

CTGF and chronic kidney fibrosis

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1. ABSTRACT

Chronic kidney fibrosis is the unifying pathological feature of diverse renal disease leading to a progressive decline in renal function and eventually end-stage kidney failure. Many growth factors are able to induce an imbalance of extracellular matrix production and degradation, leading to excessive matrix and fibrosis in both glomeruli and in the tubulointerstitium. Over the last decade the role of connective tissue growth factor (CTGF) in renal fibrosis has been intensively studied. CTGF participates in cell proliferation, migration, and differentiation and mediates profibrotic activity by acting either directly, or as a co-factor for TGF β 1, which is well characterised as a key cytokine mediating both the induction and promotion of fibrogenesis. CTGF also has the potential to modulate factors such as VEGF and bone morphogenic proteins, which are integral to both the development and repair process inherent in renal fibrogenesis. This review focuses on the role of CTGF in renal fibrosis and specifically its role in inducing fibrosis by factors integrally involved in the development of diabetic nephropathy, namely high glucose, angiotensin II, TGF β 1 and AGEs.

2. INTRODUCTION

Many diverse conditions including hypertension, diabetes mellitus, renal ischaemia, chronic infection, inflammatory disease, kidney stones and cystic disease all ultimately cause pathophysiological changes in the tubulointerstitium, vessels and glomeruli, with the ultimate consequence being a progressive loss of renal function. Podocyte dysfunction, the activation of mesangial cells and resident fibroblasts, and the process of tubular epithelial-mesenchymal transition (EMT) contribute to excess production of extracellular matrix, with CTGF being implicated in each case. Angiotensin II (AngII), transforming growth factor-beta 1 (TGF β 1) and factors inherent in diabetic nephropathy such as high glucose and increased advanced glycation end products (AGEs) are considered to be key factors contributing to renal fibrogenesis (1-5). Recent studies have indicated that CTGF either *mediates* or *facilitates* fibrogenesis in each of these pathophysiological circumstances (6). As CTGF plays a crucial role in the development of kidney fibrosis early phase studies targeting CTGF are currently being conducted.

3. CTGF EXPRESSION

CTGF/CCN2, also termed *fisp-12*, is a prototypic member of the CCN (CTGF, *cyr 61/cef 10, nov*) family that share a conserved multimodular structure but exhibit distinctive functional features (7). Other CCN family members include cysteine-rich 61 (*cyr 61*; also termed β IG-M1 or *cef 10*), *nov* (nephroblastoma overexpressed gene), *wisp-1* (also termed *elml*), CTGF-L (also termed *rCop-1* or *wisp-2*), and *wisp-3*. CTGF was classified as a mosaic protein containing four discrete structural modules, CTGF1-4. It is also a member of the IGF binding protein (IGFBP) superfamily and was initially classified as IGFBP-8 (8) on the basis that it has 30 to 38% homology to IGFBPs 1 to 6. However, this nomenclature was subsequently revised because of its relatively low affinity binding for IGFs and CTGF is now also known as IGFBP-related protein 2 (IGFBP-RP2) (9, 10). CTGF is a 36 (nonreduced) to 38 (reduced) kDa cysteine-rich peptide containing 349 amino acids. A cell-associated, long-lived and insoluble form of CTGF which is secreted inefficiently is also reported (11). CTGF fragments of varying size (10, 12, 16, 18, 19, 20 and 24 kDa) have also been found in different cell types, tissues, and body fluids (10-13).

The gene for human CTGF, which is located on chromosome 6q23.1 proximal to *c-myc*, comprises five exons and four introns and was originally cloned from a human umbilical vein endothelial cell (HUVEC) cDNA library (14). The CTGF promoter consists of various transcription factor binding sites including KLF4, TATA, GATA-1, GATA-2, AP1, NF κ B, NF-AT, Sp1, GC box, cap, CCAAT, *c-Ets-1*. It is clear that several of these transcription factors are also upregulated in chronic kidney disease including diabetic nephropathy. Variation among individuals exists in the promoter region of CTGF that may predispose to renal injury (15). Recently, an important study showed the direct genetic association between *CTGF* and systemic sclerosis and identified a *CTGF* risk genotype involving a polymorphism within a novel Sp3-dependent transcriptional repressor site (16). Those findings not only identify a novel regulatory mechanism governing *CTGF* promoter activity but also directly place *CTGF* on the genetic map of human disease (16).

