-1 expression. Thus, modulators of gout including xanthine oxidase inhibitors and colchicine are being employed in human and rodent studies to investigate progression of diabetic kidney disease.

The endoplasmic reticulum (ER) stress pathway is activated during cellular stress and is designed to repress protein synthesis and increase ER chaperone content to facilitate efficient trafficking of proteins through the ER. This pathway is activated in the diabetic

kidney, and currently efforts are under way to identify novel regulators of this pathway with the goal of improving the progression of diabetic kidney disease [54].

Conclusion

There are many new promising targets for therapy that may retard the progression and improve renal structure and function in DN. Two oral agents, pirfenidone and bardoxolone, appear especially promising as renal function improvement was found in early phase trials. These studies suggest that renal functional improvement may occur in patients even with advanced disease but will need validation in larger trials. The results of ongoing clinical studies are eagerly awaited to determine if new agents that specifically block kidney fibrosis and inflammation will delay or arrest progressive renal failure.

References

1. Nguyen G, Delarue F, Burckle C, Bouzahir L, Giller T, Sraer JD. Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest*. 2002; 109:1417–1427. [PubMed: 12045255]
2. Huang J. The important role of angiotensin receptor blocker on treatment of chronic heart failure (in Chinese). *Zhonghua Xin Xue Guan Bing Za Zhi*. 2006; 34:773–774. [PubMed: 17217679]
3. Kelly DJ, Zhang Y, Moe G, Naik G, Gilbert GE. Aliskiren, a novel renin inhibitor, is renoprotective in a model of advanced diabetic nephropathy in rats. *Diabetologia*. 2007; 50:2398–2404. [PubMed: 17828524]
4. Feldman DL, Jin L, Xuan H, Contrepas A, Zhou Y, Webb RL, Mueller DN, Feldt S, Cumin F, Maniara W, et al. albuminuria, and (pro)renin receptor expression in diabetic TG(mRen-2)27 rats. *Hypertension*. 2008; 52:130–136. [PubMed: 18490518]
5. Parving HH, Persson F, Lewis JB, Lewis EJ, Hollenberg NK. Aliskiren combined with losartan in type 2 diabetes and nephropathy. *N Engl J Med*. 2008; 358:2433–2446. [PubMed: 18525041]
6. Calhoun DA, Sharma K. The role of aldosteronism in causing obesity-related cardiovascular risk. *Cardiol Clin*. 2010; 28:517–527. [PubMed: 20621254]
7. Siragy HM, Xue C. Local renal aldosterone production induces inflammation and matrix formation in kidneys of diabetic rats. *Exp Physiol*. 2008; 93:817–824. [PubMed: 18296490]
8. Shibata S, Nagase M, Yoshida S, Kawarazaki W, Kurihara H, Tanaka H, Miyoshi J, Takai Y, Fujita T. Modification of mineralocorticoid receptor function by Rac1 GTPase: implication in proteinuric kidney disease. *Nat Med*. 2008; 14:1370–1376. [PubMed: 19029984]
9. Mehdi UF, Adams-Huet B, Raskin P, Vega GL, Toto RD. Addition of angiotensin receptor blockade or mineralocorticoid antagonism to maximal angiotensin-converting enzyme inhibition in diabetic nephropathy. *J Am Soc Nephrol*. 2009; 20:2641–2650. [PubMed: 19926893]
10. Sharma K, Jin Y, Guo Y, Ziyadeh FN. Neutralization of TGF-beta by anti-TGF-beta antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. *Diabetes*. 1996; 45:522–530. [PubMed: 8603776]
11. Ziyadeh FN, Hoffman BB, Han DC, Iglesias-De La Cruz MC, Hong SW, Isono M, Chen S, McGowan TA, Sharma K. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice. *Proc Natl Acad Sci USA*. 2000; 97:8015–8020. [PubMed: 10859350]
12. Declèves AE, Sharma K. New pharmacological treatment for improving renal outcomes in diabetes. *Nat Rev Nephrol*. 2010; 6:371–380. [PubMed: 20440278]
13. Denton CP, Merkel PA, Furst DE, Khanna D, Emery P, Hsu VM, Silliman N, Streisand J, Powell J, Akesson A, et al. Recombinant human anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebocontrolled phase I/II trial of CAT-192. *Arthritis Rheum*. 2007; 56:323–333. [PubMed: 17195236]

