The management of CKD: A look into the future

A Khwaja[1], M El Kossi[1], J Floege[2] and M El Nahas[1]

[1]Department of Academic Nephrology, Sheffield Kidney Institute, Sheffield, UK and [2]Division of Nephrology and Immunology, RWTH University, Aachen, Germany, on behalf of the European Kidney Institute (EKI)

The increasing global prevalence of chronic kidney disease (CKD) and end-stage renal disease with the associated spiraling cost has profound public health and economic implications. This has made slowing the progression of CKD, a major health-care priority. CKD is invariably characterized by progressive kidney fibrosis and at present, treatment aiming to slow the progression of CKD is limited to aggressive blood pressure control, with few therapies targeting the fibrotic process itself. In this review, we explore the potential of experimental therapeutic strategies, based on preventing or reversing the pathophysiologic steps of kidney remodeling that lead to fibrosis.

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Correspondence: *A Khwaja, Division of Nephrology, Sheffield Kidney Institute, Northern General Hospital, Herries Road, Sheffield S5 7AU, UK. E-mail: arif.khwaja@sth.nhs.uk*

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Recent reports suggest that up to 10% of the population may be affected by chronic kidney disease (CKD).[1,2] The global pandemic of CKD is fueled by population ageing as well as the rise in the numbers of those affected by diabetes, obesity, and hypertension. The rising tide of CKD has major health-care repercussions not only in terms of end-stage renal disease service provisions but also in view of its major multiplier impact on cardiovascular disease.

Irrespective of the underlying cause, progressive CKD is characterized histologically by the concurrent development of glomerulosclerosis and tubulointerstitial fibrosis. Podocyte damage and loss has been identified as a key mechanism, at which a number of glomerular pathomechanisms converge to result in glomerulosclerosis.[3,4] The mesangial cell is the major matrix forming cell in the glomerulus and is also pivotal to the glomerulosclerotic process, while the activated (α-smooth muscle actin-positive) interstitial fibroblast or myofibroblast is central to the development of tubulointerstitial fibrosis. The particular importance of tubulointerstitial damage in progressive CKD was highlighted in an extensive systematic morphometric analysis of over 1700 renal biopsies, which showed that interstitial inflammation and fibrosis were significantly associated with a more rapid decline of kidney function.[5] Furthermore, analysis of interstitial fibroblasts from diseased kidneys demonstrated that these cells played a key role in fibrogenesis due to increased proliferative activity and their ability to synthesize extracellular matrix (ECM) proteins.[6] The cellular mechanisms underlying this fibrotic process are characterized by kidney cell loss through necrosis and apoptosis, epithelial–mesenchymal transformation (EMT) (reversal to an embryonic phenotype), and proliferation of myofibroblasts with subsequent increased ECM synthesis along with decreased breakdown and clearance of ECM (Figure 1).

Currently, treatment of CKD is mostly limited to aggressive control of hypertension with a particular focus on agents inhibiting the renin–angiotensin–aldosterone system, which have been reviewed extensively elsewhere.[7] However, so far there are no clinical therapies specifically targeting kidney remodeling and the ensuing fibrotic process itself. In this review, we discuss a number of therapeutic approaches that have the potential of being translated to the clinical arena within the foreseeable future. In particular, we will focus on experimental therapies targeting the key cellular events that are central to kidney fibrosis, namely cellular loss, transformation, proliferation, and ECM accumulation.

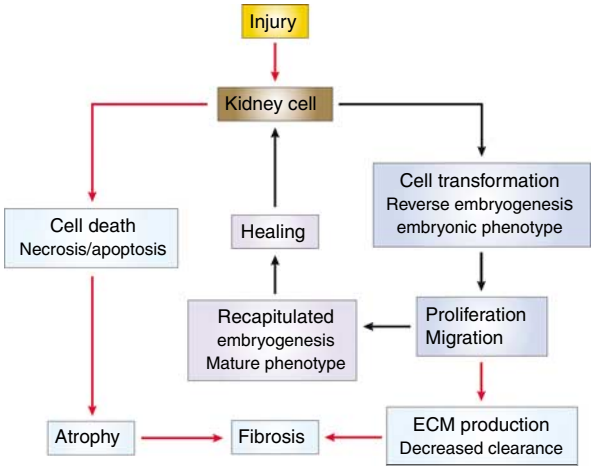

Figure 1 | The cellular mechanisms underlying this fibrotic process are characterized by kidney cell loss through necrosis and apoptosis, EMT (reversal to an embryonic phenotype), and proliferation of myofibroblasts with subsequent increased ECM synthesis along with decreased breakdown and clearance of ECM.

TARGETING CELLULAR EVENTS UNDERLYING FIBROSIS

Cell loss and proliferation

The response to glomerular and tubulointerstitial cell injury in kidney diseases invariably involves changes in cell number (i.e. cell proliferation, apoptosis, and necrosis) and/or cell size (hypertrophy). These events typically precede the accumulation of ECM, and therefore the balance between these differing processes determines whether the response to an insult to the kidney leads to resolution of glomerular/tubulointerstitial injury and healing, or results in progressive fibrosis and scarring (Figure 1). Thus pharmacological manipulation of these factors may modulate the fibrotic process in CKD.

Cell loss

Loss of intrinsic glomerular and tubular epithelial cells is likely to play a pivotal role in the initiation and progression of CKD. Loss of mesangial cells through apoptosis has been implicated in the initiation of glomerulosclerosis in a number of experimental models of glomerulonephritis.[8] In the anti-Thy1.1 glomerulonephritis model, mesangial apoptosis is followed by mesangial repair through migration into the glomerulus and proliferation of mesangial remnants.[8,9] While mesangial cell apoptosis is an essential mechanism in normalizing cell number during the reparative mesangioproliferative response to glomerular injury as in the anti-Thy1.1 nephritis model, excessive apoptosis promotes glomerulosclerosis.[10] Loss of glomerular endothelial and epithelial cells has also been associated with glomerulosclerosis.[11] Podocyte loss through apoptosis, with consequent denudement of the glomerular basement membrane and the formation of capsular adhesions, has been shown to initiate glomerulosclerosis.[3,4,12] Tubular epithelial cell apoptosis in excess of proliferation is a key factor in the development of both acute and chronic tubulointerstitial fibrosis. Indeed in both immune- and nonimmune-mediated models of CKD, we and others have noted a progressive increase in kidney cell apoptosis as tubular atrophy and kidney scarring progress.[8,13] These histological manifestations of scarring were associated with the activation of a number of pro-apoptotic pathways, the upregulation of pro-apoptotic genes and proteins, including those involved in the caspase enzymatic system, and a parallel downregulation of apoptosis inhibitors such as Bcl2.[14] Thus targeting these pro-apoptotic mediators may attenuate the fibrotic response to tissue injury. For example, administration of a pan-caspase inhibitor, B-D-FMK, in the nephrotoxic serum nephritis model, significantly decreased glomerular inflammation and reduced both glomerular and interstitial fibrosis. This was associated with the preservation of kidney cell number and a significant reduction in proteinuria.[13]