Having been initially isolated in HUVEC cells it has subsequently been detected in multiple cells and tissues (8). In the kidney it is found in mesangial cells, podocytes, parietal epithelial cells, tubular epithelial cells, fibroblasts, endothelial cells and vascular smooth muscle cells. CTGF has been shown to be strongly upregulated in proliferative and non-proliferative forms of glomerulonephritis and in non-immune disease such as obstructive and diabetic nephropathy, with the degree of expression correlating with the degree of injury in both the glomerulus and the tubulointerstitium (17, 18). The magnitude of increased expression is exemplified by experimental data in a diabetic *db/db* mouse model demonstrating that CTGF transcripts in glomeruli were increased 28-fold after approximately 3.5 mo of diabetes compared to control (13).

The CCN family has diverse functions *in vitro*, depending on the cell type studied. Relevant to the kidney, CTGF has been reported to have a mitogenic and chemotactic effects on fibroblastic and stromal cells (14, 19, 20) and enhances mRNA levels for α 1-type 1 collagen, fibronectin, and α 5 integrin in fibroblasts (21), suggesting a role in matrix production. The role of CTGF in renal fibrogenesis will be explored further in Section 5. CTGF and *cyr 61* have also been reported to regulate angiogenesis (22). CTGF has been considered to sequester the pro-angiogenic cytokine VEGF and on proteolysis by the MMP family VEGF is released, thus promoting angiogenic activity (23, 24). However, in *in vivo* models, both CTGF and *CYR61* have been shown to have intrinsically active angiogenic activity and are downstream effectors of AGE in the diabetic retina (25, 26). Conversely, the development of models of oxygen-induced retinopathy and laser-induced choroidal neovascularisation was no different in CTGF +/- or CTGF +/- neonatal and mature mice. In renal carcinoma cells both CTGF and VEGF are considered to have independent and additive proangiogenic actions (27). No data is available in models of progressive renal disease and in particular in diabetic nephropathy. Hence, the role that CTGF plays in regulating renal angiogenesis is unclear, particularly given the paradoxical proliferative vasculopathy that occurs with diabetic retinopathy and the paucity of vasculature in nephropathy.

4. CTGF AND SIGNALLING PATHWAYS

Many studies have reported that CTGF is a downstream mediator of TGF β 1 (28, 29). Early studies revealed that TGF β 1 increased CTGF mRNA and protein levels markedly in human foreskin fibroblasts but not other growth factors including PDGF, epidermal growth factor, and basic fibroblast growth factor. In these studies cycloheximide did not block the large TGF-beta stimulation of CTGF gene expression, indicating that it is directly regulated by TGF-beta (30). These results suggest that TGF β 1 is a direct stimulus for CTGF gene transcription. Conversely, CTGF activates TGF β 1 to mediate downstream extracellular matrix production (31).

TGF β 1 induces CTGF gene expression via the Smad binding elements (SBE) and a unique TGF β 1 response element in the CTGF promoter (32). In both normal and phenotypically diseased fibroblasts the TGF β 1 response element in the CTGF promoter is required for basal CTGF promoter activity. This element is distinct from other reported TGF β 1 responsive elements and is not present in other genes regulated by TGF β 1. Moreover, this element is preserved in CTGF and is not found in other CCN family members. Downstream Smads, Ras/MEK/ERK, protein kinase C, and fibroblast-enriched factors that bind GAGGAATGG (a consensus transcription enhancer factor binding element) act together to drive the TGF-beta-mediated induction of CTGF (33-38). In addition to TGF- β 1 inducing CTGF expression, other stimuli such as high glucose, vascular endothelial growth factor, oxidative stress, nitric oxide and inflammatory mediators such as TNF- α have been shown to increase CTGF expression in diverse cell types including kidney cells

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implicated in renal fibrosis such as NRK fibroblasts, mesangial cells and tubular epithelial cells (39). Conversely, nitric oxide has been suggested to act as a strong repressor of CTGF expression in cultured rat mesangial cell (40). Hence it is likely that there are both time related and disease specific regulators of CTGF expression.