14. Juarez P, Vilchis-Landeros MM, Ponce-Coria J, Mendoza V, Hernandez-Pando R, Bobadilla NA, Lopez-Casillas F. Soluble betaglycan reduces renal damage progression in db/db mice. *Am J Physiol Renal Physiol*. 2007; 292:F321–F329. [PubMed: 16954341]
15. Merline R, Lazaroski S, Babelova A, Tsalastra-Greul W, Pfeilschifter J, Schluter KD, Gunther A, Iozzo RV, Schaefer RM, Schaefer L. Decorin deficiency in diabetic mice: aggravation of nephropathy due to overexpression of profibrotic factors, enhanced apoptosis and mononuclear cell infiltration. *J Physiol Pharmacol*. 2009; 60(suppl 4):5–13. [PubMed: 20083846]
16. Daniel C, Schaub K, Amann K, Lawler J, Hugo C. Thrombospondin-1 is an endogenous activator of TGF-beta in experimental diabetic nephropathy in vivo. *Diabetes*. 2007; 56:2982–2989. [PubMed: 17878288]
17. RamachandraRao SP, Zhu Y, Ravasi T, McGowan TA, Toh I, Dunn SR, Okada S, Shaw MA, Sharma K. Pirfenidone is renoprotective in diabetic kidney disease. *J Am Soc Nephrol*. 2009; 20:1765–1775. [PubMed: 19578007]
18. Sharma K, Mathew AV, Fervenza FC, Cho M, Pflueger A, Dunn SR, Francos B, Sharma S, Falkner B, McGowan TA, Donohue M, Ramachandrarao SP, Xu R, Kopp JB. Pirfenidone for diabetic nephropathy: a randomized placebo controlled clinical trial. *J Am Soc Nephrol*. 2011 in press.
19. Peng ZZ, Hu GY, Shen H, Wang L, Ning WB, Xie YY, Wang NS, Li BX, Tang YT, Tao LJ. Fluorofenidone attenuates collagen I and transforming growth factor-beta1 expression through a nicotinamide adenine dinucleotide phosphate oxidase-dependent way in NRK-52E cells. *Nephrology (Carlton)*. 2009; 14:565–572. [PubMed: 19712256]
20. Zheng JM, Zhu JM, Li LS, Liu ZH. Rhein reverses the diabetic phenotype of mesangial cells over-expressing the glucose transporter (GLUT1) by inhibiting the hexosamine pathway. *Br J Pharmacol*. 2008; 153:1456–1464. [PubMed: 18264122]
21. Gao Q, Qin WS, Jia ZH, Zheng JM, Zeng CH, Li LS, Liu ZH. Rhein improves renal lesion and ameliorates dyslipidemia in db/db mice with diabetic nephropathy. *Planta Med*. 2010; 76:27–33. [PubMed: 19639539]
22. Sugimoto H, Grahovac G, Zeisberg M, Kalluri R. Renal fibrosis and glomerulosclerosis in a new mouse model of diabetic nephropathy and its regression by bone morphogenic protein-7 and advanced glycation end product inhibitors. *Diabetes*. 2007; 56:1825–1833. [PubMed: 17456853]
23. Mitu GM, Wang S, Hirschberg R. BMP7 is a podocyte survival factor and rescues podocytes from diabetic injury. *Am J Physiol Renal Physiol*. 2007; 293:F1641–F1648. [PubMed: 17804487]
24. Sugaru E, Nakagawa T, Ono-Kishino M, Nagamine J, Tokunaga T, Kitoh M, Hume WE, Nagata R, Taiji M. Enhanced effect of combined treatment with SMP-534 (antifibrotic agent) and losartan in diabetic nephropathy. *Am J Nephrol*. 2006; 26:50–58. [PubMed: 16508247]
25. Sugaru E, Nakagawa T, Ono-Kishino M, Nagamine J, Tokunaga T, Kitoh M, Hume WE, Nagata R, Taiji M. Amelioration of established diabetic nephropathy by combined treatment with SMP-534 (antifibrotic agent) and losartan in db/db mice. *Nephron Exp Nephrol*. 2007; 105:e45–e52. [PubMed: 17139189]
26. Soma J, Sato K, Saito H, Tsuchiya Y. Effect of tranilast in early-stage diabetic nephropathy. *Nephrol Dial Transplant*. 2006; 21:2795–2799. [PubMed: 16820373]
27. Soma J, Sugawara T, Huang YD, Nakajima J, Kawamura M. Tranilast slows the progression of advanced diabetic nephropathy. *Nephron*. 2002; 92:693–698. [PubMed: 12372957]
28. Akahori H, Ota T, Torita M, Ando H, Kaneko S, Takamura T. Tranilast prevents the progression of experimental diabetic nephropathy through suppression of enhanced extracellular matrix gene expression. *J Pharmacol Exp Ther*. 2005; 314:514–521. [PubMed: 15857946]
29. Nguyen TQ, Tarnow L, Jorsal A, Oliver N, Roestenberg P, Ito Y, Parving HH, Rossing P, van Nieuwenhoven FA, Goldschmeding R. Plasma connective tissue growth factor is an independent predictor of end-stage renal disease and mortality in type 1 diabetic nephropathy. *Diabetes Care*. 2008; 31:1177–1182. [PubMed: 18344285]
30. Yokoi H, Mukoyama M, Mori K, Kasahara M, Suganami T, Sawai K, Yoshioka T, Saito Y, Ogawa Y, Kuwabara T, et al. Overexpression of connective tissue growth factor in podocytes worsens diabetic nephropathy in mice. *Kidney Int*. 2008; 73:446–455. [PubMed: 18075496]