While such therapies appear promising it is important to recognize that under certain circumstances apoptosis may actually promote tissue healing in glomerulonephritis by clearing excessive numbers of resident glomerular cells and infiltrating inflammatory cells.[15] Thus, therapies targeting apoptosis may have a double-edged effect—on the one hand, decreasing the generation of the pro-inflammatory cytokines such as interleukin-1,[13] known to be activated by the caspase enzymatic system, while on the other simultaneously preventing the clearance of pro-inflammatory cells within damaged kidneys thereby hampering recovery.

Cell proliferation

It is widely accepted that α-smooth muscle actin-positive myofibroblasts are the principal effector cells in fibrogenesis, with increased proliferation of myofibroblasts preceding ECM expansion in both glomerular and tubulointerstitial compartments.[16] Therefore therapies targeting mediators of myofibroblast formation and proliferation such as extracellular mitogens, cell-cycle proteins, and intracellular signaling pathways may have therapeutic benefit in kidney fibrosis.

Platelet-derived growth factor (PDGF) is a potent renal glomerular cell and fibroblast mitogen. Its inhibition is effective in reducing experimental glomerular inflammatory and proliferative changes. A number of agents have been shown to target PDGF in models of proliferative glomerulonephritis including PDGF aptamers and Trapidil, an anti-platelet agent that is a potent PDGF antagonist.[17] Imatinib mesylate is a c-abl tyrosine kinase inhibitor, which also blocks the PDGF receptor and is used for the treatment of chronic myeloid leukemia. It was found to be effective in reducing kidney injury in experimental mesangioproliferative glomerulonephritis and attenuated kidney fibrosis in animal models of chronic allograft nephropathy, obstructive as well as diabetic nephropathy.[17] However, imatinib exhibits some cardiotoxicity and affects bone remodeling,[18,19] and trials in renal patients have been placed on hold by the manufacturer; later generation tyrosine kinase inhibitors are currently being

tested for potentially better safety profiles. Other more specific strategies targeting PDGF are already available. For instance, antagonism of PDGF-D with the human monoclonal antibody CR002 is effective in preventing scarring in the anti-Thy1.1 glomerulonephritis model and this antibody has already completed a phase I clinical study.[17]

Epidermal growth factor (EGF) stimulates kidney fibroblast proliferation and collagen expression *in vitro*. Inhibiting EGF preserves kidney function in a model of polycystic kidney disease and attenuates glomerular fibrosis in a model of fibrosis and hypertension.[20] A number of agents targeting EGF signaling are in therapeutic use in a variety of human cancers. *In vivo* work using these inhibitors may help clarify the role of EGF in kidney fibrosis. Of relevance is the role played by the EGF receptor (EGFR) in the progression of kidney scarring; transgenic mice harboring a dominant-negative form of EGFR is resistant to the progression of renal lesions induced by nephron reduction or angiotensin infusion.[21] Also, tissue growth factor-α (TGFα), an EGFR ligand, and its sheddase tumor necrosis factor-α converting enzyme are overexpressed after angiotensin infusion, and angiotensin-induced renal lesions are blunted in TGFα knockout mice and by pharmacological tumor necrosis factor-α converting enzyme blockade. Experimental data suggest that EGFR transactivation by angiotensin also plays a key role in renal functional deterioration and that pharmacological inhibitors of tumor necrosis factor-α converting enzyme might be useful for preventing the progression of CKD.[21]

Basic fibroblast growth factor-2 (FGF-2) is expressed in normal kidneys and is mitogenic for a variety of cells including mesangial,[22] proximal-tubular epithelial,[23] glomerular endothelial,[24] and glomerular epithelial cells.[25] Furthermore, FGF-2 expression correlates strongly with scarring in human kidneys with tubulointerstitial fibrosis and is an inducer of both fibroblast proliferation[26] and EMT.[27] In addition, FGF-2 appears to promote podocyte injury,[28] and targeting FGF-2 attenuates renal injury in anti-Thy1.1 nephritis.[29] There is increasing interest in targeting FGF-2 due to the key role it plays in angiogenesis in a variety of human cancers and inflammatory conditions such as rheumatoid arthritis.[30] The use of such agents in *in vivo* models of kidney disease will help determine their therapeutic potential in CKD.

Proliferation and cell-cycle progression is ultimately regulated by specific proteins known as cyclins, cyclin-dependent kinases, and cyclin-dependent kinases inhibitors. As well as controlling cell proliferation, cell-cycle regulatory proteins also regulate other biologic processes central to the fibrotic process including cellular hypertrophy, differentiation, and apoptosis. The synthetic cyclin-dependent kinases inhibitor roscovitine has beneficial effects on kidney function and histology in anti-Thy1.1 nephritis, and a human immunodeficiency virus-1 transgenic mouse model of collapsing glomerulopathy.[31,32] Furthermore, the inhibitory effect of roscovitine on cell-cycle progression has also been shown to be effective at inhibiting cystogenesis in a murine model of polycystic kidney disease.[33] Roscovitine is already in clinical trials for cancer, and cyclin-dependent kinases inhibitors may have a valuable therapeutic role in inhibiting myofibroblast proliferation and fibrosis.