It has been reported that CTGF enhances the TGF β 1/Smad signaling pathway by inducing induction of TIEG-1, a known repressor of Smad7 transcription which normally acts to inhibit TGF β 1 induced fibrinogenesis (37, 41). In these studies the TGF- β 1-responsive genes plasminogen activator inhibitor-1 (PAI-1) and collagen III were maximally stimulated by the combination of TGF- β 1 and CTGF. TGF- β 1-stimulated expression of these genes was markedly reduced in the presence of CTGF and TIEG-1 antisense oligonucleotides (37, 41). These findings are congruent with those of Abreu *et al* (31) where it was demonstrated that the profibrotic effects of CTGF were at least in part explained by the enhanced binding of TGF- β 1 in the presence of CTGF. These investigators reported that CTGF can directly bind BMP4 and TGF-beta 1 through its cysteine rich binding domain. Hence CTGF may antagonize BMP4 activity by preventing its binding to BMP receptors and conversely may enhance receptor binding of TGF-beta 1.

This is consistent with our data demonstrating that TGF- β 1 induced Smad7, presumably as a control mechanism to limit the effects of TGF- β 1 on renal fibrosis, but CTGF reduced Smad7 (42). Parallel *in vivo* studies in a diabetic rat model demonstrated increased renal CTGF protein expression with a concomitant reduction in Smad7 mRNA (42). Furthermore, in our *in vitro* studies, the basal expression level of Smad7 decreased in TGF- β 1 silenced cells, whilst the basal expression level of Smad7 increased in CTGF silenced cells which was further increased by exposure to TGF- β 1. Both mRNA and protein levels of TGF- β 1 decreased in CTGF silenced cells accompanied by reduction in Smad2 mRNA level. Therefore, CTGF is likely to play a key role in promoting TGF- β 1 activity by decreasing Smad7 and increasing Smad2 (42). Our studies confirmed that CTGF induced fibronectin and collagen IV secretion in primary cultures of human proximal tubule cells and cortical fibroblasts via promoting the action of TGF β 1 by demonstrating that his effect was inhibited by neutralizing antibodies to either TGF β 1 or to the TGF β type II receptor (TbetaRII). Hence we concluded that CTGF requires TGF β 1 signaling through the TGFbetaRII in both primary tubular cells and cortical fibroblasts, to exert its fibrogenic response (43).

It has been reported that CTGF exerts independent activity by binding to the PDGF type β receptor (14) with further data supporting an interaction between CTGF and PDGF type α receptors (44). Additional observations suggest that CTGF acts through a tyrosine kinase pathway as the tyrosine kinase receptor TrkA has been shown to be a ligand for CTGF. Despite the clear cascade of signalling molecules induced by CTGF a

specific receptor for CTGF remains elusive. Two classes of CTGF specific receptors on human chondrocytic cells HCS-2/8 have been demonstrated now 10 years ago, but the localization, structure and function of these CTGF-specific receptors has not been further elucidated (45).

A 280 kDa CTGF binding protein has been identified in the membrane of HCS-2y8 chondrocytic cells (46, 47), which is likely to be the low-density lipoprotein receptor-related protein/ (2-macroglobulin receptor (LRP), which has been identified as a receptor for CTGF in fibroblasts (48). It is possible that the LRP receptor signals via tyrosine kinase as CTGF was shown to induce tyrosine phosphorylation of the cytoplasmic domain of the low-density lipoprotein receptor-associated protein (LRP) in fibroblasts, which was inhibited by the LRP-antagonist, receptor-associated protein (RAP) (49). LRP has also been demonstrated to be involved in the rapid internalization of CTGF and its subsequent degradation by endosomes (48). Although the intracellular trafficking of CTGF after receptor binding is as yet unclear these observations are of interest as in glucose-stimulated mesangial cells, exogenous tagged CTGF was internalized, presumably into the endosomes, and transported to the nucleus where it stimulated the production of ribosomal RNA (50). The functional significance of these observations is unclear (39). It remains to be definitively established whether CTGF binding to LRP represents solely a clearance mechanism or if this interaction is also required as part of the CTGF signalling cascade.

CTGF rapidly activates several additional intracellular signalling molecules in human mesangial cells, including extracellular signal-related kinase 1/2, Jun NH (2)-terminal kinase, protein kinase B, CaMK II, protein kinase Calpha, and protein kinase C delta and Smad/integrin-linked kinase (ILK) (37, 39, 41, 51). Indeed inhibition of ERK activation by the MEK1 inhibitor PD98059 was associated with a reduction of CTGF-promoted alpha-SMA protein expression (49).