31. Yokoi H, Mukoyama M, Nagae T, Mori K, Suganami T, Sawai K, Yoshioka T, Koshikawa M, Nishida T, Takigawa M, et al. Reduction in connective tissue growth factor by antisense treatment ameliorates renal tubulointerstitial fibrosis. *J Am Soc Nephrol.* 2004; 15:1430–1440. [PubMed: 15153554]
32. Guha M, Xu ZG, Tung D, Lanting L, Natarajan R. Specific down-regulation of connective tissue growth factor attenuates progression of nephropathy in mouse models of type 1 and type 2 diabetes. *FASEB J.* 2007; 21:3355–3368. [PubMed: 17554073]
33. Adler SG, Schwartz S, Williams ME, Arauz-Pacheco C, Bolton WK, Lee T, Li D, Neff TB, Urquilla PR, Sewell KL. Phase I study of anti-CTGF monoclonal antibody in patients with diabetes and microalbuminuria. *Clin J Am Soc Nephrol.* 2010; 5:1420–1428. [PubMed: 20522536]
34. Mizuno S, Nakamura T. Suppressions of chronic glomerular injuries and TGF-beta 1 production by HGF in attenuation of murine diabetic nephropathy. *Am J Physiol Renal Physiol.* 2004; 286:F134–F143. [PubMed: 14519594]
35. Yang J, Dai C, Liu Y. Hepatocyte growth factor gene therapy and angiotensin II blockade synergistically attenuate renal interstitial fibrosis in mice. *J Am Soc Nephrol.* 2002; 13:2464–2477. [PubMed: 12239235]
36. Liu E, Morimoto M, Kitajima S, Koike T, Yu Y, Shiiki H, Nagata M, Watanabe T, Fan J. Increased expression of vascular endothelial growth factor in kidney leads to progressive impairment of glomerular functions. *J Am Soc Nephrol.* 2007; 18:2094–2104. [PubMed: 17554151]
37. Mendel DB, Laird AD, Smolich BD, Blake RA, Liang C, Hannah AL, Shaheen RM, Ellis LM, Weitman S, Shawver LK, et al. Development of SU5416, a selective small molecule inhibitor of VEGF receptor tyrosine kinase activity, as an anti-angiogenesis agent. *Anticancer Drug Des.* 2000; 15:29–41. [PubMed: 10888034]
38. Deelman LE, Decleves AE, Rychak JJ, Sharma K. Targeted renal therapies through microbubbles and ultrasound. *Adv Drug Deliv Rev.* 2010; 62:1369–1377. [PubMed: 20946925]
39. Yamamoto Y, Doi T, Kato I, Shinohara H, Sakurai S, Yonekura H, Watanabe T, Myint KM, Harashima A, Takeuchi M, et al. Receptor for advanced glycation end products is a promising target of diabetic nephropathy. *Ann N Y Acad Sci.* 2005; 1043:562–566. [PubMed: 16037279]
40. Jensen LJ, Denner L, Schrijvers BF, Tilton RG, Rasch R, Flyvbjerg A. Renal effects of a neutralising RAGE-antibody in long-term streptozotocin-diabetic mice. *J Endocrinol.* 2006; 188:493–501. [PubMed: 16522729]
41. Onorato JM, Jenkins AJ, Thorpe SR, Baynes JW. Pyridoxamine, an inhibitor of advanced glycation reactions, also inhibits advanced lipoxidation reactions. Mechanism of action of pyridoxamine. *J Biol Chem.* 2000; 275:21177–21184. [PubMed: 10801874]
42. Tanimoto M, Gohda T, Kaneko S, Hagiwara S, Murakoshi M, Aoki T, Yamada K, Ito T, Matsumoto M, Horikoshi S, et al. Effect of pyridoxamine (K-163), an inhibitor of advanced glycation end products, on type 2 diabetic nephropathy in KK-A(y)/Ta mice. *Metabolism.* 2007; 56:160–167. [PubMed: 17224327]
43. Williams ME, Bolton WK, Khalifah RG, Degenhardt TP, Schotzinger RJ, McGill JB. Effects of pyridoxamine in combined phase 2 studies of patients with type 1 and type 2 diabetes and overt nephropathy. *Am J Nephrol.* 2007; 27:605–614. [PubMed: 17823506]
44. Deb DK, Sun T, Wong KE, Zhang Z, Ning G, Zhang Y, Kong J, Shi H, Chang A, Li YC. Combined vitamin D analog and AT1 receptor antagonist synergistically block the development of kidney disease in a model of type 2 diabetes. *Kidney Int.* 2010; 77:1000–1009. [PubMed: 20182412]
45. Zhang Y, Deb DK, Kong J, Ning G, Wang Y, Li G, Chen Y, Zhang Z, Strugnelli S, Sabbagh Y, et al. Long-term therapeutic effect of vitamin D analog doxercalciferol on diabetic nephropathy: strong synergism with AT1 receptor antagonist. *Am J Physiol Renal Physiol.* 2009; 297:F791–F801. [PubMed: 19535571]
46. de Zeeuw D, Agarwal R, Amdahl M, Audhya P, Coyne D, Garimella T, Parving HH, Pritchett T, Remuzzi G, Ritz E, Andress D. Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial. *Lancet.* 2010; 376:1543–1551. [PubMed: 21055801]