The Ras–Raf–Mek–Erk and the Rho kinase (ROCK) signaling pathways not only play a key role in regulating cellular proliferation but also can amplify TGFβ-mediated fibrotic responses[34] (see below). Targeting the Ras–Raf–Mek–Erk pathway using the Ras antagonist farnesylthiosalicylic acid or with the Mek inhibitor UO126 has reduced kidney injury in models such as anti-Thy1.1 nephritis,[35,36] cisplatin-induced renal failure,[37] and a murine model of polycystic disease.[38] A number of inhibitors targeting this pathway are already in use in oncology,[36] these drugs may be promising therapeutics in progressive CKD. Statins also attenuate fibrogenesis in a number of *in vivo* models such as nephrotoxic nephritis,[39] and there is a body of evidence to suggest that these effects are mediated by the inhibition of Ras signaling (by depleting prenylation substrates) rather than by cholesterol reduction.[40] Clinical trials are underway to assess full potential of statins in the management of progressive CKD.

Fasudil, a ROCK inhibitor, is used in the treatment of cerebral vasospasm in Japan and clinical trials are underway to assess its efficacy in cardiovascular disease. ROCK inhibitors attenuate interstitial fibrosis in unilateral ureteric obstruction (UUO), subtotal nephrectomy, and hypertensive models of glomerulosclerosis.[41] Interestingly, ROCK1 knockout mice are not protected from kidney fibrosis following UUO,[42] suggesting that ROCK inhibitors may mediate their effects through targeting other kinases such as ROCK2. Experimental data support the evaluation of ROCK inhibitors in CKD.

Again with a plethora of anti-proliferative therapies emerging, it is important to recognize that cell proliferation may be an essential reparative response to injury (as in anti-Thy1.1 nephritis), and therefore inhibiting cellular proliferation in some circumstances could aggravate injury.

Cell transformation

While the cellular response to injury may involve cell loss and/or proliferation, some cells regress to an embryonic mesenchymal phenotype, allowing their regeneration through proliferation thereby restoring the kidney structural integrity. Other cells are transformed to a mesenchymal/myofibroblastic phenotype thus contributing to pool of fibroblasts that migrate, proliferate, secrete excess interstitial collagens, and hence promote ECM expansion and fibrosis. A large body of literature deals with such EMT of kidney cells and its role in kidney fibrogenesis.[43] A number of mediators have been implicated in the stimulation of EMT, most notably TGF-β1 but also interleukin-1, connective tissue growth factor, EGF, PDGF as well as advanced glycation end products. There is strong data suggesting that TGF-β1 mediates its effects via the focal adhesion adapter protein, Hic-5, which is RhoA/ROCK1-dependent.[44]

Consequently, interventions targeting EMT or the stimulation of mesenchymal–epithelial transformation may have therapeutic appeal. For example, inhibiting TGF-β1 activity with neutralizing antibodies,[45] receptor antagonists[46] as well as the inhibition of TGF-β1 gene transcription[47] has attenuated fibrosis in a number of experimental models such as anti-Thy1.1 nephritis and diabetic nephropathy.[48]

More recent strategies have focused on targeting TGF-β1-mediated signal transduction either through the blockade of its receptor activin-like kinase or the manipulation of its intracellular transduction mediators, the Smads. For example, IN-1130 a small molecule inhibitor of activin-like kinase-5 attenuated fibrotic changes in UUO.[49] Hepatocyte growth factor inhibits TGF-β1–Smad signal transduction by inhibiting nuclear translocation of Smads or by upregulating the expression of Smad corepressors.[50] Hepatocyte growth factor has been effective in reducing fibrosis in experimental models of CKD where EMT is thought to play a significant role in fibrogenesis such as obstructive uropathy,[51] diabetic nephrectomy,[52] and chronic allograft nephropathy.[53]

Bone morphogenetic protein-7 (BMP-7) is another endogenous mediator that inhibits TGF-β1–Smad signaling via upregulation of inhibitory Smads.[54] BMP-7 inhibits not only EMT but may reverse established fibrosis through mesenchymal–epithelial transformation in models such as serum nephrotoxic nephritis[54] and UUO.[55] Furthermore, BMP-7 may attenuate vascular[56] and skeletal complications of CKD.[57] In addition, targeting endogenous antagonists of BMP activity[58] such as uterine sensitization-associated gene-1 and sclerostin may also have therapeutic potential.[59] Uterine sensitization-associated gene-1 is abundantly expressed in the kidney. Uterine sensitization-associated gene-1 knockout mice show preserved kidney function after acute and chronic kidney damage.[59] In contrast, the cysteine-rich protein Kielin/chordin-like protein (KCP) stimulates BMP-7 signaling, and KCP-deficient mice have been found to be susceptible to developing renal interstitial fibrosis.[60] While BMP-7 and hepatocyte growth factor are in early clinical development, at present, their potential to stimulate mesenchymal–epithelial transformation represents an exciting development in the treatment of fibrotic kidney disease.

Other TGF-β1 antagonists include small leucine-rich proteoglycans such as decorin and biglycan, which can antagonize TGF-β1-mediated experimental fibrosis possibly by sequestering TGF-β1 in the ECM, thereby reducing its biological activity. Furthermore, local targeting of TGF-β1 signaling by upregulating the expression of inhibitory Smads such as Smad7 has attenuated interstitial fibrosis in animals submitted to UUO.[61] Such gene delivery is an interesting experimental tool but its clinical utility remains somewhat limited.