A recent study indicated that CTGF induces the rapid transcriptional activation and synthesis of MKP-1 (MAPK phosphatase-1) in human mesangial cells. This in turn prevents the anti-apoptotic protein, Bcl-2, from being phosphorylated, leading to the survival of the cells. Knockout of MKP-1 protein in mesangial cells treated with CTGF, using siRNA or antisense oligonucleotides, allows p38 MAPK activation and induces mesangial cell death. Hence it is suggested that CTGF prolongs mesangial survival, thus enhancing the development of the kidney fibrosis (52).

The interaction between CTGF and the Ras and Rho family of GTPases has been of recent interest as it has been increasingly recognised that prenylated proteins serve as essential molecular switches in the downstream signalling of many cytokines involved in the regulation of renal fibroblast activity. CTGF has been shown to increase the association between Rho A and p27 (Kip-1). This results in an increase in phosphorylation of LIM kinase and subsequent phosphorylation of cofilin, suggesting that

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CTGF mediated p27 (Kip-1) activation results in uncoupling of the Rho A/LIM kinase/cofilin pathway (53). As this pathway has been shown to be integrally involved in tubular albumin uptake (54), it suggests CTGF may act to increase urinary albumin excretion. Both farnesyltransferase inhibitor BMS-191563 and geranylgeranyltransferase inhibitor GGTI-298 significantly reduced CTGF protein expression (55) and TGF-beta1-dependent CTGF promoter activity is reduced by inhibiting Ras and MEK activation. Differential signalling responses may be observed in normal cells versus those that have already been 'primed' to a fibrotic phenotype. Activation of cell specific signaling pathways, by low TGF-beta1 doses, was demonstrated with predominant activation of the Rho/ROCK pathway in cells derived from fibrotic models of disease, whereas the Smad pathway was primarily activated in normal cells. These results suggest a cell-specific regulation of the CTGF gene. In cells primed for fibrosis CTGF triggers its auto-induction and low concentrations of TGF-beta1 potentiate CTGF auto-induction. Recent studies in cultured vascular smooth muscle cells atorvastatin and simvastatin inhibited Ang II-induced CTGF production. The inhibitory effect of statins on CTGF upregulation was reversed by mevalonate and geranylgeranylpyrophosphate, suggesting that RhoA inhibition was involved in this process (56). Hence these studies support our data that both specific and combinatorial roles of low TGF-beta1 dose and CTGF exist (57). Furthermore, these data provide a mechanistic basis for the potential value of HMG-CoA reductase inhibitors in improving the outcome of renal disease (58).

5. CTGF AND RENAL FIBROSIS

CTGF has been reported to significantly increase in fibrotic diseases in the kidney, including diabetic nephropathy (13, 17, 29, 59). A recent study has determined in a mouse model the tissue distribution of increased CTGF expression and the relationship of plasma, urinary, and renal CTGF levels to the development and severity of diabetic nephropathy (60). The results showed increased CTGF mRNA in the kidneys of streptozotocin (STZ)-diabetes mellitus mice, with expression mainly localized to the epithelial cells of the glomerulus. After 9 weeks of STZ-DM, CTGF was also present in Bowman's capsule and in the mesangial area, in addition to persistent expression in podocytes (60). Because of this pattern of increase in the early stages of nephropathy, CTGF has also been implicated in renal hypertrophy. CTGF protein has been demonstrated in the mesangial cells, podocytes, vascular endothelial cells, interstitial cells and epithelial cells of various tubular segments in diabetic nephropathy (13, 39). At a tissue level CTGF mRNA and protein levels have been shown to be increased in the renal cortex of rats with streptozotocin-induced diabetes, particularly in areas of tubulointerstitial damage (61-64). CTGF is likely to play a crucial role in the deposition/degradation process of ECM deposition during tubulointerstitial fibrosis. TGFβ1 upregulated CTGF gene expression precedes the expression of alpha-smooth muscle actin, fibronectin and plasminogen activator inhibitor-1 (PAI-1). These markers of interstitial fibrosis were reversed in the presence of CTGF antisense