47. Vallon V, Sharma K. Sodium-glucose transport: role in diabetes mellitus and potential clinical implications. *Curr Opin Nephrol Hypertens*. 2010; 19:425–431. [PubMed: 20539226]
48. Kodera R, Shikata K, Kataoka HU, Takatsuka T, Miyamoto S, Sasaki M, Kajitani N, Nishishita S, Sarai K, Hirota D, et al. Glucagon-like peptide-1 receptor agonist ameliorates renal injury through its anti-inflammatory action without lowering blood glucose level in a rat model of type 1 diabetes. *Diabetologia*. 2011 Epub ahead of print.
49. Park CW, Kim HW, Ko SH, Lim JH, Ryu GR, Chung HW, Han SW, Shin SJ, Bang BK, Breyer MD. Long-term treatment of glucagon-like peptide-1 analog exendin-4 ameliorates diabetic nephropathy through improving metabolic anomalies in db/db mice. *J Am Soc Nephrol*. 2007; 18:1227–1238. [PubMed: 17360951]
50. Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia*. 1998; 41:1241–1248. [PubMed: 9794114]
51. Rivero A, Mora C, Muros M, Garcia J, Herrera H, Navarro-Gonzalez JF. Pathogenic perspectives for the role of inflammation in diabetic nephropathy. *Clin Sci (Lond)*. 2009; 116:479–492. [PubMed: 19200057]
52. Ortiz-Munoz G, Lopez-Parra V, Lopez-Franco O, Fernandez-Vizarra P, Mallavia B, Flores C, Sanz A, Blanco J, Mezzano S, Ortiz A, Egido J, Gomez-Guerrero C. Suppressors of cytokine signaling abrogate diabetic nephropathy. *J Am Soc Nephrol*. 2010; 21:763–772. [PubMed: 20185635]
53. Ruster C, Wolf G. The role of chemokines and chemokine receptors in diabetic nephropathy. *Front Biosci*. 2008; 13:944–955. [PubMed: 17981602]
54. Sharma K, Cunard R. The endoplasmic reticulum stress response and diabetic kidney disease. *American Journal of Physiology Renal*. 2011 in press.