Inflammation

Experimental and clinical kidney fibrosis is often preceded by an interstitial inflammatory response as reviewed by Chatziantoniou and Dussaule.[62] It is mediated by the infiltration of the interstitium by lymphocytes, monocytes, and macrophages through the activation of a number of cytokines and chemokines. These inflammatory cells mediate tubulointerstitial injury by a number of mechanisms that include the production of reactive oxygen species, secretion of vasoactive hormones, and profibrotic growth factors such as TGF-β1. While it is beyond the scope of this review to discuss the impact of inflammatory cell modulation, and the inhibition of cytokines as well as chemokines on the development of kidney fibrosis, recent advances warrant highlighting. Many chemokines and their receptors, CXCL13, CX3CR1, CCR1, CCR2, MCP-1, and osteopontin, are upregulated in damaged kidneys, thus contributing to the initiation of the inflammatory response and fibrosis. Some of these chemokines, including MCP-1, have been shown to be released by damaged or transformed tubules cells. Blocking of CCR2, a receptor of MCP-1, decreased infiltration and activation of macrophages with subsequent amelioration of progressive fibrosis following UUO.[63] Neutralizing MCP-1 with either a specific antibody or gene therapy delayed clearance of apoptotic neutrophils in a rat model of tubulointerstitial nephritis and ameliorated progressive fibrosis.[64] It also reduced renal fibrosis associated with protein-overload proteinuria[65] and interstitial fibrosis in crescentic glomerulonephritis.[66] Blockade of CCR1 (a receptor for a number of chemokine ligands including RANTES/CCL5, MIP-1α/CCL3, and MIP-1β/CCL4) by CX471 injection substantially reduced interstitial leukocyte accumulation and the subsequent kidney fibrosis in a murine model of nephrotic syndrome and focal segmental glomerulosclerosis.[67] This approach has also proved effective in preventing the progression of lupus nephritis in MRL-Fas(lpr) mice,[68] and in reducing kidney fibrosis after UUO.[69] Blocking CX3CR1 (a receptor for the chemokine CX3CL1 or fractalkine), by both gene inactivation and target protein blockade, was capable of reducing inflammation and fibrosis following ischemic kidney injury.[70] Chemokine antagonists and receptor blockers are being developed to treat a number of inflammatory conditions and their application to the treatment of clinical kidney inflammation and fibrosis is promising.

A number of cell-signaling pathways are central in mediating the inflammatory and fibrotic responses to kidney injury. For example, the protein kinase C signaling pathway plays a key role in diabetic nephropathy by upregulating osteopontin expression, thereby promoting macrophage recruitment and TGF-β1 production.[71] Protein kinase C inhibitors have attenuated interstitial fibrosis in experimental models of diabetic nephropathy.[72] The protein kinase C inhibitor, ruboxistaurin, is currently in clinical trials and early data suggest that it may reduce albuminuria and stabilize kidney function in patients with diabetic nephropathy.[73]

A number of pro-inflammatory cytokines and pro-fibrotic factors, such as PDGF and TGF-β1, also activate the p38 mitogen-activated protein kinase (MAPK). Blockade of p38 MAPK inhibits TGF-β1-induced collagen expression in fibroblasts and mesangial cells *in vitro*.[74,75] A p38 MAPK

Table 1 | Potential therapeutic agents and targets in CKD

Target	Intervention	Mechanism of action	Evidence level
TGF-β1	Bone morphogenetic protein-7	Inhibitor of the TGF-β1–Smad signalling pathway	Experimental models of kidney disease
	Hepatocyte growth factor	Inhibits nuclear translocation of receptor-regulated Smads and upregulates the expression of Smad corepressors	Experimental models of kidney disease and trials in promoting angiogenesis
	Inhibition of connective tissue growth factor	Mediator of TGF-β1-induced fibrosis	Experimental of kidney disease and phase II trial completed in diabetic nephropathy
	Tranilast	Inhibits TGF-β1-induced ECM synthesis	Experimental models of kidney disease, early clinical data in diabetic nephropathy, and used in the treatment of hypertrophic scars and scleroderma
	Decorin	Sequesters TGF-β1 in the extracellular matrix	Experimental models of kidney disease
Proliferative mitogens	Anti-platelet-derived growth factor, for example PDGF aptamers, imatinib mesylate, CR002, trapidil	Imatinib mesylate—kinase inhibitor of PDGF transduction. CR002 monoclonal antibody targeting PDGF-D	Experimental data indicates potential of CR002 in mesnagioproliferative disease—phase I trial completed. Cardiotoxicity limits the use of imatinib mesylate in clinical use in oncology and beneficial effects in experimental models of kidney disease.
	Anti-epidermal growth factor	Inhibition of renal fibroblast proliferation and collagen expression	Experimental models of kidney disease
Intracellular transduction cascade	Ras–Raf–Mek–Erk pathway inhibition by Ras: prenylation inhibitors (statins), prenyltransferase inhibitors, farnesylthiosalicylic acid, Raf and Mek kinase inhibitors	Inhibition of cellular proliferation, differentiation, and apoptosis	Experimental models of kidney disease and phase II clinical trial in cancer treatment. Role of statins in progressive CKD not yet defined
	Rho kinase inhibition: fasudil	Interference with cell proliferation, tubulointerstitial fibrosis, and glomerular hemodynamics	Experimental models of kidney disease and phase II studies in ischemic heart disease fasudil in clinical use in Japan for cerebral vasospasm
	p38 mitogen-activated protein kinase inhibitors	Inhibition of pro-inflammatory and profibrotic mediators	Experimental models of kidney disease and clinical trials in rheumatoid arthritis and type I diabetes mellitus
	Protein kinase C inhibitors such as ruboxistaurin	Inhibition of cell growth most evident in diabetic nephropathy	Experimental models of kidney disease. Early clinical data with ruboxistaurin in patients with early diabetic nephropathy
Cell-cycle Inhibitors	Cyclin-dependent kinases inhibitors such as roscovitine	Inhibition of cell-cycle progression	Experimental models and phase II trials in cancer
Immuno-suppressive agents	Mycophenolate mofetil	Inhibitor of inosine monophosphate dehydrogenase inhibiting cell proliferation	No clear data on efficacy in progressive CKD
	Rapamycin	Interference with cell proliferation by regulating ribosomal biogenesis and protein translation	Variable data in experimental models of kidney disease but can induce proteinuria. No clinical data on efficacy in progressive CKD
Other Agents	Pentoxyfylline	Phosphodiesterase inhibitor interfering with cell proliferation and EMT	Experimental models of kidney disease. Ongoing clinical trials assessing role in proteinuric CKD
	Endothelin antagonists	Reduce cellular proliferation and intra-glomerular hypertension	Experimental models of kidney disease. Ongoing clinical trials in diabetic nephropathy
	Pirfenidone	Inhibits ECM accumulation	Experimental models of CKD. Phase II trials in diabetic nephropathy and phase III in pulmonary fibrosis
	Peroxisome proliferator-activated receptor-γ agonists	Reduce cell growth, inflammation. Antiproteinuric effect	Experimental models of kidney disease and clinical data. Reduces proteinuria in diabetic nephropathy. Ongoing clinical trials in diabetic and non-diabetic CKD

Table 1 continued on following page.