oligonucleotides (ODN) (65-68). This has been confirmed in a further study where the progression of interstitial fibrosis and myofibroblasts expressing alpha-smooth muscle actin were attenuated by CTGF antisense ODN (69). Both *in vitro* experiments and animal studies showed that tubular epithelium is an important source of CTGF in the fibrosing kidney. Intravenous administration of CTGF antisense ODN can significantly block CTGF expression in the proximal tubular epithelial cells in the remnant kidney of animals despite sustained levels of TGFβ1 mRNA. This reduced CTGF level paralleled a reduction of matrix molecules as well as PAI-1 and tissue inhibitor of metalloproteinase-1, which resulted in suppressed renal interstitial fibrogenesis (70). Our own group has demonstrated that TGFβ1-induced increases in extracellular matrix protein was inhibited in the presence of the effect of the anti-fibrotic compound tranilast. Tranilast reduced TGFβ1 induced CTGF mRNA and phosphorylation of Smad2 in proximal tubular cells and cortical fibroblasts (71).

In vivo studies conducted in an obstructive nephropathy model in rats demonstrated that TGFβ1 and CTGF mRNA expression in the obstructed kidney was upregulated in a co-ordinated manner from the early stage of interstitial fibrosis, followed by marked induction of fibronectin and alpha1 (I) collagen mRNA expression (29). CTGF antisense ODN transfection in cultured normal rat kidney fibroblast (NRK-49F) cells significantly attenuated TGFβ1-induced fibronectin and alpha1 (I) collagen mRNA expression (29). Further studies suggest that TGFβ1 also mediates both fibrosis and cytokine production in the proximal tubule of the kidney. However, CTGF plays a more specific role as a downstream mediator of TGFβ1 induced fibrosis (72, 73).

5.1. CTGF and epithelial to mesenchymal transition (EMT)

Tubular EMT is a complex process involving the disruption of polarized tubular epithelial cell morphology into cells with spindle-shaped mesenchymal morphology, formation of actin stress fibers, loss of cell-cell adhesion through downregulation of E-cadherin, destruction of basement membrane, and increased cell migration and invasion (74). During renal fibrosis new fibroblasts are derived mainly through EMT (75) and are hence reprogrammed to secrete and accumulate extracellular matrix (76). Many reviews have summarized the role of EMT in fibrotic renal disease (51, 74, 75, 77, 78). Recently CTGF has been implicated in EMT in the kidney. It has been suggested that growth factors TGFβ1/CTGF and bone morphogenic protein-7 (BMP-7)/hepatocyte growth factor (HGF) are the key determinants that influence the cellular phenotypes (79). As described, TGFβ and BMP-7 signalling pathways are tightly regulated through both positive and negative mechanisms and one of these negative feedback mechanisms is the production of inhibitory Smads (Smad6/7) (79). Smad6 preferentially inhibits BMP signalling, while Smad7 inhibits the TGFβ/activin signalling pathway (80). TGFβ1 and BMP-7 counteract the activity of each other by cross-inducing their respective inhibitory Smads (79). As discussed above

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CTGF reduces Smad7 thereby amplifying the downstream profibrotic consequences of TGF β 1 induced Smad2/3 signalling. HGF is a potent antifibrotic cytokine, which blocks TGF β 1 induced tubular EMT by upregulating the Smad transcriptional co-repressor SnoN expression (81). It has been reported that recombinant human BMP-7 leads to repair of renal tubular epithelial cells, in association with reversal of chronic renal injury by limiting EMT. BMP-7 has also been shown to block TGF β 1-dependent upregulation of PAI-1, leading to enhanced activation of MMP-2 and subsequent degradation of the fibrotic matrix (82) and release of VEGF from CTGF to promote angiogenesis (24).

In vivo, increased CTGF expression in the diabetic kidney co-localizes on the tubular epithelium with sites of EMT (63). It is now considered that at least part of the EMT induced by high glucose, AGEs and TGF β 1 are mediated through CTGF. *In vitro* exposure of the rat kidney tubular epithelial cell line (NRK-52E) to AGE modified bovine serum albumin increased the numbers of cells stained for the mesenchymal markers α -SMA and vimentin, which subsequently were reduced after treatment with CTGF siRNA. The role of CTGF as a downstream effector of TGF β 1 was confirmed as siRNA also reduced α -SMA protein expression in NRK-52E cells exposed to TGF β 1 (63).