Table 1 | Continued

Target	Intervention	Mechanism of action	Evidence level
	Prolyl hydroxylase domain inhibitors, for example cobalt chloride, FG-2216	Upregulation of HIF-regulated genes such as VEGF and EPO	Experimental models of kidney disease. Phase II clinical trials of FG-2216 underway for the treatment of anaemia
	N-acetyl-cysteine	Antioxidant	Experimental models of kidney disease. No clinical evidence in CKD
	Tocopherols	Antioxidant	Experimental models of kidney disease. Tocopherols and α- lipoic acid in clinical trials in CKD

CKD, chronic kidney disease; ECM, extracellular matrix; EMT, epithelial–mesenchymal transformation; PDGF, platelet-derived growth factor; TGF-α, tissue growth factor-α; VEGF, vascular endothelial growth factor.

inhibitor reduced ECM accumulation and renal fibrosis in UUO[76] and the co-administration of a p38 MAPK inhibitor (SB203580) along with a TGF-β1 receptor inhibitor (activin-like kinase-5 inhibitor) resulted in a downregulation of TGF-β1 with reduced myofibroblast accumulation and ECM deposition.[77] A number of p38 MAPK inhibitors are in clinical trials for the treatment of inflammatory diseases and these data suggest that such compounds may have useful anti-fibrotic and anti-inflammatory actions in CKD.

Besides chemokine antagonists and receptor blockers, advances in the inhibition of the complement system, another phlogistic pathway, may hold promise. A number of experimental models have highlighted the role of the complement system in kidney fibrogenesis. Complement-deficient mice have proved resistant to the development and progression of fibrosis following a number of insults.[78] Lack of the naturally occurring complement regulatory protein Crry led to unrestricted complement activation, marked inflammation, and progressive kidney failure in mice.[79] Inhibition of Crry also exacerbated kidney injury in the puromycin-induced model of nephrotic syndrome.[80] On the other hand, the systemic administration of the complement membrane attack complex inhibitor CD59 has been shown to have therapeutic potential in experimental proteinuric states.[80] Progressive membranous nephropathy has been associated with raised urinary membrane attack complex excretion. It would be tempting to speculate that agents capable of membrane attack complex inhibition, such as CD59, may have a therapeutic role in such a nephropathy. Finally, recent data indicate a role of C5 and specifically the C5a receptor, which is amenable to therapeutic intervention, in the progression of kidney tubulointerstitial fibrosis.[81]

There is also increasing interest in vascular endothelial growth factor-A (VEGF-A or VEGF) that acts as a key regulator of angiogenesis and vascular permeability. Evidence is now emerging that begins to elucidate the pathophysiological role of VEFG. For example, both experimental and clinical data suggest that VEGF is upregulated in diabetic nephropathy.[82] In contrast, the podocyte-specific deletion of VEGF in mice is associated with failure of glomerular development and death.[83] Interestingly, overexpression of VEGF resulted in podocyte proliferation and a collapsing glomerulopathy as seen in HIV infection, while intermediate levels of VEGF expression were characterized by proteinuria and endotheliosis analogous to lesions seen in pre-eclampsia.[83] Data from *in vivo* models are conflicting. For example, exogenous VEGF improved endothelial repair in models of glomerulonephritis[84] and had ameliorated fibrosis and stabilized kidney function in a remnant kidney model of progressive CKD. In contrast, blockade of VEGF had beneficial effects in experimental diabetes[85] though interestingly administration of a VEGF antibody in patients with renal cell cancer, induced proteinuria in 64% patients.[86] Given complexity of the VEGF system and the potential adverse pro-angiogenic effects of VEGF therapy, it is not clear whether VEGF administration or blockade will have a therapeutic role in CKD.

Targeting ECM deposition

Targeting ECM deposition would rely on the inhibition of ECM synthesis or stimulation of its breakdown and clearance. Not only does TGF-β1 play a pivotal role in EMT, but it is also a key driver of ECM accumulation by (i) qualitatively and quantitatively altering the synthesis of key ECM molecules, (ii) decreasing matrix degradation by inhibiting proteases as well as activating protease inhibitors (e.g. plasminogen activator inhibitor-1), and (iii) promoting cell–matrix interactions by upregulating integrin expression.[87] Therefore, the strategies targeting TGF-β1 highlighted above may also have beneficial effects on ECM accumulation.

Increased clearance of ECM may depend on the activation of collagenolytic pathways including metalloproteinases, which have been shown to be suppressed during the course of renal fibrosis.[88] Alternatively, blockade of tissue inhibitors of metalloproteinases may be of therapeutic benefit. Little experimental data are available on interventions directly targeting these enzymes or the plasminogen activator collagenolytic pathway though enhancing metalloproteinase and plasminogen activator activity is a mechanism by which hepatocyte growth factor enhances ECM clearance.

Work from our own group has highlighted the role of tissue transglutaminase in the pathogenesis of renal fibrosis. Tissue transglutaminase irreversibly crosslinks proteins and collagens rendering ECM resistant to the proteolytic activities of metalloproteinases. We have observed an upregulation of tissue transglutaminase expression in a number of experi-

mental models of fibrosis,[89,90] while in human nephropathies tissue transglutaminase expression correlates with the severity of interstitial fibrosis.[91] Our own research has shown the potential of specific tissue transglutaminase inhibitors to attenuate kidney fibrosis in a number of experimental models of CKD.[92] Ongoing work is exploring the clinical potential of these compounds.

CONCLUSION

In addition to the strategies highlighted above a number of other therapies summarized in Table 1 may have a role in progressive CKD. However, nephrologists have so far failed to address the pathological steps involved in renal remodeling when attempting to slow the progression of CKD; instead they have focused their efforts to treating hypertension, a complication of CKD. This has met with some success; however, many patients with CKD (EGFR < 60 ml/min) continue to progress to end-stage renal disease. Treatment strategies based on the manipulation of the underlying kidney remodeling and scarring process may prove effective and complementary to current management interventions.

REFERENCES

1. Coresh J, Astor BC, Greene T *et al.* Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis* 2003; **41**: 1–12.
2. Lameire N, Jager K, Van Biesen W *et al.* Chronic kidney disease: a European perspective. *Kidney Int Suppl* 2005; **99**: S30–S38.
3. Kriz W, Gretz N, Lemley KV. Progression of glomerular diseases: is the podocyte the culprit? *Kidney Int* 1998; **54**: 687–697.
4. Wharram BL, Goyal M, Wiggins JE *et al.* Podocyte depletion causes glomerulosclerosis: diphtheria toxin-induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. *J Am Soc Nephrol* 2005; **16**: 2941–2952.
5. Bohle A, Wehrmann M, Bogenschutz O *et al.* The long-term prognosis of the primary glomerulonephritides. A morphological and clinical analysis of 1747 cases. *Pathol Res Pract* 1992; **188**: 908–924.
6. Rodemann HP, Muller GA. Characterization of human renal fibroblasts in health and disease: II. *In vitro* growth, differentiation, and collagen synthesis of fibroblasts from kidneys with interstitial fibrosis. *Am J Kidney Dis* 1991; **17**: 684–686.
7. Remuzzi G, Perico N, Macia M *et al.* The role of renin–angiotensin–aldosterone system in the progression of chronic kidney disease. *Kidney Int Suppl* 2005; **99**: S57–S65.
8. Thomas GL, Yang B, Wagner BE *et al.* Cellular apoptosis and proliferation in experimental renal fibrosis. *Nephrol Dial Transplant* 1998; **13**: 2216–2226.
9. Hugo C, Shankland SJ, Bowen-Pope DF *et al.* Extraglomerular origin of the mesangial cell after injury. A new role of the juxtaglomerular apparatus. *J Clin Invest* 1997; **100**: 786–794.
10. Sakai N, Iseki K, Suzuki S *et al.* Uninephrectomy induces progressive glomerulosclerosis and apoptosis in anti-Thy1 glomerulonephritis. *Pathol Int* 2005; **55**: 19–26.
11. Wolthuis A, van Goor H, Weening JJ *et al.* Pathobiology of focal sclerosis. *Curr Opin Nephrol Hypertens* 1993; **2**: 458–464.
12. Kriz W. The pathogenesis of 'classic' focal segmental glomerulosclerosis-lessons from rat models. *Nephrol Dial Transplant* 2003; **18**(Suppl 6): vi39–vi44.
13. Yang B, Johnson TS, Haylor JL *et al.* Effects of caspase inhibition on the progression of experimental glomerulonephritis. *Kidney Int* 2003; **63**: 2050–2064.
14. Yang B, Johnson TS, Thomas GL *et al.* A shift in the Bax/Bcl-2 balance may activate caspase-3 and modulate apoptosis in experimental glomerulonephritis. *Kidney Int* 2002; **62**: 1301–1313.
15. Hughes J, Savill JS. Apoptosis in glomerulonephritis. *Curr Opin Nephrol Hypertens* 2005; **14**: 389–395.
16. Badid C, Vincent M, Fouque D *et al.* Myofibroblast: a prognostic marker and target cell in progressive renal disease. *Ren Fail* 2001; **23**: 543–549.
17. Floege J, Eitner F, Alpers C. A new look at platelet-derived growth factor and renal disease. *J Am Soc Nephrol* 2007 (in press).
18. Kerkela R, Grazette L, Yacobi R *et al.* Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nat Med* 2006; **12**: 908–916.
19. Berman E, Nicolaides M, Maki RG *et al.* Altered bone and mineral metabolism in patients receiving imatinib mesylate. *N Engl J Med* 2006; **354**: 2006–2013.
20. Francois H, Placier S, Flamant M *et al.* Prevention of renal vascular and glomerular fibrosis by epidermal growth factor receptor inhibition. *FASEB J* 2004; **18**: 926–928.
21. Lautrette A, Li S, Alili R *et al.* Angiotensin II and EGF receptor cross-talk in chronic kidney diseases: a new therapeutic approach. *Nat Med* 2005; **11**: 867–874.
22. Floege J, Eng E, Young BA *et al.* Factors involved in the regulation of mesangial cell proliferation *in vitro* and *in vivo*. *Kidney Int Suppl* 1993; **39**: S47–S54.
23. Kanda S, Nomata K, Saha PK *et al.* Growth factor regulation of the renal cortical tubular cells by epidermal growth factor, insulin-like growth factor-I, acidic and basic fibroblast growth factor, and transforming growth factor-beta in serum free culture. *Cell Biol Int Rep* 1989; **13**: 687–699.
24. Ballermann BJ. Regulation of bovine glomerular endothelial cell growth *in vitro*. *Am J Physiol* 1989; **256**: C182–C189.
25. Takeuchi A, Yoshizawa N, Yamamoto M *et al.* Basic fibroblast growth factor promotes proliferation of rat glomerular visceral epithelial cells *in vitro*. *Am J Pathol* 1992; **141**: 107–116.
26. Strutz F, Zeisberg M, Hemmerlein B *et al.* Basic fibroblast growth factor expression is increased in human renal fibrogenesis and may mediate autocrine fibroblast proliferation. *Kidney Int* 2000; **57**: 1521–1538.
27. Strutz F, Zeisberg M, Ziyadeh FN *et al.* Role of basic fibroblast growth factor-2 in epithelial-mesenchymal transformation. *Kidney Int* 2002; **61**: 1714–1728.
28. Floege J, Kriz W, Schulze M *et al.* Basic fibroblast growth factor augments podocyte injury and induces glomerulosclerosis in rats with experimental membranous nephropathy. *J Clin Invest* 1995; **96**: 2809–2819.
29. Floege J, Burg M, Hugo C *et al.* Endogenous fibroblast growth factor-2 mediates cytotoxicity in experimental mesangioproliferative glomerulonephritis. *J Am Soc Nephrol* 1998; **9**: 792–801.
30. Ziche M, Donnini S, Morbidelli L. Development of new drugs in angiogenesis. *Curr Drug Targets* 2004; **5**: 485–493.
31. Gherardi D, D'Agati V, Chu TH *et al.* Reversal of collapsing glomerulopathy in mice with the cyclin-dependent kinase inhibitor CYC202. *J Am Soc Nephrol* 2004; **15**: 1212–1222.
32. Pippin JW, Qu Q, Meijer L *et al.* Direct *in vivo* inhibition of the nuclear cell cycle cascade in experimental mesangial proliferative glomerulonephritis with roscovitine, a novel cyclin-dependent kinase antagonist. *J Clin Invest* 1997; **100**: 2512–2520.
33. Bukanov NO, Smith LA, Klinger KW *et al.* Long-lasting arrest of murine polycystic kidney disease with CDK inhibitor roscovitine. *Nature* 2006; **444**: 949–952.
34. Phanish MK, Wahab NA, Hendry BM *et al.* TGF-beta1-induced connective tissue growth factor (CCN2) expression in human renal proximal tubule epithelial cells requires Ras/MEK/ERK and Smad signalling. *Nephron Exp Nephrol* 2005; **100**: e156–e165.
35. Bokemeyer D, Panek D, Kramer HJ *et al. In vivo* identification of the mitogen-activated protein kinase cascade as a central pathogenic pathway in experimental mesangioproliferative glomerulonephritis. *J Am Soc Nephrol* 2002; **13**: 1473–1480.
36. Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 2003; **3**: 11–22.
37. Jo SK, Cho WY, Sung SA *et al.* MEK inhibitor, U0126, attenuates cisplatin-induced renal injury by decreasing inflammation and apoptosis. *Kidney Int* 2005; **67**: 458–466.
38. Omori S, Hida M, Fujita H *et al.* Extracellular signal-regulated kinase inhibition slows disease progression in mice with polycystic kidney disease. *J Am Soc Nephrol* 2006; **17**: 1604–1614.
39. Christensen M, Su AW, Snyder RW *et al.* Simvastatin protection against acute immune-mediated glomerulonephritis in mice. *Kidney Int* 2006; **69**: 457–463.
40. Khwaja A, O'Connolly J, Hendry BM. Prenylation inhibitors in renal disease. *Lancet* 2000; **355**: 741–744.
41. Hayashi K, Wakino S, Kanda T *et al.* Molecular mechanisms and therapeutic strategies of chronic renal injury: role of rho-kinase in the development of renal injury. *J Pharmacol Sci* 2006; **100**: 29–33.
42. Fu P, Liu F, Su S *et al.* Signaling mechanism of renal fibrosis in unilateral ureteral obstructive kidney disease in ROCK1 knockout mice. *J Am Soc Nephrol* 2006; **17**: 3105–3114.