Cross-sectional studies in both animal models and humans have shown that the magnitude of urinary CTGF-N (CTGF NH (2)-terminal fragment) excretion is related to the severity of diabetic nephropathy (83). A more recent study also evaluated urinary CTGF excretion in a large cross-sectional study of patients with type I diabetes (84). The observed association of urinary CTGF with urinary albumin excretion and glomerular filtration rate provides further evidence for its pathological role in renal disease. The correlate that interventions known to slow the progression of renal disease are associated with reduced renal CTGF expression was studied by assessing the effect of the angiotensin II receptor blocker Losartan on urinary CTGF levels in hypertensive patients with type I diabetes mellitus and nephropathy. This 3-year study demonstrated that Losartan persistently reduced urinary CTGF excretion, which was associated with a slower rate of decline in glomerular filtration rate (85).

5.2. CTGF and angiotensin II in kidney fibrosis

Both *in vivo* and *in vitro* evidence suggest that CTGF is involved in Ang II-induced renal pathology, as occurs in diabetic nephropathy. Ang II has been shown to induce CTGF expression in human kidney (HK2) cells in association with EMT, cell cycle arrest and cellular hypertrophy. Co-treatment with anti-CTGF antibody and CTGF antisense ODN attenuated these phenotypic changes (86-89). Several intracellular signals elicited by Ang II are involved in CTGF synthesis, including protein kinase C activation, reactive oxygen species, and TGF β 1 production. *In vivo*, rats infused with Ang II overexpress CTGF in glomeruli, tubules, and renal arteries, as well as demonstrating tubular injury and elevated fibronectin deposition (88, 89). A recent study showed that hypertension induced vascular and renal damage is

associated with stimulation of CTGF gene and protein content and CTGF is likely to be a downstream mediator of Ang II (90). In further models of proteinuric renal disease known to be associated with excess AngII production (immune complex nephritis), renal overexpression of CTGF is also diminished by treatment with an ACE inhibitor (88, 89).

5.3. CTGF and AGEs in kidney fibrosis

AGE-dependent pathways may play a role in the development of tubulointerstitial fibrosis in the diabetic kidney (91). AGEs interact with specific receptors (RAGE) and binding proteins to influence the expression of growth factors and cytokines, including TGF β 1 and CTGF, thereby regulating the growth and proliferation of the various renal cell types (92). Intravenous administration of AGE to rats induced significant matrix accumulation in glomeruli in association with increased CTGF, and direct exposure of rat mesangial cells to AGEs *in vitro* increased fibronectin and collagen IV production, which could be completely prevented by pretreatment with anti-CTGF antibody. However, inhibition of TGF β 1 mRNA expression by shRNA or neutralization of TGF β 1 protein by anti-TGF β 1 antibody did not prevent AGE-increased expression of CTGF, suggesting that AGE induced CTGF production was independent of TGF β 1 (93). It has been reported that AGE-induced mitogenesis and type I collagen production are dependent on the Ang II-JAK2-CTGF pathway in the rat proximal tubular line NRK-49F cells (94). AGE increased CTGF expression and angiotensinogen protein which was attenuated by a JAK2 inhibitor. Moreover, the ACE inhibitor captopril attenuated AGE-induced CTGF mRNA/protein expression while also attenuating AGE-induced mitogenesis and type I collagen production. The JAK2 inhibitor and CTGF antisense ODN attenuated Ang II and AGE-induced mitogenesis and type I collagen production (94). Hence it is likely that AGE-induced matrix production is a consequence of complex interactions with CTGF and the renin-angiotensin system.

6. CONCLUSION

CTGF has been definitively shown to be elaborated by almost all cells resident in the kidney under pathological conditions. This results in an altered cellular phenotype that contributes to renal fibrosis and ultimately proteinuria and impaired angiogenesis. The end result is renal fibrogenesis with progressive loss of renal function. CTGF is known to be directly stimulated by factors inherent in the diabetic milieu, such as angiotensin II and advanced glycation endproducts, and also elaborated downstream of the multifunctional cytokine TGF β 1, both mediating and amplifying its profibrotic effects. Speculation still exists as to whether a specific receptor exists for CTGF and the downstream signalling pathways. Whether targeting CTGF is a therapeutic option for progressive renal diseases is currently being assessed.

7. ACKNOWLEDGEMENTS

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