43. Neilson EG. Mechanisms of disease: fibroblasts—a new look at an old problem. *Nat Clin Pract Nephrol* 2006; **2**: 101–108.
44. Tumbarello DA, Turner CE. Hic-5 contributes to epithelial–mesenchymal transformation through a RhoA/ROCK-dependent pathway. *J Cell Physiol* 2007; **211**: 736–747.
45. Yu L, Border WA, Anderson I *et al.* Combining TGF-beta inhibition and angiotensin II blockade results in enhanced antifibrotic effect. *Kidney Int* 2004; **66**: 1774–1784.
46. Juarez P, Vilchis-Landeros MM, Ponce-Coria J *et al.* Soluble betaglycan reduces renal damage progression in db/db mice. *Am J Physiol Renal Physiol* 2007; **292**: F321–F329.
47. Akagi Y, Isaka Y, Arai M *et al.* Inhibition of TGF-beta 1 expression by antisense oligonucleotides suppressed extracellular matrix accumulation in experimental glomerulonephritis. *Kidney Int* 1996; **50**: 148–155.
48. Ziyadeh FN, Hoffman BB, Han DC *et al.* 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.
49. Moon JA, Kim HT, Cho IS *et al.* IN-1130, a novel transforming growth factor-beta type I receptor kinase (ALK5) inhibitor, suppresses renal fibrosis in obstructive nephropathy. *Kidney Int* 2006; **70**: 1234–1243.
50. Dai C, Liu Y. Hepatocyte growth factor antagonizes the profibrotic action of TGF-beta1 in mesangial cells by stabilizing Smad transcriptional corepressor TGIF. *J Am Soc Nephrol* 2004; **15**: 1402–1412.
51. Yang J, Liu Y. Delayed administration of hepatocyte growth factor reduces renal fibrosis in obstructive nephropathy. *Am J Physiol Renal Physiol* 2003; **284**: F349–F357.
52. 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.
53. Azuma H, Takahara S, Matsumoto K *et al.* Hepatocyte growth factor prevents the development of chronic allograft nephropathy in rats. *J Am Soc Nephrol* 2001; **12**: 1280–1292.
54. Zeisberg M, Hanai J, Sugimoto H *et al.* BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat Med* 2003; **9**: 964–968.
55. Klahr S, Morrissey J. Obstructive nephropathy and renal fibrosis: the role of bone morphogenic protein-7 and hepatocyte growth factor. *Kidney Int Suppl* 2003; **87**: S105–S112.
56. Davies MR, Lund RJ, Mathew S *et al.* Low turnover osteodystrophy and vascular calcification are amenable to skeletal anabolism in an animal model of chronic kidney disease and the metabolic syndrome. *J Am Soc Nephrol* 2005; **16**: 917–928.
57. Gonzalez EA, Lund RJ, Martin KJ *et al.* Treatment of a murine model of high-turnover renal osteodystrophy by exogenous BMP-7. *Kidney Int* 2002; **61**: 1322–1331.
58. Yanagita M. Modulator of bone morphogenetic protein activity in the progression of kidney diseases. *Kidney Int* 2006; **70**: 989–993.
59. Yanagita M, Okuda T, Endo S *et al.* Uterine sensitization-associated gene-1 (USAG-1), a novel BMP antagonist expressed in the kidney, accelerates tubular injury. *J Clin Invest* 2006; **116**: 70–79.
60. Lin J, Patel SR, Cheng X *et al.* Kielin/chordin-like protein, a novel enhancer of BMP signaling, attenuates renal fibrotic disease. *Nat Med* 2005; **11**: 387–393.
61. Lan HY, Mu W, Tomita N *et al.* Inhibition of renal fibrosis by gene transfer of inducible Smad7 using ultrasound-microbubble system in rat UUO model. *J Am Soc Nephrol* 2003; **14**: 1535–1548.
62. Chatziantoniou C, Dussaule JC. Insights into the mechanisms of renal fibrosis: is it possible to achieve regression? *Am J Physiol Renal Physiol* 2005; **289**: F227–F234.
63. Kitagawa K, Wada T, Furuichi K *et al.* Blockade of CCR2 ameliorates progressive fibrosis in kidney. *Am J Pathol* 2004; **165**: 237–246.
64. Li P, Garcia GE, Xia Y *et al.* Blocking of monocyte chemoattractant protein-1 during tubulointerstitial nephritis resulted in delayed neutrophil clearance. *Am J Pathol* 2005; **167**: 637–649.
65. Shimizu H, Maruyama S, Yuzawa Y *et al.* Anti-monocyte chemoattractant protein-1 gene therapy attenuates renal injury induced by protein-overload proteinuria. *J Am Soc Nephrol* 2003; **14**: 1496–1505.
66. Lloyd CM, Minto AW, Dorf ME *et al.* RANTES and monocyte chemoattractant protein-1 (MCP-1) play an important role in the inflammatory phase of crescentic nephritis, but only MCP-1 is involved in crescent formation and interstitial fibrosis. *J Exp Med* 1997; **185**: 1371–1380.
67. Vielhauer V, Berning E, Eis V *et al.* CCR1 blockade reduces interstitial inflammation and fibrosis in mice with glomerulosclerosis and nephrotic syndrome. *Kidney Int* 2004; **66**: 2264–2278.
68. Anders HJ, Belemezova E, Eis V *et al.* Late onset of treatment with a chemokine receptor CCR1 antagonist prevents progression of lupus nephritis in MRL-Fas(lpr) mice. *J Am Soc Nephrol* 2004; **15**: 1504–1513.
69. Anders HJ, Vielhauer V, Frink M *et al.* A chemokine receptor CCR-1 antagonist reduces renal fibrosis after unilateral ureter ligation. *J Clin Invest* 2002; **109**: 251–259.
70. Furuichi K, Gao JL, Murphy PM. Chemokine receptor CX3CR1 regulates renal interstitial fibrosis after ischemia–reperfusion injury. *Am J Pathol* 2006; **169**: 372–387.
71. Kelly DJ, Chanty A, Gow RM *et al.* Protein kinase Cbeta inhibition attenuates osteopontin expression, macrophage recruitment, and tubulointerstitial injury in advanced experimental diabetic nephropathy. *J Am Soc Nephrol* 2005; **16**: 1654–1660.
72. Kelly DJ, Zhang Y, Hepper C *et al.* Protein kinase C beta inhibition attenuates the progression of experimental diabetic nephropathy in the presence of continued hypertension. *Diabetes* 2003; **52**: 512–518.
73. Tuttle KR, Bakris GL, Toto RD *et al.* The effect of ruboxistaurin on nephropathy in type 2 diabetes. *Diabetes Care* 2005; **28**: 2686–2690.
74. Sato M, Shegogue D, Gore EA *et al.* Role of p38 MAPK in transforming growth factor beta stimulation of collagen production by scleroderma and healthy dermal fibroblasts. *J Invest Dermatol* 2002; **118**: 704–711.
75. Chin BY, Mohsenin A, Li SX *et al.* Stimulation of pro-alpha(1)(I) collagen by TGF-beta(1) in mesangial cells: role of the p38 MAPK pathway. *Am J Physiol Renal Physiol* 2001; **280**: F495–F504.
76. Stambe C, Atkins RC, Tesch GH *et al.* The role of p38alpha mitogen-activated protein kinase activation in renal fibrosis. *J Am Soc Nephrol* 2004; **15**: 370–379.
77. Li J, Campanale NV, Liang RJ *et al.* Inhibition of p38 mitogen-activated protein kinase and transforming growth factor-beta1/Smad signaling pathways modulates the development of fibrosis in adriamycin-induced nephropathy. *Am J Pathol* 2006; **169**: 1527–1540.
78. Matsuo S, Morita Y, Mizuno M *et al.* Proteinuria and damage to tubular cells—is complement a culprit? *Nephrol Dial Transplant* 1998; **13**: 2723–2726.
79. Bao L, Wang Y, Chang A *et al.* Unrestricted C3 activation occurs in Crry-deficient kidneys and rapidly leads to chronic renal failure. *J Am Soc Nephrol* 2007; **18**: 811–822.
80. He C, Imai M, Song H *et al.* Complement inhibitors targeted to the proximal tubule prevent injury in experimental nephrotic syndrome and demonstrate a key role for C5b-9. *J Immunol* 2005; **174**: 5750–5757.
81. Boor P, Konieczny A, Villa L *et al.* Complement C5 mediates experimental tubulointerstitial fibrosis. *J Am Soc Nephrol* 2007; **18**: 1508–1515.
82. Schrijvers BF, Flyvbjerg A, De Vriese AS. The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. *Kidney Int* 2004; **65**: 2003–2017.
83. Eremina V, Sood M, Haigh J *et al.* Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest* 2003; **111**: 707–716.
84. Masuda Y, Shimizu A, Mori T *et al.* Vascular endothelial growth factor enhances glomerular capillary repair and accelerates resolution of experimentally induced glomerulonephritis. *Am J Pathol* 2001; **159**: 599–608.
85. de Vriese AS, Tilton RG, Elger M *et al.* Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. *J Am Soc Nephrol* 2001; **12**: 993–1000.
86. Yang JC, Haworth L, Sherry RM *et al.* A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med* 2003; **349**: 427–434.
87. Ziyadeh FN. Mediators of diabetic renal disease: the case for tgf-beta as the major mediator. *J Am Soc Nephrol* 2004; **15**(Suppl 1): S55–S57.
88. Catania JM, Chen G, Parrish AR. Role of matrix metalloproteinases in renal pathophysiologies. *Am J Physiol Renal Physiol* 2007; **292**: F905–F911.
89. Johnson TS, Griffin M, Thomas GL *et al.* The role of transglutaminase in the rat subtotal nephrectomy model of renal fibrosis. *J Clin Invest* 1997; **99**: 2950–2960.
90. Johnson TS, Abo-Zenah H, Skill JN *et al.* Tissue transglutaminase: a mediator and predictor of chronic allograft nephropathy? *Transplantation* 2004; **77**: 1667–1675.
91. Johnson TS, El-Koraie AF, Skill NJ *et al.* Tissue transglutaminase and the progression of human renal scarring. *J Am Soc Nephrol* 2003; **14**: 2052–2062.
92. Skill NJ, Johnson TS, Coutts IG *et al.* Inhibition of transglutaminase activity reduces extracellular matrix accumulation induced by high glucose levels in proximal tubular epithelial cells. *J Biol Chem* 2004; **279**: 47754–47762.